Innovation, Sciences et
Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and
Economic Development Canada
Canadian Intellectual Property Office

CA 3024643 C 2021/08/03

(11)(21) **3 024 643**

(12) BREVET CANADIEN
CANADIAN PATENT

(13) **C**

(22) **Date de dépôt/Filing Date:** 2010/03/12

(41) **Mise à la disp. pub./Open to Public Insp.:** 2010/09/16

(45) **Date de délivrance/Issue Date:** 2021/08/03

(62) **Demande originale/Original Application:** 2 755 301

(30) **Priorités/Priorities:** 2009/03/12 (US12/403,369);
2009/08/26 (US61/237,198)

(51) **Cl.Int./Int.Cl.** *G01N 33/48* (2006.01),
G01N 33/53 (2006.01)

(72) **Inventeurs/Inventors:**
STREEPER, ROBERT T., US;
BAEK, SUNG H., US;
IZBICKA, EIZBIETA, US

(73) **Propriétaire/Owner:**
CANCER PREVENTION AND CURE, LTD., US

(74) **Agent:** BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PROCEDES D'IDENTIFICATION, D'EVALUATION, DE PREVENTION ET DE THERAPIE DE MALADIES DES POUMONS ET LEURS KITS COMPRENANT UNE IDENTIFICATION, UNE EVALUATION, UNE PREVENTION ET UNE THERAPIE DE MALADIES BASEES SUR LE SEXE

(54) Title: METHODS OF IDENTIFICATION, ASSESSMENT, PREVENTION AND THERAPY OF LUNG DISEASES AND KITS THEREOF INCLUDING GENDER-BASED DISEASE IDENTIFICATION, ASSESSMENT, PREVENTION AND THERAPY

(57) **Abrégé/Abstract:**

The invention provides biomarkers and combinations of biomarkers useful in diagnosing lung diseases such as non-small cell lung cancer or reactive airway disease. The invention also provides methods of differentiating lung disease, methods of monitoring therapy, and methods of predicting a subjects response to therapeutic intervention based on the extent of expression of the biomarkers and combinations of biomarkers. Kits comprising agents for detecting the biomarkers and combination of biomarkers are also provided.

ABSTRACT

The invention provides biomarkers and combinations of biomarkers useful in diagnosing lung diseases such as non-small cell lung cancer or reactive airway disease. The invention also provides methods of differentiating lung disease, methods of monitoring therapy, and methods of predicting a subjects response to therapeutic intervention based on the extent of expression of the biomarkers and combinations of biomarkers. Kits comprising agents for detecting the biomarkers and combination of biomarkers are also provided.

METHODS OF IDENTIFICATION, ASSESSMENT, PREVENTION AND THERAPY OF LUNG DISEASES AND KITS THEREOF INCLUDING GENDER-BASED DISEASE IDENTIFICATION, ASSESSMENT, PREVENTION AND THERAPY

BACKGROUND OF THE INVENTION

(a) Field of the Invention

[001] The invention relates to the detection, identification, assessment, prevention, diagnosis, and treatment of lung disease using biomarkers and kits thereof. More specifically, the invention relates to the diagnosis of non-small cell lung cancers and reactive airway diseases by measuring and quantifying expression levels of specific biomarkers. The invention also relates to the identification of biomarkers present in human serum or other biological fluids, which, when found to be expressed at levels different from those found in the normal population, are indicative of pathologies associated with human lung tissues and the human respiratory system. By identifying the biomarkers associated with such pathologies, quantifying the expression levels of those biomarkers, and comparing the expression levels with those levels generally expected to present in a normal person's serum, it is possible to detect the presence of the pathologies early on in their progression through simple blood tests and characterize the progression of the pathology, as well as to differentiate among the pathologies.

(b) Description of the Related Art

[002] Pathologies of the respiratory system, such as asthma and lung cancer, affect millions of Americans. In fact, the American Lung Association® reports that almost 20 million Americans suffer from asthma. The American Cancer Society, Inc. estimated 229,400 new cancer cases of the respiratory system and 164,840 deaths from cancers of the respiratory system in 2007 alone. While the five year survival rate of all cancer cases when the cancer is detected while still localized is 46%, the five year survival rate of lung cancer patients is only 13%. Correspondingly, only 16% of lung cancers are discovered before the disease has spread. Lung cancers are generally categorized as two main types based on the pathology of the cancer cells. Each type is named for the types of cells that were transformed to become cancerous. Small cell lung cancers are derived from small cells in the human lung tissues, whereas non-small-cell lung cancers generally encompass all lung cancers that are not small-cell type. Non-small cell lung cancers are grouped together because the treatment is generally the same for all non-small-cell types. Together, non-small-cell lung cancers, or NSCLCs, make up about 75% of all lung cancers.

[003] A major factor in the low survival rate of lung cancer patients is the fact that lung cancer is difficult to diagnose early. Current methods of diagnosing lung cancer or identifying its existence in a human are restricted to taking X-rays, Computed Tomography (CT) scans and similar tests of the lungs to physically determine the presence or absence of a tumor. Therefore, the diagnosis of lung cancer is often made only in response to symptoms which have presented for a significant period of time, and after the disease has been present in the human long enough to produce a physically detectable mass.

[004] Similarly, current methods of detecting asthma are typically performed long after the presentation of symptoms such as recurrent wheezing, coughing, and chest tightness. Current methods of detecting asthma are typically restricted to lung function tests such as spirometry tests or challenge tests. Moreover, these tests are often ordered by the physician to be performed along with a multitude of other tests to rule out other pathologies or reactive airway diseases such as chronic obstructive pulmonary disease (COPD), bronchitis, pneumonia, and congestive heart failure.

[005] There does not exist in the art a simple, reliable method of diagnosing pathologies of human lung tissues early in their development. Furthermore, there is not a blood test available today which is capable of indicating the presence of a particular lung tissue pathology. It is therefore desirable to develop a method to determine the existence of lung cancers early in the disease progression. It is likewise desirable to develop a method to diagnose asthma and non-small cell lung cancer, and to differentiate them from each other and from other lung diseases such as infections, at the earliest appearance of symptoms. It is further desirable to identify specific proteins present in human blood which, when altered in terms of relative intensities of expression, are indicative of the presence of non-small cell lung cancers and/or reactive airway disease.

SUMMARY OF THE INVENTION

[006] The present inventors have identified a number of biomarkers which are useful for characterizing the physiologic state of a subject with regard to lung diseases, such as non-small cell lung cancer or reactive airway disease. These biomarkers are presented in Tables 1-23.

[007] Table 1A lists biomarkers whose expression level has been found to be different from the level in normal individuals when measured in individuals with one or more lung diseases. Table 1B lists biomarkers whose expression level has been found to be different from the level in normal individuals when measured in individuals with either non-small cell lung cancer or reactive airway disease, and to show a differential expression level between non-small cell lung cancer and reactive airway disease. Table 1C lists biomarkers whose expression has been found to be different from the level in normal individuals when measured in individuals with non-small cell lung cancer or with reactive airway disease.

[008] Table 2 lists biomarkers whose expression has been found to be different from the level in normal individuals when measured in individuals with reactive airway disease. Table 3 lists biomarkers whose expression has been found to be different from the level in normal individuals when measured in individuals with non-small cell lung cancer. Table 4 lists biomarkers whose expression levels have been found to be different when measured between individuals with non-small cell lung cancer and reactive airway disease.

[009] Table 5A lists biomarkers whose expression level has been found to be different from the level in normal males when measured in males with one or more lung diseases. Table 5B lists biomarkers whose expression level has been found to be different from the level in normal males when measured in males with either non-small cell lung cancer or reactive airway disease, and to show a differential expression level between non-small cell lung cancer and reactive airway disease. Table 5C lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with non-small cell lung cancer and reactive airway disease.

[010] Table 6 lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with reactive airway disease. Table 7 lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with non-small cell lung cancer. Table 8 lists biomarkers whose expression levels have been found to be different when measured between males with non-small cell lung cancer and reactive airway disease.

[011] Table 9A lists biomarkers whose expression level has been found to be different from the level in normal females when measured in females with one or more lung diseases. Table 9B lists biomarkers whose expression level has been found to be different from the level in normal females when measured in females with either non-small cell lung cancer or reactive airway disease, and to show a differential expression level between non-small cell lung cancer and reactive airway disease. Table 9C lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with non-small cell lung cancer and reactive airway disease.

[012] Table 10 lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with reactive airway disease. Table 11 lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with non-small cell lung cancer. Table 12 lists biomarkers whose expression levels have been found to be different when measured between females with non-small cell lung cancer and reactive airway disease.

[013] Table 13A lists biomarkers whose expression significantly differs between male and female reactive airway disease populations. Table 13B lists biomarkers whose expression does not significantly differ between male and female reactive airway disease populations. Table 14A lists biomarkers whose expression significantly differs between male and female non-small cell lung cancer populations. Table 14B lists biomarkers whose expression does not significantly differ between male and female non-small cell lung cancer populations. Table 15A lists biomarkers ranked by relative standard deviation in fluorescence intensity for the normal population. Table 15B lists biomarkers ranked by relative standard deviation in fluorescence intensity for the normal female population. Table 15C lists biomarkers ranked by relative standard deviation in fluorescence intensity for the normal male population.

[014] Table 16A lists biomarkers whose expression level has been found to be different from the level in normal males when measured in males with one or more lung diseases. Table 16B lists biomarkers whose expression level has been found to be different from the level in normal males when measured in males with either non-small cell lung cancer or reactive airway disease, and to show a differential expression level between non-small cell lung cancer and reactive airway disease. Table 16C lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with non-small cell lung cancer and reactive airway disease.

[015] Table 17 lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with reactive airway disease. Table 18 lists biomarkers whose expression has been found to be different from the level in normal males when measured in males with non-small cell lung cancer. Table 19 lists biomarkers whose expression levels have been found to be different when measured between males with non-small cell lung cancer and reactive airway disease.

[016] Table 20A lists biomarkers whose expression level has been found to be different from the level in normal females when measured in females with one or more lung diseases. Table 20B lists biomarkers whose expression level has been found to be different from the level in normal females when measured in females with either non-small cell lung cancer or reactive airway disease, and to show a differential expression level between non-small cell lung cancer and reactive airway disease. Table 20C lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with non-small cell lung cancer and reactive airway disease.

[017] Table 21 lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with reactive airway disease. Table 22 lists biomarkers whose expression has been found to be different from the level in normal females when measured in females with non-small cell lung cancer. Table 23 lists biomarkers whose expression levels have been found to be different when measured between females with non-small cell lung cancer and reactive airway disease.

[018] Significance for Tables 1-15 were determined using the Student's t test. Significance for Tables 16-23 were determined using the Kruskal-Wallis method.

[019] Polypeptides comprising SEQ ID NOS: 1-17 are additional biomarkers whose expression has been found to change with one or more lung diseases.

[020] The present invention provides various diagnostic, prognostic and therapeutic methods which depend on the identification of these biomarkers.

[021] The invention provides for a method of physiological characterization in a subject comprising determining the extent of expression of at least one biomarker from any number of Tables 1-12 or 16-23 in a physiological sample of the subject, wherein the extent of expression of said at least one biomarker is indicative of a lung disease, such as of non-small cell lung cancer or reactive airway disease, or can assist in distinguishing lung diseases, such as of non-small cell lung cancer or reactive airway disease. The invention also provides for methods of physiological characterization in a subject comprising determining the extent of expression of at least one biomarker from Tables 13B, 14B, or 15B, which also appears on Tables 1-12 or 16-23 in a physiological sample of the subject, preferably the biomarker is at least one of biomarker nos. 1-10 of Tables 1-12 or 16-23, wherein the extent of expression of said at least one biomarker is indicative of a lung disease, such as of non-small cell lung cancer or reactive airway disease. Alternatively, or additionally, the extent of expression of the first order interactors of these biomarkers may be determined.

[022] The invention provides for a method of physiological characterization in a subject comprising determining the extent of expression of SEQ ID NO: 12 in a physiological sample of the subject, wherein the extent of expression of SEQ ID NO: 12 is indicative of a lung disease, such as non-small cell lung cancer or reactive airway disease.

[023] The invention provides for a method of physiological characterization in a subject comprising determining the extent of expression of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17 in a physiological sample of the subject, and determining the extent of expression of at least one biomarker from any number of Tables 1-12

or 16-23, wherein the extent of expression of said at least one polypeptide and said at least one biomarker from any number of Tables 1-12 or 16-23 is indicative of a lung disease, such as non-small cell lung cancer or reactive airway disease.

[024] The invention provides for a method of diagnosing reactive airway disease in a subject comprising determining the extent of expression of at least one biomarker from Table 2, Table 6, Table 10, Table 17, and Table 21 in a physiological sample of the subject, wherein the extent of expression of said at least one biomarker is indicative of reactive airway disease.

[025] The invention provides for a method of diagnosing non-small cell lung cancer in a subject comprising determining the extent of expression at least one biomarker from Table 3, Table 7, Table 11, Table 18, or Table 22 in a physiological sample of the subject, wherein the extent of expression of said at least one biomarker is indicative of the presence or development of non-small cell lung cancer.

[026] The invention provides a diagnostic method to assist in differentiating the likelihood that a subject is at-risk of non-small cell lung cancer or of reactive airway disease comprising determining the extent of expression of at least one biomarker from Table 4, Table 8, Table 12, Table 19, or Table 23 in a physiological sample of the subject who is at-risk for at least one of non-small cell lung cancer or reactive airway disease, wherein the extent of expression of said at least one biomarker from Table 4, Table 8, Table 12, Table 19, or Table 23 assists in differentiating the likelihood that said subject is at-risk of non-small cell lung cancer or of reactive airway disease.

[027] The invention provides a method for predicting the likelihood that a subject will respond to therapeutic intervention comprising determining the extent of expression of at least one biomarker described herein in a physiological sample of the subject, wherein the extent of expression of said at least one biomarker assists in predicting a subject's response to said therapeutic intervention.

[028] The invention also provides a method of monitoring a subject comprising determining a first extent of expression of at least one biomarker described herein in a physiological sample of the subject, a second extent of expression of said at least one biomarker in a physiological sample of the subject at a subsequent time to said first determination, and comparing said first extent of expression and said second extent of expression.

[029] The invention also provides for methods of designing kits comprising selecting at least one biomarker described herein, selecting a means for determining the extent of expression of said at least one biomarker, and designing a kit comprising said means for determining the extent of expression.

[030] The invention also provides for methods of designing kits comprising selecting at least one biomarker described herein, selecting detection agents for detecting said at least one biomarker, and designing a kit comprising said detection agents for detecting at least one biomarker.

[031] The invention also provides kits comprising at least one biomarker described herein.

[032] The invention also provides a kit comprising a means for determining the extent of expression of at least one polypeptide selected from the group consisting of SEQ ID NO: 12.

[033] The invention also provides a kit comprising, detection agents for detecting at least one polypeptide selected from the group consisting of SEQ ID NO: 12.

[034] The invention also provides a kit comprising, (a) means for determining the extent of expression of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and (b) means for determining the extent of expression of at least one biomarker from anyone of Tables 1-12 or Tables 16-23.

[035] The invention also provides a kit comprising, (a) detection agents for detecting at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and (b) detection agents for detecting at least one biomarker from anyone of Tables 1-12 or Tables 16-23.

[036] The invention further provides for kits containing biomarkers and/or polypeptides from a plurality of the above Tables.

BRIEF DESCRIPTION OF THE DRAWINGS

[037] FIG. 1A shows the average fluorescence intensity level of the biomarkers in the normal (NO) population from Example 1, as well as the standard deviation and relative standard deviation.

[038] FIG. 1B shows the average fluorescence intensity level of the biomarkers in the non-small cell lung cancer (LC) population from Example 1, as well as the standard deviation and relative standard deviation.

[039] FIG. 1C shows the average fluorescence intensity level of the biomarkers in the asthma (AST) population from Example 1, as well as the standard deviation and relative standard deviation.

[040] FIG. 1D shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the LC population v. NO population, AST population v. NO population, and the LC population v. AST population from Example 1.

[041] FIG. 1E shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the populations from Example 1, where the populations to be compared are LC population v. NO population, AST population v. NO population, and the LC population v. AST population, respectively.

[042] FIG. 2A shows the average fluorescence intensity level of the biomarkers in the normal (NO) population from Example 2, as well as the standard deviation and relative standard deviation.

[043] FIG. 2B shows the average fluorescence intensity level of the biomarkers in the non-small cell lung cancer (LC) population from Example 2, as well as the standard deviation and relative standard deviation.

[044] FIG. 2C shows the average fluorescence intensity level of the biomarkers in the asthma (AST) population from Example 2, as well as the standard deviation and relative standard deviation.

[045] FIG. 2D shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the LC population v. NO population, AST population v. NO population, and the AST v. LC population from Example 2.

[046] FIG. 2E shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the populations from Example 2, where the populations to be compared are LC population v. NO population, AST population v. NO population, and the AST population v. LC population, respectively.

[047] FIG. 3A shows the average fluorescence intensity level of the biomarkers in the normal (NO) population from Example 3, as well as the standard deviation and relative standard deviation.

[048] FIG. 3B shows the average fluorescence intensity level of the biomarkers in the non-small cell lung cancer (LC) population from Example 3, as well as the standard deviation and relative standard deviation.

[049] FIG. 3C shows the average fluorescence intensity level of the biomarkers in the asthma (AST) population from Example 3, as well as the standard deviation and relative standard deviation.

[050] FIG. 3D shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the AST population v. NO population, LC population v. NO populations, and the AST population v. LC population from Example 3.

[051] FIG. 3E shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the populations from Example 3, where the populations to be compared are AST population v. NO population, LC population v. NO population, and the AST v. LC population, respectively.

[052] FIG. 4A shows the average fluorescence intensity level of the biomarkers in the normal (NO) female population from Example 3, as well as the standard deviation and relative standard deviation.

[053] FIG. 4B shows the average fluorescence intensity level of the biomarkers in the non-small cell lung cancer (LC) female population from Example 3, as well as the standard deviation and relative standard deviation.

[054] FIG. 4C shows the average fluorescence intensity level of the biomarkers in the asthma (AST) female population from Example 3, as well as the standard deviation and relative standard deviation.

[055] FIG. 4D shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the AST population v. NO female population, LC population v. NO female population, and the AST population v. LC female population from Example 3.

[056] FIG. 4E shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the female populations from Example 3, where the populations to be compared are AST population v. NO female population, LC population v. NO female population, and the AST population v. LC female population, respectively.

[057] FIG. 5A *shows the average fluorescence intensity level of the biomarkers in the* normal (NO) male population from Example 3, as well as the standard deviation and relative standard deviation.

[058] FIG. 5B shows the average fluorescence intensity level of the biomarkers in the non-small cell lung cancer (LC) male population from Example 3, as well as the standard deviation and relative standard deviation.

[059] FIG. 5C shows the average fluorescence intensity level of the biomarkers in the asthma (AST) male population from Example 3, as well as the standard deviation and relative standard deviation.

[060] FIG. 5D shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the AST population v. NO male population, LC population v. NO male population, and the AST population v. LC male population from Example 3.

[061] FIG. 5E shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the male populations from Example 3, where the populations to be compared are AST v. NO male populations, LC v. NO male populations, and the LC v. AST male populations, respectively.

[062] FIG. 6A shows the percent change in the mean of fluorescence intensity for each of the biomarkers in the AST male population compared to the AST female population, the LC male population compared to the LC female population, and the NO male population compared to the NO female population from Example 3.

[063] FIG. 6B shows the probability associated with Student's t values obtained by comparing the mean fluorescence intensity and variability measured for each biomarker in the male and female populations from Example 3, where the populations to be compared are the AST male and female populations, LC male and female populations, and the NO male and female populations, respectively.

[064] Figure 7A shows the percent change in the mean concentration of each of the biomarkers in the LC v. NO female populations, AST v. NO female populations, and the AST v. LC female populations of Example 3.

[065] FIG. 7B shows the probability associated with the Kruskal-Wallis test calculated by comparing the concentration measured for each biomarker in the female populations of Example 3, where the populations to be compared are AST v. NO female populations, LC v. NO female populations, and the AST v. LC female populations, respectively.

[066] Figure 8A shows the percent change in the mean concentration of each of the biomarkers in the LC v. NO male populations, AST v. NO male populations, and the AST v. LC male populations of Example 3.

[067] FIG. 8B shows the probability associated with the Kruskal-Wallis test calculated by comparing the concentration measured for each biomarker in the male populations of Example 3, where the populations to be compared are AST v. NO male populations, LC v. NO male populations, and the AST v. LC male populations, respectively.

[068] FIG 9 shows relationships between the biomarkers of Table 16B.

DETAILED DESCRIPTION OF THE INVENTION

[069] The invention relates to various methods of detection, identification, assessment, prevention, diagnosis, and treatment of lung disease using biomarkers including gender-based disease detection, identification, assessment, prevention, and diagnosis, and treatment. These methods involve determining the extent of expression of specific biomarkers for which an altered expression is indicative of non-small cell lung cancer and/or reactive airway disease (*e.g.*, asthma, chronic obstructive pulmonary disease, etc.). The invention also provides for various kits comprising detection agents for detecting these biomarkers, or means for determining the extent of expression of these biomarkers.

Definitions

[070] As used herein, a "biomarker" or "marker" is a biological molecule that is objectively measured as a characteristic indicator of the physiological status of a biological system. For purposes of the present disclosure biological molecules include ions, small molecules, peptides, proteins, peptides and proteins bearing post-translational modifications, nucleosides, nucleotides and polynucleotides including RNA and DNA, glycoproteins, lipoproteins, as well as various covalent and non-covalent modifications of these types of molecules. Biological molecules include any of these entities native to, characteristic of, and/or essential to the function of a biological system. The majority of biomarkers are polypeptides, although they may also be mRNA or modified mRNA which represents the pre-translation form of the gene product expressed as the polypeptide, or they may include post-translational modifications of the polypeptide.

[071] As used herein, a "subject" means any animal, but is preferably a mammal, such as, for example, a human. In many embodiments, the subject will be a human patient having, or at-risk of having, a lung disease.

[072] As used herein, a "physiological sample" includes samples from biological fluids and tissues. Biological fluids include whole blood, blood plasma, blood serum, sputum, urine, sweat, lymph, and alveolar lavage. Tissue samples include biopsies from solid lung tissue or other solid tissues, lymph node biopsy tissues, biopsies of metastatic foci. Method of obtaining physiological samples are well known.

[073] As used herein, "therapeutic intervention" includes administration of one or more therapeutic agents such as a small molecule or macromolecule, radiation, surgery, or any combinations thereof.

[074] As used herein, "detection agents" include reagents and systems that specifically detect the biomarkers described herein. Detection agents include reagents such as antibodies, nucleic acid probes, aptamers, lectins, or other reagents that have specific affinity for a particular marker or markers sufficient to discriminate between the particular marker and other markers which might be in samples of interest, and systems such as sensors, including sensors making use of bound or otherwise immobilized ligands as described above.

Identification of Biomarkers

[075] The biomarkers of the invention were identified using two methods. First, identification of biomarkers indicative of non-small cell lung cancers and/or asthma was made by comparing the measured expression levels of fifty-nine selected biomarkers in the plasma of patients from populations who had been diagnosed with those respective pathologies to a population who had not been diagnosed with the pathologies, as confirmed by a physician. This method is detailed in Examples 1-3.

[076] Second, biomarkers were identified using mass spectrometry. Identification of proteins indicative of non-small cell lung cancers and/or asthma was made by comparing the mass spectral data for tryptic peptide digests of samples obtained from patients in different physiological states. In particular, the data was the mass of peptide fragments, represented as graphical indications of the intensities of the pseudo or protonated molecular ion signals of peptides and proteins containing those fragments expressed across time in a single dimension. The expression levels of thousands of proteins were compared, resulting in the identification of seventeen proteins which were expressed in substantially differing intensities between populations of individuals not having any diagnosed lung tissue pathologies, populations of individuals having asthma, as diagnosed by a physician, and populations of individuals having non-small cell lung cancers, as diagnosed by a physician. This method is detailed in Examples 6 and 7.

First Order Interactors

[077] To promote and control the multitude of cellular and organismal physiological functions necessary to maintain life, biological molecules must interact with each other. These interactions can be considered a type of communication. In this communication the various biological molecules can be considered messages. These molecules, as a necessary part of their signal transduction functions, necessarily interact with a broad variety of targets including other types of biological molecules.

[078] One type of interacting molecule is commonly known as a receptor. Another type of direct intermolecular interaction is the binding of a co-factor to an enzyme. These intermolecular interactions form networks of signaling molecules that work together to carry out and control the essential life functions of cells and organisms. The particular biomarkers of this invention are linked physiologically to other biomarkers whose level increases or decreases in a fashion coordinated with the level of particular biomarkers. These other biomarkers are called "first order interactors" with respect to the particular biomarkers of the invention.

[079] "First order interactors" are those molecular entities that interact directly with a particular biological molecule. For instance the drug morphine interacts directly with opiate receptors resulting ultimately in the diminishment of the sensation of pain. Thus, the opiate receptors are first order interactors under the definition of "first order interactor." First order interactors include both upstream and downstream direct neighbors for said biomarkers in the communication pathways in which they interact. These entities encompass proteins, nucleic acids and small molecules which may be connected by relationships that include but are not limited to direct (or indirect) regulation, expression, chemical reaction, molecular synthesis, binding, promoter binding, protein modification and molecular transport. Groups of biomarkers whose levels are coordinated are well known to those skilled in the art and those knowledgeable in physiology and cellular biology. Indeed, first order interactors for a particular biomarker are known in the art and can found using various databases and available bioinformatics software such as ARIADNE PATHWAY STUDIO, ExPASY Proteomics Server Qlucore Omics Explorer, Protein Prospector, PQuad, ChEMBL, and others. (*see, e.g.*, ARIADNE PATHWAY STUDIO, Ariadne, Inc., <www.ariadne.genomics.com> or ChEMBL Database, European Bioinformatics Institute, European Molecular Biology Laboratory, <www.ebi.ac.uk>).

[080] When the levels of the particular biomarkers of this invention are abnormal, levels of first order interactor biomarkers whose expression is coordinated with the particular biomarkers are also abnormal. Therefore, determination that levels of a particular biomarker are abnormal may be accomplished by measuring the level of a first order interactor coordinated therewith. The skilled person will of course confirm that the level of a first order interactor which is used in lieu or in addition to a particular biomarker will vary in a defined and reproducible way consistent with the behavior of the particular biomarker.

[081] The invention provides that for any of the methods described herein, the methods to be performed with a particular biomarker may alternatively be performed with the first order interactors of that particular biomarker. For example, the invention provides for methods of physiological characterization comprising determining the extent of expression of HGF. As such, the invention also provides for methods of physiological characterization comprising determining the extent of expression of a first order interactor of HGF. The first order interactors of HGF include, but are not limited to those identified in Example 12.

Tables Identifying Significant Biomarkers

[082] Table 1A lists biomarkers whose expression levels have a significant or marginally significant difference between at least one of AST v. NO populations, LC v. NO populations, and AST v. LC populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the significance and magnitude of the difference in fluorescence intensity.

TABLE 1A

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE			
No.	Biomarker	No.	Biomarker
1	IL-13	28	MMP-12
2	I-TAC	29	PAI-1
3	MCP-1	30	Amylin (Total)
4	MMP-1	31	IL-1α
5	MPO	32	sFSl
6	HGF	33	IL-4
7	Eotaxin	34	MIP-1β
8	MMP-9	35	IL-10
9	MMP-7	36	SE-selectin
10	IP-10	37	IL-17
11	SAA	38	GM-CSF
12	Resistin	39	G-CSF
13	IL-5	40	TGF-α
14	Leptin	41	IFN-γ
15	sVCAM-1	42	Fractalkine
16	Adiponectin	43	VEGF
17	CRP	44	IL-7
18	C-Peptide	45	IL-12 (p40)
19	MMP-3	46	Sfas
20	SAP	47	MIF
21	IL-1ra	48	IL-1β
22	IL-15	49	IL-2
23	EGF	50	MIP-1α
24	IL12 (p70)	51	Insulin
25	MMP-8	52	GLP-1
26	IL-8	53	sCD40 ligand
27	IL-6		

[083] Table 1B lists biomarkers whose expression levels have a significant difference between the AST v. NO populations, LC v. NO populations, and AST v. LC populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 1B

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE	
No.	Biomarker
1	IL-13
2	I-TAC
3	MCP-1
4	MMP-1
5	MPO
6	HGF
7	Eotaxin
8	MMP-9
9	MMP-7
10	IP-10
11	SAA
12	Resistin
13	IL-5
14	Leptin
15	sVCAM-1
16	Adiponectin
17	CRP
18	C-Peptide
19	MMP-3
20	SAP
21	IL-1ra
22	IL-15

[084] Table 1C lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO populations and LC v. NO populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 1C

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE	
No.	Biomarker
1	EGF
2	IL12 (p70)
3	IL-8
4	IL-6
5	MMP-12
6	PAI-1
7	Amylin (Total)
8	IL-4
9	MIP-1β
10	IL-10
11	SE-selectin
12	IL-17
13	GM-CSF
14	G-CSF
15	TGF-α
16	IFN-γ
17	Fractalkine
18	VEGF
19	IL-12 (p40)
20	IL-7
21	Insulin

[085] Table 2 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 2

<table>
<tr><th colspan="4">SIGNIFICANT BIOMARKERS INDICATIVE OF REACTIVE AIRWAY DISEASE</th></tr>
<tr><th><u>No.</u></th><th><u>Biomarker</u></th><th><u>No.</u></th><th><u>Biomarker</u></th></tr>
<tr><td>1</td><td>IL-13</td><td>24</td><td>IL-7</td></tr>
<tr><td>2</td><td>I-TAC</td><td>25</td><td>sVCAM-1</td></tr>
<tr><td>3</td><td>EGF</td><td>26</td><td>SE-selectin</td></tr>
<tr><td>4</td><td>MCP-1</td><td>27</td><td>Leptin</td></tr>
<tr><td>5</td><td>HGF</td><td>28</td><td>Adiponectin</td></tr>
<tr><td>6</td><td>MPO</td><td>29</td><td>IL-17</td></tr>
<tr><td>7</td><td>IL12 (p70)</td><td>30</td><td>CRP</td></tr>
<tr><td>8</td><td>MMP-9</td><td>31</td><td>GM-CSF</td></tr>
<tr><td>9</td><td>IL-8</td><td>32</td><td>MIP-1β</td></tr>
<tr><td>10</td><td>Eotaxin</td><td>33</td><td>TGF-α</td></tr>
<tr><td>11</td><td>IL-6</td><td>34</td><td>IL-10</td></tr>
<tr><td>12</td><td>IP-10</td><td>35</td><td>Fractalkine</td></tr>
<tr><td>13</td><td>IL-1α</td><td>36</td><td>IFN-γ</td></tr>
<tr><td>14</td><td>PAI-1</td><td>37</td><td>C-Peptide</td></tr>
<tr><td>15</td><td>Resistin</td><td>38</td><td>VEGF</td></tr>
<tr><td>16</td><td>sFSI</td><td>39</td><td>G-CSF</td></tr>
<tr><td>17</td><td>IL-5</td><td>40</td><td>IL-1ra</td></tr>
<tr><td>18</td><td>Amylin (Total)</td><td>41</td><td>IL-15</td></tr>
<tr><td>19</td><td>MMP-1</td><td>42</td><td>MMP-3</td></tr>
<tr><td>20</td><td>MMP-12</td><td>43</td><td>IL-12 (p40)</td></tr>
<tr><td>21</td><td>IL-4</td><td>44</td><td>SAP</td></tr>
<tr><td>22</td><td>SAA</td><td>45</td><td>Insulin</td></tr>
<tr><td>23</td><td>MMP-7</td><td></td><td></td></tr>
</table>

[086] Table 3 lists biomarkers whose expression levels have a significant or marginally significant difference between the LC v. NO populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 3

SIGNIFICANT BIOMARKERS FOR NON-SMALL CELL LUNG CANCER (NSCLC)			
No.	Biomarker	No.	Biomarker
1	**IL-13**	25	**IL-5**
2	**EGF**	26	**IL-4**
3	**I-TAC**	27	**Leptin**
4	**MMP-1**	28	**SE-selectin**
5	**IL12 (p70)**	29	**MIP-1α**
6	**Eotaxin**	30	**C-Peptide**
7	**MMP-8**	31	**IL-1ra**
8	**MCP-1**	32	**SAP**
9	**MPO**	33	**G-CSF**
10	**IP-10**	34	**IL-17**
11	**SAA**	35	**MMP-3**
12	**HGF**	36	**IFN-γ**
13	**MMP-9**	37	**TGF-α**
14	**MMP-12**	38	**sVCAM-1**
15	**Amylin (Total)**	39	**IL-15**
16	**PAI-1**	40	**GM-CSF**
17	**MMP-7**	41	**Fractalkine**
18	**IL-6**	42	**IL-1β**
19	**MIP-1β**	43	**VEGF**
20	**Adiponectin**	44	**GLP-1**
21	**IL-10**	45	**IL-7**
22	**CRP**	46	**Insulin**
23	**Resistin**	47	**IL-12 (p40)**
24	**MIF**	48	**IL-8**

[087] Table 4 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. LC populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 4

SIGNIFICANT BIOMARKERS DISTINGUISHING BETWEEN REACTIVE AIRWAY DISEASE AND NSCLC			
No.	**Biomarker**	**No.**	**Biomarker**
1	**MMP-7**	17	**MMP-3**
2	**MMP-1**	18	**IL-5**
3	**SAA**	19	**SAP**
4	**MMP-8**	20	**Eotaxin**
5	**IL-8**	21	**MMP-9**
6	**MCP-1**	22	**CRP**
7	**Leptin**	23	**Adiponectin**
8	**IL-1α**	24	**IP-10**
9	**HGF**	25	**IL-1ra**
10	**I-TAC**	26	**Sfas**
11	**sVCAM-1**	27	**IL-2**
12	**MPO**	28	**IL-15**
13	**sFSl**	29	**IL12 (p70)**
14	**C-Peptide**	30	**IL-6**
15	**IL-13**	31	**sCD40 ligand**
16	**Resistin**	32	**VEGF**

[088] Table 5A lists biomarkers whose expression levels have a significant or marginally significant difference between at least one of AST v. NO male populations, LC v. NO male populations, and AST v. LC male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the significance and magnitude of the difference in fluorescence intensity.

TABLE 5A

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	I-TAC	27	IL-5
2	MPO	28	Resistin
3	HGF	29	IL-1β
4	MMP-1	30	IL-7
5	MMP-8	31	IL-4
6	Eotaxin	32	MIP-1β
7	IL-8	33	Leptin
8	MMP-7	34	GM-CSF
9	PAI-1	35	G-CSF
10	IP-10	36	TGF-α
11	sVCAM-1	37	IL-17
12	IL-10	38	CRP
13	Adiponectin	39	IL-15
14	SAP	40	VEGF
15	IFN-γ	41	Fractalkine
16	IL-13	42	MMP-3
17	EGF	43	IL-12 (p40)
18	MCP-1	44	C-Peptide
19	MIF	45	IL-1ra
20	IL-12(p70)	46	GLP-1
21	MMP-9	47	MIP-1α
22	IL-6	48	sFSl
23	Amylin (Total)	49	Insulin
24	SAA	50	Sfas
25	IL-1α	51	SE-selectin
26	TNF-α	52	MMP-12

[0089] Table 5B lists biomarkers whose expression levels have a significant difference between the AST v. NO male populations, LC v. NO male populations, and AST v. LC male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 5B

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION	
No.	**Biomarker**
1	**I-TAC**
2	**MPO**
3	**HGF**
4	**MMP-1**
5	**MMP-8**
6	**Eotaxin**
7	**IL-8**
8	**MMP-7**
9	**PAI-1**
10	**IP-10**
11	**sVCAM-1**
12	**IL-10**
13	**Adiponectin**
14	**SAP**
15	**IFN-γ**

[090] Table 5C lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO male populations and LC v. NO male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 5C

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION	
No.	**Biomarker**
1	IL-13
2	EGF
3	MCP-1
4	MIF
5	IL-12(p70)
6	MMP-9
7	IL-6
8	TNF-α
9	IL-5
10	Resistin
11	IL-1β
12	IL-7
13	IL-4
14	MIP-1β
15	Leptin
16	GM-CSF
17	G-CSF
18	TGF-α
19	IL-17
20	IL-15
21	VEGF
22	Fractalkine
23	IL-12 (p40)
24	MIP-1α

[091] Table 6 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 6

<table>
<tr><th colspan="4">SIGNIFICANT BIOMARKERS FOR REACTIVE AIRWAY DISEASE IN THE MALE POPULATION</th></tr>
<tr><th>No.</th><th>Biomarker</th><th>No.</th><th>Biomarker</th></tr>
<tr><td>1</td><td>IL-13</td><td>23</td><td>IL-4</td></tr>
<tr><td>2</td><td>I-TAC</td><td>24</td><td>IP-10</td></tr>
<tr><td>3</td><td>MPO</td><td>25</td><td>MIP-1β</td></tr>
<tr><td>4</td><td>HGF</td><td>26</td><td>GM-CSF</td></tr>
<tr><td>5</td><td>EGF</td><td>27</td><td>G-CSF</td></tr>
<tr><td>6</td><td>MCP-1</td><td>28</td><td>TGF-α</td></tr>
<tr><td>7</td><td>IL-8</td><td>29</td><td>Leptin</td></tr>
<tr><td>8</td><td>MIF</td><td>30</td><td>IL-17</td></tr>
<tr><td>9</td><td>IL-6</td><td>31</td><td>sVCAM-1</td></tr>
<tr><td>10</td><td>MMP-9</td><td>32</td><td>GLP-1</td></tr>
<tr><td>11</td><td>IL-12(p70)</td><td>33</td><td>IL-15</td></tr>
<tr><td>12</td><td>Eotaxin</td><td>34</td><td>MMP-7</td></tr>
<tr><td>13</td><td>IL-1α</td><td>35</td><td>VEGF</td></tr>
<tr><td>14</td><td>PAI-1</td><td>36</td><td>IL-10</td></tr>
<tr><td>15</td><td>MMP-8</td><td>37</td><td>Fractalkine</td></tr>
<tr><td>16</td><td>TNF-α</td><td>38</td><td>IL-12 (p40)</td></tr>
<tr><td>17</td><td>IL-5</td><td>39</td><td>IFN-γ</td></tr>
<tr><td>18</td><td>MMP-1</td><td>40</td><td>Adiponectin</td></tr>
<tr><td>19</td><td>IL-1β</td><td>41</td><td>SE-selectin</td></tr>
<tr><td>20</td><td>sFS1</td><td>42</td><td>SAP</td></tr>
<tr><td>21</td><td>Resistin</td><td>43</td><td>MIP-1α</td></tr>
<tr><td>22</td><td>IL-7</td><td></td><td></td></tr>
</table>

[092] Table 7 lists biomarkers whose expression levels have a significant or marginally significant difference between the LC v. NO male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 7

SIGNIFICANT BIOMARKERS FOR NSCLC IN THE MALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**IL-13**	25	**CRP**
2	**I-TAC**	26	**IL-10**
3	**EGF**	27	**Adiponectin**
4	**MPO**	28	**IL-7**
5	**HGF**	29	**IL-4**
6	**MMP-1**	30	**MMP-3**
7	**MMP-8**	31	**G-CSF**
8	**MIF**	32	**MIP-1α**
9	**Eotaxin**	33	**IL-17**
10	**IL-12(p70)**	34	**IFN-γ**
11	**MCP-1**	35	**IL-1ra**
12	**MMP-9**	36	**C-Peptide**
13	**PAI-1**	37	**TGF-α**
14	**SAA**	38	**IL-15**
15	**IP-10**	39	**Fractalkine**
16	**Amylin (Total)**	40	**IL-1β**
17	**MMP-7**	41	**GM-CSF**
18	**Resistin**	42	**sVCAM-1**
19	**IL-6**	43	**SAP**
20	**MIP-1β**	44	**VEGF**
21	**TNF-α**	45	**IL-12 (p40)**
22	**Leptin**	46	**Insulin**
23	**IL-8**	47	**MMP-12**
24	**IL-5**		

[093] Table 8 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. LC male populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 8

SIGNIFICANT BIOMARKERS DISTINGUISHING BETWEEN REACTIVE AIRWAY DISEASE AND NSCLC IN THE MALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**MMP-1**	15	**SAP**
2	**MMP-8**	16	**HGF**
3	**MMP-7**	17	**C-Peptide**
4	**Amylin (Total)**	18	**I-TAC**
5	**SAA**	19	**Sfas**
6	**IL-8**	20	**PAI-1**
7	**Insulin**	21	**IL-1ra**
8	**IL-1α**	22	**Adiponectin**
9	**sVCAM-1**	23	**IFN-γ**
10	**IP-10**	24	**IL-10**
11	**CRP**	25	**GLP-1**
12	**MPO**	26	**IL-6**
13	**MMP-3**	27	**IL-13**
14	**Eotaxin**	28	**IL-15**

[094] Table 9A lists biomarkers whose expression levels have a significant or marginally significant difference between at least one of AST v. NO female populations, LC v. NO female populations, and AST v. LC female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the significance and magnitude of the difference in fluorescence intensity.

TABLE 9A

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	I-TAC	27	G-CSF
2	Leptin	28	SAP
3	IP-10	29	MMP-3
4	MMP-7	30	GM-CSF
5	SAA	31	sICAM-1
6	MPO	32	TNF-α
7	Eotaxin	33	IL-10
8	MMP-9	34	MIP-1β
9	Adiponectin	35	IL-1α
10	CRP	36	sCD40 ligand
11	C-Peptide	37	IL-6
12	sVCAM-1	38	MMP-12
13	IL-15	39	MMP-2
14	IL-1ra	40	IL-5
15	IL-13	41	IL-4
16	EGF	42	Sfas
17	IL-12(p70)	43	MMP-8
18	MCP-1	44	IL-1β
19	MMP-1	45	IL-12 (p40)
20	HGF	46	IL-2
21	IL-8	47	VEGF
22	Resistin	48	TGF-α
23	sFSl	49	IFN-γ
24	PAI-1	50	GLP-1
25	MIF	51	Amylin (Total)
26	SE-selectin	52	Insulin

[095] Table 9B lists biomarkers whose expression levels have a significant difference between the AST v. NO female populations, LC v. NO female populations, and AST v. LC female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 9B

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION	
No.	**Biomarker**
1	**I-TAC**
2	**Leptin**
3	**IP-10**
4	**MMP-7**
5	**SAA**
6	**MPO**
7	**Eotaxin**
8	**MMP-9**
9	**Adiponectin**
10	**CRP**
11	**C-Peptide**
12	**sVCAM-1**
13	**IL-15**
14	**IL-1ra**

[096] Table 9C lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO female populations and LC v. NO female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 9C

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION	
No.	**Biomarker**
1	**IL-13**
2	**EGF**
3	**IL-12(p70)**
4	**MCP-1**
5	**PAI-1**
6	**MIF**
7	**SE-selectin**
8	**G-CSF**
9	**GM-CSF**
10	**sICAM-1**
11	**IL-2**
12	**TGF-α**

[097] Table 10 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. NO female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 10

SIGNIFICANT BIOMARKERS FOR REACTIVE AIRWAY DISEASE IN THE FEMALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**IL-13**	24	**C-Peptide**
2	**I-TAC**	25	**IL-4**
3	**EGF**	26	**Adiponectin**
4	**MCP-1**	27	**Sfas**
5	**Leptin**	28	**TNF-α**
6	**IL-12(p70)**	29	**G-CSF**
7	**IP-10**	30	**MIP-1β**
8	**MPO**	31	**MMP-3**
9	**HGF**	32	**IL-15**
10	**MMP-9**	33	**IL-12 (p40)**
11	**Eotaxin**	34	**IL-2**
12	**SAA**	35	**sICAM-1**
13	**Resistin**	36	**IL-1β**
14	**sFSI**	37	**GM-CSF**
15	**PAI-1**	38	**IL-1ra**
16	**MMP-2**	39	**VEGF**
17	**MMP-7**	40	**GLP-1**
18	**CRP**	41	**Amylin (Total)**
19	**sCD40 ligand**	42	**IL-1α**
20	**MIF**	43	**Insulin**
21	**SE-selectin**	44	**IL-6**
22	**sVCAM-1**	45	**TGF-α**
23	**IL-5**		

[098] Table 11 lists biomarkers whose expression levels have a significant or marginally significant difference between the LC v. NO female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 11

SIGNIFICANT BIOMARKERS FOR NSCLC IN THE FEMALE POPULATION			
No.	Biomarker	No.	Biomarker
1	IL-13	17	MMP-12
2	EGF	18	MIF
3	IL-12(p70)	19	SE-selectin
4	I-TAC	20	PAI-1
5	SAA	21	SAP
6	IP-10	22	IL-1ra
7	MMP-1	23	C-Peptide
8	MCP-1	24	sICAM-1
9	Eotaxin	25	sVCAM-1
10	Leptin	26	IL-15
11	MMP-9	27	G-CSF
12	Adiponectin	28	GM-CSF
13	MMP-7	29	IFN-γ
14	MPO	30	IL-2
15	IL-8	31	TGF-α
16	CRP		

[099] Table 12 lists biomarkers whose expression levels have a significant or marginally significant difference between the AST v. LC female populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 12

SIGNIFICANT BIOMARKERS DISTINGUISHING BETWEEN REACTIVE AIRWAY DISEASE AND NSCLC IN THE FEMALE POPULATION

No.	Biomarker	No.	Biomarker
1	MMP-7	16	MMP-8
2	MMP-1	17	IL-15
3	IL-8	18	SAP
4	IL-10	19	MMP-3
5	SAA	20	MMP-9
6	HGF	21	Eotaxin
7	I-TAC	22	IL-1ra
8	Leptin	23	CRP
9	Resistin	24	IP-10
10	sFSl	25	IL-6
11	MPO	26	MIP-1β
12	C-Peptide	27	IL-13
13	sVCAM-1	28	IL-5
14	IL-1α	29	PAI-1
15	Adiponectin	30	IFN-γ

[0100] Table 13A lists biomarkers whose expression levels have a significant or marginally difference between male and female AST populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 13A

BIOMARKERS WITH SIGNIFICANT DIFFERENCES BETWEEN MALE AND FEMALE REACTIVE AIRWAY DISEASE POPULATIONS			
No.	Biomarker	No.	Biomarker
1	IL-6	18	Sfas
2	IL-1α	19	Resistin
3	IL-5	20	I-TAC
4	G-CSF	21	IL-17
5	IL-4	22	HGF
6	IL-7	23	MMP-9
7	Leptin	24	IP-10
8	GM-CSF	25	CRP
9	MIF	26	C-Peptide
10	IL-15	27	sVCAM-1
11	TGF-α	28	PAI-1
12	MIP-1β	29	SAP
13	MMP-1	30	IL-10
14	sCD40 ligand	31	Fractalkine
15	MMP-2	32	Amylin (Total)
16	VEGF	33	MPO
17	IL-12 (p40)		

[0101] Table 13B lists biomarkers whose expression levels have an insignificant difference between male and female AST populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 13B

BIOMARKERS WITH INSIGNIFICANT DIFFERENCES BETWEEN MALE AND FEMALE REACTIVE AIRWAY DISEASE POPULATIONS	
No.	Biomarkers
1	Adiponectin
2	MMP-3
3	IL-1ra
4	IFN-γ
5	SE-selectin
6	IL-2
7	IL-13
8	SAA
9	Eotaxin
10	sICAM-1
11	EGF
12	MMP-7
13	IL-12(p70)
14	MMP-12
15	sFSl
16	IL-8
17	MMP-13
18	Insulin
19	MMP-8
20	MCP-1
21	GLP-1
22	IL-1β
23	TNF-α
24	MIP-1α

[0102] Table 14A lists biomarkers whose expression levels have a significant or marginally significant difference between male and female LC populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 14A

BIOMARKERS WITH SIGNIFICANT DIFFERENCES BETWEEN MALE AND FEMALE NSCLC POPULATIONS	
No.	Biomarker
1	HGF
2	MMP-1
3	Leptin
4	PAI-1
5	Resistin
6	IP-10
7	Adiponectin
8	MIF
9	IL-8
10	IL-10
11	MIP-1α
12	SAA
13	I-TAC
14	MMP-3
15	IL-1β

[0103] Table 14B lists biomarkers whose expression levels have an insignificant difference between male and female LC populations. Significance was determined as shown in Examples 1-3 using a Student's t test. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 14B

BIOMARKERS WITH INSIGNIFICANT DIFFERENCES BETWEEN MALE AND FEMALE NSCLC POPULATIONS			
No.	**Biomarker**	**No.**	**Biomarker**
1	IL-15	22	Sfas
2	Eotaxin	23	IL-6
3	Fractalkine	24	SE-selectin
4	sICAM-1	25	EGF
5	IL-1ra	26	MMP-9
6	GM-CSF	27	Insulin
7	IL-12 (p40)	28	MMP-8
8	TGF-α	29	GLP-1
9	MPO	30	IL-5
10	IL-13	31	MMP-2
11	MMP-7	32	IL-4
12	IL-17	33	MIP-1β
13	IL-2	34	IL-12(p70)
14	SAP	35	sCD40 ligand
15	IFN-γ	36	IL-1α
16	sVCAM-1	37	IL-7
17	CRP	38	MMP-12
18	MCP-1	39	TNF-α
19	VEGF	40	Amylin (Total)
20	C-Peptide	41	sFSl
21	G-CSF	42	MMP-13

[0104] Table 15A lists biomarkers ranked, in ascending order, by the relative standard deviation in fluorescence intensity for the normal population.

TABLE 15A

BIOMARKERS RANKED BY RELATIVE STANDARD DEVIATION IN FLUORESCENCE INTENSITY FOR THE NORMAL POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**G-CSF**	30	**Eotaxin**
2	**IL-15**	31	**PAI-1**
3	**Fractalkine**	32	**sFSl**
4	**TGF-α**	33	**Leptin**
5	**SAP**	34	**IL-6**
6	**IL-10**	35	**MMP-9**
7	**VEGF**	36	**IP-10**
8	**IL-12 (p40), free**	37	**Insulin**
9	**sVCAM-1**	38	**EGF**
10	**IL-17**	39	**MMP-1**
11	**TNF-α**	40	**GLP-1**
12	**MMP-3**	41	**SAA**
13	**IFN-γ**	42	**IL-1α**
14	**IL-1β**	43	**MIF**
15	**C-Peptide**	44	**MMP-12**
16	**IL-7**	45	**Amylin (Total)**
17	**GM-CSF**	46	**Sfas**
18	**MIP-1β**	47	**MPO**
19	**sICAM-1**	48	**IL-8**
20	**MMP-7**	49	**sCD40 ligand**
21	**IL-4**	50	**MMP-2**
22	**MCP-1**	51	**HGF**
23	**Adiponectin**	52	**MMP-13**
24	**MIP-1α**	53	**IL-2**
25	**Resistin**	54	**MMP-8**
26	**CRP**	55	**IL12 p40**
27	**SE-selectin**	56	**IL-2**
28	**IL-1ra**	57	**I-TAC**
29	**IL-5**		

[0105] Table 15B lists biomarkers ranked, in ascending order, by the relative standard deviation in fluorescence intensity for the normal female population.

TABLE 15B

BIOMARKERS RANKED BY RELATIVE STANDARD DEVIATION IN FLUORESCENCE INTENSITY FOR THE NORMAL FEMALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**G-CSF**	30	**MIP-1α**
2	**IL-15**	31	**sFSl**
3	**GM-CSF**	32	**Eotaxin**
4	**IL-1ra**	33	**PAI-1**
5	**Fractalkine**	34	**IP-10**
6	**IL-10**	35	**IL-5**
7	**IL-2**	36	**MMP-2**
8	**TGF-α**	37	**MMP-9**
9	**VEGF**	38	**IL-6**
10	**IL-12 (p40)**	39	**MMP-1**
11	**SAP**	40	**EGF**
12	**TNF-α**	41	**IL-12(p70)**
13	**sVCAM-1**	42	**MIF**
14	**IL-17**	43	**Leptin**
15	**MMP-3**	44	**sCD40 ligand**
16	**IL-7**	45	**HGF**
17	**MIP-1β**	46	**Insulin**
18	**C-Peptide**	47	**MPO**
19	**sICAM-1**	48	**SAA**
20	**IFN-γ**	49	**GLP-1**
21	**MMP-7**	50	**IL-1α**
22	**IL-1β**	51	**MMP-8**
23	**IL-4**	52	**I-TAC**
24	**Adiponectin**	53	**IL-8**
25	**Resistin**	54	**MMP-12**
26	**Sfas**	55	**IL-13**
27	**MCP-1**	56	**Amylin (Total)**
28	**CRP**	57	**MMP-13**
29	**SE-selectin**		

[0106] Table 15C lists biomarkers ranked, in ascending order, by the relative standard deviation in fluorescence intensity for the normal male population.

TABLE 15C

BIOMARKERS RANKED BY RELATIVE STANDARD DEVIATION IN FLUORESCENCE INTENSITY FOR THE NORMAL MALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**IL-1β**	30	**HGF**
2	**IL-15**	31	**Leptin**
3	**G-CSF**	32	**IL-5**
4	**MIP-1α**	33	**Eotaxin**
5	**TGF-α**	34	**MMP-9**
6	**Fractalkine**	35	**IL-1ra**
7	**SAP**	36	**PAI-1**
8	**IFN-γ**	37	**sFSl**
9	**IL-10**	38	**IL-6**
10	**sVCAM-1**	39	**Insulin**
11	**TNF-α**	40	**EGF**
12	**VEGF**	41	**Amylin (Total)**
13	**IL-12 (p40)**	42	**MMP-1**
14	**MCP-1**	43	**IL-8**
15	**MIP-1β**	44	**IP-10**
16	**C-Peptide**	45	**SAA**
17	**MMP-3**	46	**GLP-1**
18	**IL-17**	47	**MMP-12**
19	**IL-7**	48	**IL-1α**
20	**sICAM-1**	49	**MMP-13**
21	**MIF**	50	**sCD40 ligand**
22	**GM-CSF**	51	**MMP-2**
23	**MMP-7**	52	**Sfas**
24	**IL-4**	53	**MPO**
25	**Adiponectin**	54	**IL-2**
26	**SE-selectin**	55	**I-TAC**
27	**CRP**	56	**IL-12(p70)**
28	**Resistin**	57	**IL-13**
29	**MMP-8**		

[0107] Table 16A lists biomarkers whose expression levels have a significant difference between at least one of AST v. NO male populations, LC v. NO male populations, and AST v. LC male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the significance and magnitude of the difference in fluorescence intensity.

TABLE 16A

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION			
No.	Biomarker	No.	Biomarker
1	HGF	19	MIP-1α
2	MMP-8	20	MMP-13
3	I-TAC	21	G-CSF
4	EGF	22	IFN-γ
5	PAI-1	23	MMP-7
6	MMP-1	24	IP-10
7	MPO	25	CRP
8	MIF	26	Insulin
9	Eotaxin	27	VEGF
10	MMP-12	28	SAP
11	SAA	29	Adiponectin
12	Resistin	30	sVCAM-1
13	sFSl	31	Sfas
14	Leptin	32	IL-1ra
15	C-Peptide	33	IL-12 (p40)
16	MMP-9	34	MIP-1β
17	MCP-1	35	sICAM-1
18	MMP-3		

[0108] Table 16B lists biomarkers whose expression levels have a significant difference between the AST v. NO male populations, LC v. NO male populations, and AST v. LC male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 16B

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION	
No.	Biomarker
1	HGF
2	MMP-8
3	I-TAC
4	EGF
5	PAI-1
6	MMP-1
7	MPO
8	MIF
9	Eotaxin
10	MMP-12
11	SAA
12	Resistin
13	sFSl
14	Leptin
15	C-Peptide

[0109] Table 16C lists biomarkers whose expression levels have a significant difference between the AST v. NO male populations and LC v. NO male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 16C

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE MALE POPULATION	
No.	**Biomarker**
1	**HGF**
2	**MMP-8**
3	**I-TAC**
4	**MMP-9**
5	**EGF**
6	**PAI-1**
7	**MMP-1**
8	**MPO**
9	**MIF**
10	**MCP-1**
11	**Eotaxin**
12	**MMP-3**
13	**MIP-1α**
14	**MMP-12**
15	**MMP-13**
16	**IP-10**
17	**VEGF**
18	**Resistin**
19	**sFSl**
20	**C-Peptide**
21	**Sfas**
22	**SAA**
23	**Insulin**
24	**SAP**
25	**Leptin**

[0110] Table 17 lists biomarkers whose expression levels have a significant difference between the AST v. NO male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 17

SIGNIFICANT BIOMARKERS FOR REACTIVE AIRWAY DISEASE IN THE MALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**HGF**	16	**sFSl**
2	**I-TAC**	17	**MMP-13**
3	**EGF**	18	**VEGF**
4	**MMP-8**	19	**C-Peptide**
5	**PAI-1**	20	**Resistin**
6	**MPO**	21	**sVCAM-1**
7	**MMP-9**	22	**G-CSF**
8	**MCP-1**	23	**Sfas**
9	**MIP-1α**	24	**sICAM-1**
10	**Eotaxin**	25	**Leptin**
11	**MMP-1**	26	**SAP**
12	**MIF**	27	**Insulin**
13	**MMP-3**	28	**IFN-γ**
14	**MMP-12**	29	**SAA**
15	**IP-10**		

[0111] Table 18 lists biomarkers whose expression levels have a significant difference between the LC v. NO male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 18

SIGNIFICANT BIOMARKERS FOR NSCLC IN THE MALE POPULATION			
No.	Biomarker	No.	Biomarker
1	HGF	17	MMP-7
2	MMP-8	18	Resistin
3	MMP-9	19	CRP
4	I-TAC	20	VEGF
5	EGF	21	SAA
6	MMP-1	22	Adiponectin
7	PAI-1	23	IL-1ra
8	MPO	24	Sfas
9	MIF	25	MIP-1β
10	MMP-3	26	sFSl
11	MMP-12	27	C-Peptide
12	Eotaxin	28	Insulin
13	MMP-13	29	SAP
14	MCP-1	30	Leptin
15	MIP-1α	31	IL-12 (p40)
16	IP-10	32	

[0112] Table 19 lists biomarkers whose expression levels have a significant difference between the AST v. LC male populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 19

SIGNIFICANT BIOMARKERS DISTINGUISHING BETWEEN REACTIVE AIRWAY DISEASE AND NSCLC IN THE MALE POPULATION			
No.	Biomarker	No.	Biomarker
1	**I-TAC**	12	**Resistin**
2	**HGF**	13	**Adiponectin**
3	**MPO**	14	**MMP-12**
4	**sFSl**	15	**MMP-7**
5	**PAI-1**	16	**CRP**
6	**C-Peptide**	17	**G-CSF**
7	**sVCAM-1**	18	**IFN-γ**
8	**Eotaxin**	19	**SAA**
9	**EGF**	20	**MMP-1**
10	**Leptin**	21	**MMP-8**
11	**MIF**	22	

[0113] Table 20A lists biomarkers whose expression levels have a significant difference between at least one of AST v. NO female populations, LC v. NO female populations, and AST v. LC female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the significance and magnitude of the difference in fluorescence intensity.

TABLE 20A

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION			
No.	Biomarker	No.	Biomarker
1	I-TAC	22	MMP-1
2	PAI-1	23	Fractalkine
3	MMP-7	24	IL-1α
4	MMP-3	25	CRP
5	IL-8	26	MIP-1β
6	MPO	27	IP-10
7	Leptin	28	IL-1ra
8	sFSl	29	MIP-1α
9	HGF	30	VEGF
10	Resistin	31	IFN-γ
11	C-Peptide	32	Adiponectin
12	MMP-13	33	Eotaxin
13	SAP	34	IL-6
14	sVCAM-1	35	MMP-12
15	MMP-8	36	sICAM-1
16	IL-10	37	MIF
17	MMP-9	38	Sfas
18	G-CSF	39	IL-12 (p40)
19	EGF	40	IL-4
20	MCP-1	41	Insulin
21	SAA		

[0114] Table 20B lists biomarkers whose expression levels have a significant difference between the AST v. NO female populations, LC v. NO female populations, and AST v. LC female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 20B

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION	
No.	**Biomarker**
1	**I-TAC**
2	**PAI-1**
3	**MMP-7**
4	**MMP-3**
5	**IL-8**
6	**MPO**
7	**Leptin**
8	**sFSl**
9	**HGF**
10	**Resistin**
11	**C-Peptide**
12	**MMP-13**
13	**SAP**
14	**sVCAM-1**
15	**MMP-8**

[0115] Table 20C lists biomarkers whose expression levels have a significant difference between the AST v. NO female populations and LC v. NO female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 20C

SIGNIFICANT BIOMARKERS FOR LUNG DISEASE IN THE FEMALE POPULATION			
No.	Biomarker	No.	Biomarker
1	MMP-9	17	HGF
2	G-CSF	18	IL-8
3	I-TAC	19	Resistin
4	EGF	20	IL-6
5	MCP-1	21	Sfas
6	PAI-1	22	C-Peptide
7	SAA	23	MMP-7
8	MPO	24	sVCAM-1
9	MMP-3	25	sICAM-1
10	CRP	26	MMP-8
11	IP-10	27	MIF
12	Leptin	28	MMP-13
13	sFSl	29	SAP
14	IFN-γ	30	MIP-1α
15	Adiponectin	31	VEGF
16	Eotaxin	32	IL-1ra

[0116] Table 21 lists biomarkers whose expression levels have a significant difference between the AST v. NO female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 21

SIGNIFICANT BIOMARKERS FOR REACTIVE AIRWAY DISEASE IN THE FEMALE POPULATION			
No.	**Biomarker**	**No.**	**Biomarker**
1	**MMP-9**	18	**Adiponectin**
2	**I-TAC**	19	**Eotaxin**
3	**EGF**	20	**C-Peptide**
4	**PAI-1**	21	**IL-6**
5	**MCP-1**	22	**sVCAM-1**
6	**G-CSF**	23	**IL-4**
7	**IL-1α**	24	**MMP-3**
8	**MPO**	25	**Sfas**
9	**IL-8**	26	**MMP-8**
10	**Leptin**	27	**sICAM-1**
11	**sFSl**	28	**MIF**
12	**HGF**	29	**MMP-13**
13	**IP-10**	30	**SAP**
14	**Resistin**	31	**MMP-7**
15	**IFN-γ**	32	**MIP-1α**
16	**SAA**	33	**VEGF**
17	**CRP**	34	**IL-1ra**

[0117] Table 22 lists biomarkers whose expression levels have a significant difference between the LC v. NO female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 22

SIGNIFICANT BIOMARKERS FOR NSCLC IN THE FEMALE POPULATION			
No.	Biomarker	No.	Biomarker
1	MMP-9	20	IL-6
2	G-CSF	21	Sfas
3	EGF	22	sICAM-1
4	IL-10	23	Resistin
5	MCP-1	24	MMP-8
6	SAA	25	sFSl
7	MMP-3	26	sVCAM-1
8	PAI-1	27	Fractalkine
9	I-TAC	28	HGF
10	CRP	29	MIF
11	MMP-1	30	MMP-13
12	MPO	31	C-Peptide
13	IP-10	32	SAP
14	Adiponectin	33	Insulin
15	MMP-7	34	IL-8
16	Eotaxin	35	MIP-1α
17	IFN-γ	36	MIP-1β
18	Leptin	37	VEGF
19	MMP-12	38	IL-1ra

[0118] Table 23 lists biomarkers whose expression levels have a significant difference between the AST v. LC female populations. Significance was determined as shown in Example 4 using the Kruskal-Wallis method. Marginally significant biomarkers are not included. Markers are listed in descending order based on the magnitude of the difference in fluorescence intensity.

TABLE 23

SIGNIFICANT BIOMARKERS DISTINGUISHING BETWEEN REACTIVE AIRWAY DISEASE AND NSCLC IN THE FEMALE POPULATION			
No.	Biomarker	No.	Biomarker
1	IL-8	12	MPO
2	HGF	13	MMP-8
3	sFSl	14	MMP-12
4	I-TAC	15	SAP
5	C-Peptide	16	MMP-13
6	IL-1α	17	MIP-1β
7	Resistin	18	MMP-1
8	IL-12 (p40)	19	MMP-3
9	Leptin	20	Fractalkine
10	sVCAM-1	21	MMP-7
11	PAI-1	22	IL-10

Determining The Extent Of Expression

[0119] Extent of expression generally relates to a quantitative measurement of an expression product which is typically a protein or polypeptide. The invention contemplates determining the extent of expression at the RNA (pre-translational) or protein level (which may include post-translational modification). In particular, the invention contemplates determining changes in biomarker concentrations reflected in an increase or decrease in the level of transcription, translation, post-transcriptional modification, or the extent or degree of degradation of protein, where these changes are associated with a particular disease state or disease progression.

[0120] Samples are collected to ensure that the extent of expression in a subject is proportional to the concentration of said biomarker in the sample. Measurements are made so that the measured value is proportional to the concentration of the biomarker in the sample. Thus, the measured value is proportional to the extent of expression. Selecting sampling techniques and measurement techniques which meet these requirements is within the skill of the art.

[0121] Typically, the extent of expression of at least one biomarker indicative of a lung disease is a level of at least one biomarker that differs by a statistically significant degree from the average expression level in normal individuals; in other words, at least one biomarker is statistically deviant from the normal. Statistical significance and deviation may be determined using any known method for comparing means of populations or comparing a measured value to the mean value for a population. Such methods include the Student's t tests for single and multiple markers considered together, analysis of variance (ANOVA), etc.

[0122] As an alternative to, or in combination with determining the extent of expression, methods described herein involve determining whether the level of a biomarker falls within a normal level (e.g., range) or is outside the normal level (i.e., abnormal). Those who measure levels of biological molecules in physiological samples routinely determine the normal level of a particular biomarker in the population they regularly measure, typically described as the normal range of values as determined by the particular laboratory. Thus, the skilled person will inevitable be familiar with normal levels of a particular biomarker and can determine whether the level of the biomarker is outside of the normal level or range.

[0123] More typically, the extent of expression of at least one biomarker indicative of a lung disease is a level of at least one biomarker that also differs by a magnitude sufficient such that the differences are analytically significant from the average expression level in normal individuals such that a diagnosis, prognosis, and/or assessment of a lung disease may be determined. Those of skill in the art understand that greater differences in magnitude are preferred to assist in the diagnosis, prognosis, and/or assessment of a lung disease. *See* Instrumental Methods of Analysis, Seventh Edition, 1988.

[0124] Many proteins expressed by a normal subject will be expressed to a greater or lesser extent in subjects having a disease or condition, such as non-small cell lung cancer or asthma. One of skill in the art will appreciate that most diseases manifest changes in multiple, different biomarkers. As such, disease may be characterized by a pattern of expression of a plurality of markers. Indeed, changes in a pattern of expression for a plurality of biomarkers may be used in various diagnostic and prognostic methods, as well as monitoring, therapy selection, and patient assessment methods. The invention provides for such methods. These methods comprise determining a pattern of expression of a plurality of markers for a particular physiologic state, or determining changes in such a pattern which correlate to changes in physiologic state, as characterized by any technique for suitable pattern recognition.

[0125] Numerous methods of determining the extent of expression are known in the art. Means for determining expression include but are not limited to radio-immuno assay, enzyme-linked immunosorbent assay (ELISA), high pressure liquid chromatography with radiometric or spectrometric detection via absorbance of visible or ultraviolet light, mass spectrometric qualitiative and quantitative analysis, western blotting, 1 or 2 dimensional gel electrophoresis with quantitative visualization by means of detection of radioactive, fluorescent or chemiluminescent probes or nuclei, antibody-based detection with absorptive or fluorescent photometry, quantitation by luminescence of any of a number of chemiluminescent reporter systems, enzymatic assays, immunoprecipitation or immuno-capture assays, solid and liquid phase immunoassays, protein arrays or chips, DNA arrays or chips, plate assays, assays that use molecules having binding affinity that permit discrimination such as aptamers and molecular imprinted polymers, and any other quantitative analytical determination of the concentration of a biomarker by any other suitable technique, instrumental actuation of any of the described detection techniques or instrumentation.

[0126] The step of determining the extent of expression may be performed by any means for determining expression known in the art, especially those means discussed herein. In preferred embodiments, the step of determining the extent of expression comprises performing an immunoassays with antibodies.

Selection of Biomarkers for Determination

[0127] One of skill in the art would readily be able to select appropriate antibodies for use in the present invention. The antibody chosen is preferably selective for an antigen of interest, possesses a high binding specificity for said antigen, and has minimal cross-reactivity with other antigens. The ability of an antibody to bind to an antigen of interest may be determined, for example, by known methods such as enzyme-linked immunosorbent assay (ELISA), flow cytometry, and immunohistochemistry. Preferably, the antigen of interest to which the antibody binds is differentially present in cells or biological samples taken from diseased patients as opposed to cells or biological samples taken from healthy patients. The differential presence of the antigen in different populations may be determined by comparing the binding of the antibody to samples taken from each of the populations of interest (e.g., the diseased population versus the healthy population). *See, e.g.*, Examples 1-4; *see also* Figures 1-8. For example, the antigen of interest may be determined to be expressed at higher levels in cancer cells than in non-cancer cells. *See, e.g.*, Examples 1-4; *see also* Figures 1-8. Furthermore, the antibody should have a relatively high binding specificity for the antigen of interest. The binding specificity of the antibody may be determined by known methods such as immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or ELISA. Disclosure of methods for selecting antibodies capable of binding antigens of interest with high binding specificity and minimal cross-reactivity are provided, for example, in U.S. Pat, No. 7,288,249.

[0128] The invention provides for various methods comprising the step of determining the extent of expression of one or more biomarkers described herein. In one embodiment, the method comprises determining the extent of expression of any of the biomarkers from any number of Tables 1-14 or 16-23. The biomarkers in Tables 1-14 and 16-23 are generally listed in decreasing order of the extent of expression. The biomarkers closer to the top of these Tables generally show more sensitivity (e.g., detect differences at lower levels). Using such biomarkers may assist in discriminating between disease conditions. The biomarkers in Table 15 are listed in ascending order based on the relative standard deviation in fluorescence intensity. The biomarkers closer to the top of Table 15 are also generally more sensitive due to a lower degree of variance other than the variance which is due to the presence of a disease state. In particular, these biomarkers have less overall variability and thus are helpful in reducing background noise when comparing the extent of expression of diseased individuals as compared to the extent of expression in normal individuals.

[0129] Consequently, a preferred method comprises determining the extent of expression of biomarker nos. 1-20 of a particular Table, or the total list of biomarkers if the Table contains less than 20. Alternatively, this mode comprises determining the extent of expression of biomarker nos. 1-10, more preferably biomarker nos. 1-8, even more preferably biomarker nos. 1-6, and most preferably biomarker nos. 1-4, or a subset of the biomarkers in any of these groups. In another embodiment, the method comprises determining the extent of expression of any combination of biomarkers from a particular Table. In another embodiment, the method comprises determining the extent of expression of any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) of a particular Table, preferably any combination of a plurality of biomarkers from biomarker nos. 1-10, more preferably any combination of a plurality of biomarkers from biomarker nos. 1-8, even more preferably any combination of biomarkers from biomarker nos. 1-6, and most preferably any combination of a plurality of biomarkers from biomarker nos. 1-4, or a subset of the biomarkers in any of these groups. In a preferred mode, the method comprises determining the extent of expression of any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 of a particular Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a particular Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a particular Table. Of course, the skilled person will recognize that it is within the contemplation of this invention to contemporaneously determine the extent of expression of other biomarkers whether or not associated with the disease of interest.

[0130] The determination of expression levels for a plurality of biomarkers facilitates the observation of a pattern of changes in expression, and such patterns provide for more sensitive and more accurate diagnoses than detection of individual biomarkers. For example, a pattern of changes would include a plurality of particular biomarkers that are simultaneously expressed at abnormal levels. A pattern of changes may also comprise abnormal elevation of some particular biomarkers simultaneously with abnormal reduction in other particular biomarkers. The skilled person will observe such patterns in the data presented in the Figures included herein. (*see* Discussion in Example 4 below). Such determination may be performed in a multiplex or matrix-based format such as a multiplexed immunoassay.

[0131] In another embodiment, the method comprises determining the extent of expression of any of the biomarkers from at least two Tables (e.g., Table 2 and Table 3). In another embodiment, the method comprises determining the extent of expression of biomarker nos. 1-20 (or the maximum list if less than 20) of a particular Table and biomarker nos. 1-20 (or the maximum list if less than 20) from a different Table, preferably biomarker nos. 1-10 from one or both Tables, more preferably biomarker nos. 1-8 from one or both Tables, even more preferably biomarker nos. 1-6 from one or both Tables, and most preferably biomarker nos. 1-4 from one or both Tables, or a subset of the biomarkers in any of these groups. In another embodiment, the method comprises determining the extent of expression of any combination of a plurality of biomarkers from a particular Table and a different Table. In another embodiment, the method comprises determining the extent of expression of any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) of a particular Table and any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) from a different Table, preferably any combination of a plurality of biomarkers from biomarker nos. 1-10 from one or both Tables, more preferably any combination of a plurality of biomarkers from biomarker nos. 1-8 from one or both Tables, even more preferably any combination of a plurality of biomarkers from biomarker nos. 1-6 from one or both Tables, and most preferably any combination of a plurality of biomarkers from biomarker nos. 1-4 from one or both Tables, or a subset of the biomarkers in any of these groups. In another embodiment, the plurality of biomarker(s) from one Table are not present in any of the other Tables. In a preferred mode, the method comprises determining the extent of expression of any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 of a particular Table and any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 from a different Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a particular Table and any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a different Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a particular Table and any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a different Table. Of course, the skilled person will recognize that it is within the contemplation of this invention to contemporaneously determine the extent of expression of other biomarkers whether or not associated with the disease of interest.

[0132] It will be understood that the same types of combinations are applicable when the method comprises determining the extent of expression of any of the biomarkers from at least three different Tables (e.g., Table 2, Table 3, and Table 4). For example, in one embodiment, the method comprises determining the extent of expression of any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) of a first Table, any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) from a second Table, and any combination of a plurality of biomarkers from biomarker nos. 1-20 (or the maximum list if less than 20) of a third Table, preferably any combination of a plurality of biomarkers from biomarker nos. 1-10 from each Table, more preferably any combination of a plurality of biomarkers from biomarker nos. 1-8 from each Table, even more preferably any combination of a plurality of biomarkers from biomarker nos. 1-6 from each Table, and most preferably any combination of a plurality of biomarkers from biomarker nos. 1-4 from each Table. In a preferred mode, the method comprises determining the extent of expression of any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 of a first Table, any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 of a second Table, and any of a particular subset of three biomarkers selected from biomarker nos. 1-6, 1-8, 1-10, 1-15, or 1-20 of a third Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a first Table, any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a second Table, and any of a particular subset of four, five, six, or seven biomarkers selected from biomarker nos. 1-8, 1-10, 1-15, or 1-20 of a third Table. Alternatively, the method comprises determining the extent of expression of any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a first Table, any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a second Table, and any of a particular subset of eight, nine, ten, eleven, twelve, or thirteen biomarkers selected from biomarker nos. 1-15 or 1-20 of a third Table. Of course, the skilled person will recognize that it is within the contemplation of this invention to contemporaneously determine the extent of expression of other biomarkers whether or not associated with the disease of interest.

[0133] The determination of expression levels for a plurality of biomarkers facilitates the observation of a pattern of changes in expression, and such patterns provide for more sensitive and more accurate diagnoses than detection of individual biomarkers. This determination may be performed in a multiplex or matrix-based format such as a multiplexed immunoassay.

[0134] In other embodiments, the extent of expression of no more than 5, 10, 15, 20, 25, 30, 35, or 40 are determined.

[0135] Selection of biomarkers for use in a diagnostic or prognostic assay may be facilitated using known relationships between particular biomarkers and their first order interactors. Many, if not all, of the biomarkers identified by the present inventors (see Tables 1-23) participate in various communications pathways of the cell or the organism. Deviation of one component of a communication pathway from normal is expected to be accompanied by related deviations in other members of the communication pathway. The skilled worker can readily link members of a communication pathway using various databases and available bioinformatics software (*see, e.g.*, ARIADNE PATHWAY STUDIO, Ariadne, Inc., <www.ariadne.genomics.com> or ChEMBL Database, European Bioinformatics Institute, European Molecular Biology Laboratory, <www.ebi.ac.uk>). A diagnostic method based on determining whether the levels of a plurality of biomarkers are abnormal where the plurality of biomarkers includes some biomarkers which are not in the same communication pathway as others in the plurality is likely to maximize the information collected by measuring the biomarker levels.

[0136] It will also be understood that the various combination of biomarkers previously discussed are also applicable to methods for designing kits and the kits described herein.

[0137] It will be appreciated that the selection criteria discussed above, including the preference for selecting particular subsets of markers, may be employed for any of the methods described herein with respect to those Tables associated with the particular methods.

Methods of Physiological Characterization

[0138] The present invention is directed to methods for physiological characterization of individuals in various populations as described below. As used herein, a method of physiological characterization according to the methods of this invention include methods of diagnosing particular diseases, methods of predicting the likelihood that an individual will respond to therapeutic intervention, methods of monitoring an individual's reaction to therapeutic intervention, methods of determining whether an individual is at-risk for an individual disease, methods for determining the degree of risk for a particular disease; methods of categorizing a patient's degree of severity of disease, and methods for differentiating between diseases having some symptoms in common. In general, these methods rely on determining the extent of expression of particular biomarkers as described above.

A. General Population

[0139] The invention provides for methods of physiological characterization in a subject. In one embodiment, the invention provides for a method of physiological characterization in a subject comprising determining the extent of expression of at least one biomarker from Table 1A in a physiological sample of the subject where the extent of expression of the at least one biomarker is indicative of lung disease such as reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 1B where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 1C where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer.

[0140] In another embodiment, the method comprises determining the extent of expression of SEQ ID NO: 12. In another embodiment, the method comprises determining the extent of expression of SEQ NO: 12 and any one of SEQ ID NOS: 1-11 and 13-17.

[0141] In a preferred embodiment, the invention provides for methods of physiological characterization in a subject comprising determining the extent of expression of a plurality of biomarkers from Table 1A in a physiological sample of the subject, where a pattern of expression of the plurality of markers correlate to a physiologic state or condition, or changes in a disease state (*e.g.*, stages in non-small cell lung cancer) or condition. In another preferred embodiment, a pattern of expression of a plurality of biomarkers from Table 1A is indicative of a lung disease such as non-small cell lung cancer or reactive airway disease, or assists in distinguishing between reactive airway disease or non-small cell lung cancer. Preferably, the plurality of biomarkers are selected based on the low probability of erroneous pattern classification based on the value of Student's t as calculated in the Examples. In another preferred embodiment, patterns of expression of biomarkers from Table 1A correlate to an increased likelihood that a subject has or may have a particular disease or condition. In a more preferred embodiment, methods of determining the extent of expression of a plurality of biomarkers from Table 1A in a subject detect an increase in the likelihood that a subject is developing, has or may have a lung disease such as non-small cell lung cancer or reactive airway disease (*e.g.*, asthma). Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 1A.

[0142] The invention also provides for a method of physiological characterization in a subject comprising determining the extent of expression of SEQ ID NO: 12 in a physiological sample of the subject, wherein the extent of expression of SEQ ID NO: 12 is indicative of the lung disease of non-small cell lung cancer or reactive airway disease. In a preferred embodiment, a pattern of expression of a plurality of markers of SEQ ID NO: 12 and any one of SEQ ID NOS: 1-11 and 13-17 are determined and used as described herein.

[0143] In another aspect, the invention provides for a method of physiological characterization in a subject comprising, (a) obtaining a physiological sample of the subject; (b) determining the extent of expression in said subject of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and (c) determining the extent of expression in said subject of at least one biomarker from Table 1A, wherein the extent of expression of both the polypeptide and the biomarker from Table 1A is indicative of a lung disease of non-small cell lung cancer or reactive airway disease. In another embodiment, a pattern of expression of a plurality of markers of SEQ ID NOS: 1-17, and a plurality of biomarkers from Table 1A are determined and used as described herein.

[0144] In one embodiment, the subject is at-risk for the lung disease of non-small cell cancer or reactive airway disease (*e.g.*, asthma, chronic obstructive pulmonary disease, etc.). Subjects "at-risk" include those individuals who are asymptomatic but are more likely than the bulk of the population to develop the disease, because of personal or family history, behavior, exposure to disease causing agents (*e.g.*, carcinogens), or some other reason. "At-risk" individuals are traditionally identified by aggregating the risk factors determined for the individual. The present invention provides for enhanced detection of "at-risk" individuals by determining the extent of expression of relevant biomarkers. In one embodiment, levels of particular biomarkers associated with the disease (particularly biomarkers from Table 2 or Table 3) are determined for an individual, and levels which differ from those expected for the normal population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers (from Table 2 or Table 3 as appropriate to the disease) which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk.

[0145] The embodiments described above refer to the biomarkers of Table 1A. It will be appreciated, however, that the biomarkers of Table 1B or 1C may be substituted for the biomarkers of Table 1A in any of the described embodiments. It will also be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

B. Male Population

[0146] The invention provides for a method of physiological characterization in a male subject. In one embodiment, the invention provides for a method of physiological characterization in a male subject comprising obtaining a sample from said male subject, and determining the extent of expression of at least one biomarker from Table 5A or 16A in a physiological sample of the male subject where the extent of expression of the at least one biomarker is indicative of lung disease such as reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 5B or 16B where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 5C or 16C where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer.

[0147] In a preferred embodiment, the invention provides for methods of physiological characterization in a male subject comprising determining the extent of expression of a plurality of biomarkers from Table 5A or 16A in a physiological sample of the male subject, where a pattern of expression of the plurality of markers correlate to a physiologic state or condition, or changes in a disease state (*e.g.*, stages in non-small cell lung cancer) or condition. In another preferred embodiment, a pattern of expression of a plurality of biomarkers from Table 5A or 16A is indicative of a lung disease such as non-small cell lung cancer or reactive airway disease, or assists in distinguishing between reactive airway disease or non-small cell lung cancer. Preferably, the plurality of biomarkers are selected based on the low probability of erroneous pattern classification based on the value of Student's t as calculated in the Examples. In another preferred embodiment, patterns of expression of biomarkers from Table 5A or 16A correlate to an increased likelihood that a male subject has or may have a particular disease or condition. In a more preferred embodiment, methods of determining the extent of expression of a plurality of biomarkers from Table 5A or 16A in a male subject detect an increase in the likelihood that a male subject is developing, has or may have a lung disease such as non-small cell lung cancer or reactive airway disease (*e.g.*, asthma). Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 5A or 16A.

[0148] In another aspect, the invention provides for a method of physiological characterization in a male subject comprising, (a) obtaining a physiological sample of the male subject; (b) determining the extent of expression in said subject of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and (c) determining the extent of expression in said subject of at least one biomarker from Table 5A or 16A, wherein the extent of expression of both the polypeptide and the biomarker from Table 5A or 16A is indicative of a lung disease of non-small cell lung cancer or reactive airway disease. In another embodiment, a pattern of expression of a plurality of markers of SEQ ID NOS: 1-17, and a plurality of biomarkers from Table 5A or 16A are determined and used as described herein.

[0149] In one embodiment, the male subject is at-risk for the lung disease of non-small cell cancer or reactive airway disease (*e.g.*, asthma, chronic obstructive pulmonary disease, etc.). "At-risk" subjects and individuals are discussed above. In one embodiment, levels of particular biomarkers associated with the disease (particularly biomarkers from Tables 6, 7, 17 or 18) are determined for an male individual, and levels which differ from those expected for the normal population suggest that the male individual is "at-risk." In another embodiment, the number of relevant biomarkers (from Tables 6, 7, 17 or 18 as appropriate to the disease) which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk.

[0150] The embodiments described above refer to the biomarkers of Table 5A or 16A. It will be appreciated, however, that the biomarkers of Table 5B or 5C may be substituted for the biomarkers of Table 5A, and that the biomarkers of Table 16B or 16C may be substituted for the biomarkers of Table 16A in any of the described embodiments. It will also be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

C. Female Population

[0151] The invention provides for a method of physiological characterization in a female subject. In one embodiment, the invention provides for a method of physiological characterization in a female subject comprising obtaining a sample from said female subject, and determining the extent of expression of at least one biomarker from Table 9A or 20A in a physiological sample of the female subject where the extent of expression of the at least one biomarker is indicative of lung disease such as reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 9B or 20B where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer, or assists in distinguishing between reactive airway disease and non-small cell lung cancer. In another embodiment, the method comprises determining the extent of expression of at least one biomarker from Table 9C or 20C where the extent of expression of the at least one biomarker is indicative of reactive airway disease or non-small cell lung cancer.

[0152] In a preferred embodiment, the invention provides for methods of physiological characterization in a female subject comprising determining the extent of expression of a plurality of biomarkers from Table 9A or 20A in a physiological sample of the female subject, where a pattern of expression of the plurality of markers correlate to a physiologic state or condition, or changes in a disease state (*e.g.*, stages in non-small cell lung cancer) or condition. In another preferred embodiment, a pattern of expression of a plurality of biomarkers from Table 9A or 20A is indicative of a lung disease such as non-small cell lung cancer or reactive airway disease, or assists in distinguishing between reactive airway disease or non-small cell lung cancer. Preferably, the plurality of biomarkers are selected based on the low probability of erroneous pattern classification based on the value of Student's t as calculated in the Examples. In another preferred embodiment, patterns of expression of biomarkers from Table 9A or 20A correlate to an increased likelihood that a female subject has or may have a particular disease or condition. In a more preferred embodiment, methods of determining the extent of expression of a plurality of biomarkers from Table 9A or 20A in a female subject detect an increase in the likelihood that a female subject is developing, has or may have a lung disease such as non-small cell lung cancer or reactive airway disease (*e.g.*, asthma). Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 9A or 20A.

[0153] In another aspect, the invention provides for a method of physiological characterization in a female subject comprising, (a) obtaining a physiological sample of the female subject; (b) determining the extent of expression in said subject of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and (c) determining the extent of expression in said subject of at least one biomarker from Table 9A or 20A, wherein the extent of expression of both the polypeptide and the biomarker from Table 9A or 20A is indicative of a lung disease of non-small cell lung cancer or reactive airway disease. In another embodiment, a pattern of expression of a plurality of markers of SEQ ID NOS: 1-17, and a plurality of biomarkers from Table 9A or 20A are determined and used as described herein.

[0154] In one embodiment, the female subject is at-risk for the lung disease of non-small cell cancer or reactive airway disease (*e.g.*, asthma, chronic obstructive pulmonary disease, etc.). "At-risk" subjects and individuals are discussed above. In one embodiment, levels of particular biomarkers associated with the disease (particularly biomarkers from Tables 10, 11, 21, or 22) are determined for an female individual, and levels which differ from those expected for the normal population suggest that the male individual is "at-risk." In another embodiment, the number of relevant biomarkers (from Tables 10, 11, 21, or 22 as appropriate to the disease) which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk.

[0155] The embodiments described above refer to the biomarkers of Table 9A or 20A. It will be appreciated, however, that the biomarkers of Table 9B or 9C may be substituted for the biomarkers of Table 9A, and that the biomarkers of Table 20B or 20C may be substituted for the biomarkers of Table 9A in any of the described embodiments. It will also be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

Lung Disease

[0156] The invention provides for various diagnostic and prognostic methods for lung disease. In particular, the invention provides methods of diagnosing reactive airway disease and in particular diseases associated with over reactive TH_2 and TH_{17} cells. Reactive airway diseases include asthma, chronic obstructive pulmonary disease, allergic rhinitis, cystic fibrosis, bronchitis, or other diseases manifesting hyper-reactivity to various physiological and/or environmental stimuli. In particular, the invention provides for methods of diagnosing asthma and chronic obstructive pulmonary disease, more particularly diagnosing asthma.

[0157] The invention also provides methods of diagnosing non-small cell lung cancer. These methods include determining the extent of expression of at least one biomarker described herein, wherein the biomarker(s) is indicative of the presence or development of non-small lung cancer. For example, the extent of expression of biomarkers described herein may be used to determine the extent of progression of non-small lung cancer, the presence of pre-cancerous lesions, or staging of non-small lung cancer.

[0158] In particular embodiments, the subject is selected from those individuals who exhibit one or more symptoms of non-small cell lung cancer or reactive airway disease. Symptoms may include cough, shortness of breath, wheezing, chest pain, and hemoptysis; shoulder pain that travels down the outside of the arm or paralysis of the vocal cords leading to hoarseness; invasion of the esophagus may lead to difficulty swallowing. If a large airway is obstructed, collapse of a portion of the lung may occur and cause infections leading to abscesses or pneumonia. Metastases to the bones may produce excruciating pain. Metastases to the brain may cause neurologic symptoms including blurred vision headaches, seizures, or symptoms commonly associated with stroke such as weakness or loss of sensation in parts of the body. Lung cancers often produce symptoms that result from production of hormone-like substances by the tumor cells. A common paraneoplastic syndrome seen in NSCLC is the production parathyroid hormone like substances which cause calcium in the bloodstream to be elevated. Asthma typically produces symptoms such as coughing, especially at night, wheezing, shortness of breath and feelings of chest tightness, pain or pressure. Thus, it is apparent that many of the symptoms of asthma are common to NSCLC.

Methods of Diagnosing Reactive Airway Disease

[0159] The present invention is directed to methods of diagnosing reactive airway disease in individuals in various populations as described below. In general, these methods rely on determining the extent of expression of particular biomarkers as described herein.

A. General Population

[0160] The invention provides for a method of diagnosing reactive airway disease in a subject comprising, (a) obtaining a physiological sample of the subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 2, wherein the extent of expression of said at least one biomarker is indicative of reactive airway disease.

[0161] In a preferred embodiment, the invention provides for methods of diagnosing reactive airway disease in a subject comprising determining the extent of expression of a plurality of biomarkers from Table 2 in a physiological sample of the subject, wherein a pattern of expression of the plurality of markers are indicative of reactive airway disease or correlate to changes in a reactive airway disease state. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a subject has or may have reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 2. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0162] In one embodiment, the subject is at-risk for reactive airway disease. In one embodiment, levels of particular biomarkers associated with reactive airway disease are determined for an individual, and levels which differ from those expected for the normal population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 2 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of reactive airway disease. In another embodiment, the subject is selected from those individuals who exhibit one or more symptoms of reactive airway disease.

[0163] In any of the above embodiments, the preferred biomarkers for use in this method comprise at least one biomarker from Table 13B. More preferably, all of the biomarkers in this embodiment are found in Table 13B.

B. Male Population

[0164] The invention provides for a method of diagnosing reactive airway disease in a male subject comprising, (a) obtaining a physiological sample of the male subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 6 or 17, wherein the extent of expression of said at least one biomarker is indicative of reactive airway disease.

[0165] In a preferred embodiment, the invention provides for methods of diagnosing reactive airway disease in a male subject comprising determining the extent of expression of a plurality of biomarkers from Table 6 or 17 in a physiological sample of the male subject, wherein a pattern of expression of the plurality of markers are indicative of reactive airway disease or correlate to changes in a reactive airway disease state. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has or may have reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 6 or 17. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0166] In one embodiment, the male subject is at-risk for reactive airway disease. In one embodiment, levels of particular biomarkers associated with reactive airway disease are determined for a male individual, and levels which differ from those expected for the normal male population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 6 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of reactive airway disease. In another embodiment, the male subject is selected from those individuals who exhibit one or more symptoms of reactive airway disease.

[0167] In another embodiment, the biomarkers for use in this method comprise at least one biomarker from Table 13A.

C. Female Population

[0168] The invention provides for a method of diagnosing reactive airway disease in a female subject comprising, (a) obtaining a physiological sample of the female subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 10 or 21, wherein the extent of expression of said at least one biomarker is indicative of reactive airway disease.

[0169] In a preferred embodiment, the invention provides for methods of diagnosing reactive airway disease in a female subject comprising determining the extent of expression of a plurality of biomarkers from Table 10 or 21 in a physiological sample of the female subject, wherein a pattern of expression of the plurality of markers are indicative of reactive airway disease or correlate to changes in a reactive airway disease state. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a female subject has or may have reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 10 or 21. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0170] In one embodiment, the female subject is at-risk for reactive airway disease. In one embodiment, levels of particular biomarkers associated with reactive airway disease are determined for a female individual, and levels which differ from those expected for the normal female population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 10 or 21 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of reactive airway disease. In another embodiment, the female subject is selected from those individuals who exhibit one or more symptoms of reactive airway disease.

[0171] In another embodiment, the biomarkers for use in this method comprise at least one biomarker from Table 13A.

Methods of Diagnosing Non-Small Cell Lung Cancer

[0172] The present invention is directed to methods of diagnosing non-small cell lung cancer in individuals in various populations as described below. In general, these methods rely on determining the extent of expression of particular biomarkers as described herein.

A. General Population

[0173] The invention provides for a method of diagnosing non-small cell lung cancer in a subject comprising, (a) obtaining a physiological sample of the subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 3, wherein the extent of expression of said at least one biomarker is indicative of the presence or development of non-small cell lung cancer.

[0174] In a preferred embodiment, the invention provides for methods of diagnosing non-small cell lung cancer in a subject comprising determining the extent of expression of a plurality of biomarkers from Table 3 in a physiological sample of the subject, wherein a pattern of expression of the plurality of markers are indicative of non-small cell lung cancer or correlate to a changes in a non-small cell lung cancer disease state (*i.e.*, clinical or diagnostic stages). In another preferred embodiment, patterns of expression correlate to an increased likelihood that a subject has or may have non-small cell lung cancer. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 3. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0175] In one embodiment, the subject is at-risk for non-small cell lung cancer. In one embodiment, levels of particular biomarkers associated with non-small cell cancer are determined for an individual, and levels which differ from those expected for the normal population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 3 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of non-small cell cancer. In another embodiment, the subject is selected from those individuals who exhibit one or more symptoms of non-small cell lung cancer.

[0176] In any of the above embodiments, the preferred biomarkers for use in this method comprise at least one biomarker from Table 14B. More preferably, all of the biomarkers in this embodiment are found in Table 14B.

B. Male Population

[0177] The invention also provides for a method of diagnosing non-small cell lung cancer in a male subject comprising, (a) obtaining a physiological sample of the male subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 7 or

18, wherein the extent of expression of said at least one biomarker is indicative of the presence or development of non-small cell lung cancer.

[0178] In a preferred embodiment, the invention provides for methods of diagnosing non-small cell lung cancer in a male subject comprising determining the extent of expression of a plurality of biomarkers from Table 7 or 18 in a physiological sample of the male subject, wherein a pattern of expression of the plurality of markers are indicative of non-small cell lung cancer or correlate to a changes in a non-small cell lung cancer disease state (*e.g.*, stages). In another preferred embodiment, patterns of expression correlate to an increased likelihood that a subject has or may have non-small cell lung cancer. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 7 or 18. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0179] In one embodiment, the male subject is at-risk for non-small cell lung cancer. In one embodiment, levels of particular biomarkers associated with non-small cell cancer are determined for a male individual, and levels which differ from those expected for the normal male population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 7 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of non-small cell cancer. In another embodiment, the male subject is selected from those individuals who exhibit one or more symptoms of non-small cell lung cancer.

[0180] In another embodiment, the biomarkers for use in this method comprise at least one biomarker from Table 14A.

C. Female Population

[0181] The invention also provides for a method of diagnosing non-small cell lung cancer in a female subject comprising, (a) obtaining a physiological sample of the female subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 11 or 22, wherein the extent of expression of said at least one biomarker is indicative of the presence or development of non-small cell lung cancer.

[0182] In a preferred embodiment, the invention provides for methods of diagnosing non-small cell lung cancer in a female subject comprising determining the extent of expression of a plurality of biomarkers from Table 11 or 22 in a physiological sample of the female subject, wherein a pattern of expression of the plurality of markers are indicative of non-small cell lung cancer or correlate to a changes in a non-small cell lung cancer disease state (*e.g.*, stages). In another preferred embodiment, patterns of expression correlate to an increased likelihood that a female subject has or may have non-small cell lung cancer. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 11 or 22. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0183] In one embodiment, the female subject is at-risk for non-small cell lung cancer. In one embodiment, levels of particular biomarkers associated with non-small cell cancer are determined for a female individual, and levels which differ from those expected for the normal female population suggest that the individual is "at-risk." In another embodiment, the number of relevant biomarkers from Table 11 or 22 which deviate statistically from normal is determined, with a greater number of deviant markers indicating greater risk of non-small cell cancer. In another embodiment, the female subject is selected from those individuals who exhibit one or more symptoms of non-small cell lung cancer.

[0184] In another embodiment, the biomarkers for use in this method comprise at least one biomarker from Table 14A.

Methods of Discriminating Between Non-Small Cell Lung Cancer and Reactive Airway Disease

[0185] The present invention is directed to methods of diagnosing lung disease in individuals in various populations as described below. In general, these methods rely on determining the extent of expression of particular biomarkers that discriminate between the indication of reactive airway disease and non-small cell lung cancer.

A. General Population

[0186] The invention also provides for a method of diagnosing a lung disease in a subject comprising determining the extent of expression in said subject of at least one biomarker from Table 4, wherein the extent of expression of said at least one biomarker from Table 4 assists in discriminating between the indication of reactive airway disease and non-small cell lung cancer. In one embodiment, the subject has been diagnosed as having reactive airway disease and/or non-small cell lung cancer. For example, the diagnosis may have been determined by the extent of expression of at least one biomarker in a physiological sample of the subject, where the extent of expression of the at least one biomarker is indicative of reactive airway disease and/or non-small cell lung cancer.

[0187] The invention also provides for a method of diagnosing a lung disease in a subject comprising, (a) obtaining a physiological sample of the subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 4, at least one biomarker from Table 2, and at least one biomarker from Table 3, wherein (i) said at least one biomarker from each of Table 2, Table 3, and Table 4 is not identical, (ii) the extent of expression of said at least one biomarker from Table 2 and Table 3 is indicative of the lung disease of reactive airway disease and non-small cell lung cancer, respectively; and (iii) the extent of expression of said at least one biomarker from Table 4 assists in discriminating between the indication of non-small cell lung cancer and reactive airway disease. Preferably, the method includes at least one marker from each Table which is not present in either of the other Tables.

[0188] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 4, and preferably also a plurality of biomarkers from Table 2, and a plurality of biomarkers from Table 3. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 2, Table 3, and Table 4. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0189] In one embodiment, the subject is at-risk for non-small cell lung cancer and/or reactive airway disease. In another embodiment, the subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer and/or reactive airway disease.

[0190] The invention also provides a diagnostic method to assist in differentiating the likelihood that a subject is at-risk of developing or suffering from non-small cell lung cancer or reactive airway disease comprising, (a) obtaining a physiological sample of the subject who is at-risk for non-small cell lung cancer or reactive airway disease; and (b) determining the extent of expression in said subject of at least one biomarker from Table 4, wherein the extent of expression of said at least one biomarker from Table 4 assists in differentiating the likelihood that said subject is at risk of non-small cell lung cancer or reactive airway disease.

[0191] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 4. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 4. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0192] In one embodiment, the subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer or reactive airway disease. Methods of relating to "at-risk" subjects are described above and methods related thereto are contemplated herein.

B. Male Population

[0193] The invention also provides for a method of diagnosing a lung disease in a male subject comprising determining the extent of expression in said subject of at least one biomarker from Table 8 or 19, wherein the extent of expression of said at least one biomarker from Table 8 or 19 assists in discriminating between the indication of reactive airway disease and non-small cell lung cancer. In one embodiment, the male subject has been diagnosed as having reactive airway disease and/or non-small cell lung cancer. For example, the diagnosis may have been determined by the extent of expression of at least one biomarker in a physiological sample of the male subject, where the extent of expression of the at least one biomarker is indicative of reactive airway disease and/or non-small cell lung cancer.

[0194] The invention also provides for a method of diagnosing a lung disease in a male subject comprising, (a) obtaining a physiological sample of the male subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 8, at least one biomarker from Table 6, and at least one biomarker from Table 7, wherein (i) said at least one biomarker from each of Table 6, Table 7, and Table 8 is not identical, (ii) the extent of expression of said at least one biomarker from Table 6 and Table 7 is indicative of the lung disease of reactive airway disease and non-small cell lung cancer, respectively; and (iii) the extent of expression of said at least one biomarker from Table 8 assists in discriminating between the indication of non-small cell lung cancer and reactive airway disease. Preferably, the method includes at least one marker from each Table which is not present in either of the other Tables.

[0195] The invention also provides for a method of diagnosing a lung disease in a male subject comprising, (a) obtaining a physiological sample of the male subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 19, at least one biomarker from Table 18, and at least one biomarker from Table 17, wherein (i) said at least one biomarker from each of Table 17, Table 18, and Table 19 is not identical, (ii) the extent of expression of said at least one biomarker from Table 17 and Table 18 is indicative of the lung disease of reactive airway disease and non-small cell lung cancer, respectively; and (iii) the extent of expression of said at least one biomarker from Table 19 assists in discriminating between the indication of non-small cell lung cancer and reactive airway disease. Preferably, the method includes at least one marker from each Table which is not present in either of the other Tables.

[0196] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 8, and preferably also a plurality of biomarkers from Table 6, and a plurality of biomarkers from Table 7. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 6, Table 7, and Table 8. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0197] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 19, and preferably also a plurality of biomarkers from Table 17, and a plurality of biomarkers from Table 18. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 17, Table 18, and Table 19. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0198] In one embodiment, the male subject is at-risk for non-small cell lung cancer and/or reactive airway disease. In another embodiment, the male subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer and/or reactive airway disease.

[0199] The invention also provides a diagnostic method to assist in differentiating the likelihood that a male subject is at-risk of developing or suffering from non-small cell lung cancer or reactive airway disease comprising, (a) obtaining a physiological sample of the male subject who is at-risk for non-small cell lung cancer or reactive airway disease; and (b) determining the extent of expression in said subject of at least one biomarker from Table 8 or 19, wherein the extent of expression of said at least one biomarker from Table 8 or 19 assists in differentiating the likelihood that said subject is at risk of non-small cell lung cancer or reactive airway disease.

[0200] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 8. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 8 or 19. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0201] In one embodiment, the male subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer or reactive airway disease. Methods of relating to "at-risk" subjects are described above and methods related thereto are contemplated herein.

B. Female Population

[0202] The invention also provides for a method of diagnosing a lung disease in a female subject comprising determining the extent of expression in said subject of at least one biomarker from Table 12 or 23, wherein the extent of expression of said at least one biomarker from Table 12 or 23 assists in discriminating between the indication of reactive airway disease and non-small cell lung cancer. In one embodiment, the female subject has been diagnosed as having reactive airway disease and/or non-small cell lung cancer. For example, the diagnosis may have been determined by the extent of expression of at least one biomarker in a physiological sample of the female subject, where the extent of expression of the at least one biomarker is indicative of reactive airway disease and/or non-small cell lung cancer.

[0203] The invention also provides for a method of diagnosing a lung disease in a female subject comprising, (a) obtaining a physiological sample of the female subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 12, at least one biomarker from Table 10, and at least one biomarker from Table 11, wherein (i) said at least one biomarker from each of Table 10, Table 11, and Table 12 is not identical, (ii) the extent of expression of said at least one biomarker from Table 10 and Table 11 is indicative of the lung disease of reactive airway disease and non-small cell lung cancer, respectively; and (iii) the extent of expression of said at least one biomarker from Table 12 assists in discriminating between the indication of non-small cell lung cancer and reactive airway disease. Preferably, the method includes at least one marker from each Table which is not present in either of the other Tables.

[0204] The invention also provides for a method of diagnosing a lung disease in a female subject comprising, (a) obtaining a physiological sample of the female subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 23, at least one biomarker from Table 21, and at least one biomarker from Table 22, wherein (i) said at least one biomarker from each of Table 21, Table 22, and Table 23 is not identical, (ii) the extent of expression of said at least one biomarker from Table 21 and Table 22 is indicative of the lung disease of reactive airway disease and non-small cell lung cancer, respectively; and (iii) the extent of expression of said at least one biomarker from Table 23 assists in discriminating between the indication of non-small cell lung cancer and reactive airway disease. Preferably, the method includes at least one marker from each Table which is not present in either of the other Tables.

[0205] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 12, and preferably also a plurality of biomarkers from Table 10, and a plurality of biomarkers from Table 11. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 10, Table 11, and Table 12. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0206] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 23, and preferably also a plurality of biomarkers from Table 21, and a plurality of biomarkers from Table 22. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a male subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 21, Table 22, and Table 23. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0207] In one embodiment, the female subject is at-risk for non-small cell lung cancer and/or reactive airway disease. In another embodiment, the female subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer and/or reactive airway disease.

[0208] The invention also provides a diagnostic method to assist in differentiating the likelihood that a female subject is at-risk of developing or suffering from non-small cell lung cancer or reactive airway disease comprising, (a) obtaining a physiological sample of the female subject who is at-risk for non-small cell lung cancer or reactive airway disease; and (b) determining the extent of expression in said subject of at least one biomarker from Table 12 or 23, wherein the extent of expression of said at least one biomarker from Table 12 or 23 assists in differentiating the likelihood that said subject is at risk of non-small cell lung cancer or reactive airway disease.

[0209] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 12 or 23. In another preferred embodiment, patterns of expression correlate to an increased likelihood that a female subject has non-small lung cancer or reactive airway disease. Patterns of expression may be characterized by any technique known in the art for pattern recognition. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 12 or 23. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0210] In one embodiment, the female subject is selected from those individuals who exhibit one or more symptoms of non-small lung cancer or reactive airway disease. Methods of relating to "at-risk" subjects are described above and methods related thereto are contemplated herein.

[0211] In any of the methods described herein which use biomarkers selected from more than one Table for the purpose of discriminating between, e.g., different disease states or different populations, analysis of the results for the biomarkers from individuals may be performed simultaneously or sequentially.

Methods Of Monitoring Therapy

[0212] The present invention is directed to methods of monitoring therapy in individuals in various populations as described below. In general, these methods rely on determining the extent of expression of particular biomarkers.

A. General Population

[0213] The invention also provides a method of monitoring a subject comprising (a) determining a first extent of expression in said subject of at least one biomarker from Table 1A in a sample obtained from the subject; (b) determining a second extent of expression in said subject of said at least one biomarker from Table 1A using a second sample obtained from the subject at a different time than said first extent of expression; and (d) comparing said first extent of expression and said second extent of expression. Typically, the subject has experienced therapeutic intervention between the time the first and second samples were obtained. Detecting of changes in the pattern of expression between the first and second determinations may be considered to reflect effects of the therapeutic intervention. This embodiment is also useful to identify particular biomarkers which exhibit changes in their extent of expression in response to particular therapeutic interventions.

[0214] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 1A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 1A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0215] The embodiments described above refer to the biomarkers of Table 1A. It will be appreciated, however, that the biomarkers of Table 1B, Table 1C, Table 2, Table 3, or Table 4 may be substituted for the biomarkers of Table 1A in any of the described embodiments.

B. Male Population

[0216] The invention also provides a method of monitoring a male subject comprising (a) determining a first extent of expression in said male subject of at least one biomarker from Table 5A or 16A in a sample obtained from the male subject; (b) determining a second extent of expression in said male subject of said at least one biomarker from Table 1A or 16A using a second sample obtained from the male subject at a different time than said first extent of expression; and (d) comparing said first extent of expression and said second extent of expression. Typically, the male subject has experienced therapeutic intervention between the time the first and second samples were obtained. Detecting of changes in the pattern of expression between the first and second determinations may be considered to reflect effects of the therapeutic intervention. This embodiment is also useful to identify particular biomarkers which exhibit changes in their extent of expression in response to particular therapeutic interventions.

[0217] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 5A or 16A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 5A or 16A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0218] The embodiments described above refer to the biomarkers of Table 5A or 16A. It will be appreciated, however, that the biomarkers of Table 5B, Table 5C, Table 6, Table 7, Table 8, or Table 16B, Table 16C, Table 17, Table 18, or Table 19 may be substituted for the biomarkers of Table 5A or 16A in any of the described embodiments.

C. Female Population

[0219] The invention also provides a method of monitoring a female subject comprising (a) determining a first extent of expression in said female subject of at least one biomarker from Table 9A or 20A in a sample obtained from the female subject; (b) determining a second extent of expression in said female subject of said at least one biomarker from Table 9A or 20A using a second sample obtained from the female subject at a different time than said first extent of expression; and (d) comparing said first extent of expression and said second extent of expression. Typically, the female subject has experienced therapeutic intervention between the time the first and second samples were obtained. Detecting of changes in the pattern of expression between the first and second determinations may be considered to reflect effects of the therapeutic intervention. This embodiment is also useful to identify particular biomarkers which exhibit changes in their extent of expression in response to particular therapeutic interventions.

[0220] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 9A or 20A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 9A or 20A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0221] The embodiments described above refer to the biomarkers of Table 9A or 20A. It will be appreciated, however, that the biomarkers of Table 9B, Table 9C, Table 10, Table 11, Table 12, Table 20B, Table 20C, Table 21, Table 22, or Table 23 may be substituted for the biomarkers of Table 9A or 20A in any of the described embodiments.

Methods Of Predicting A Subject's Response To Therapeutic Intervention

[0222] The present invention is directed to methods of predicting a subject's response to therapeutic intervention in various populations as described below. In general, these methods rely on determining the extent of expression of particular biomarkers.

A. General Population

[0223] The invention also provides a method for predicting a subject's response to therapeutic intervention comprising, (a) obtaining a physiological sample of the subject; and (b) determining the extent of expression in said subject of at least one biomarker from Table 1A, wherein the extent of expression of said at least one biomarker from Table 1A assists in predicting a subject's response to said therapeutic intervention. Preferred biomarkers for use in this embodiment are those biomarkers shown to be responsive to the therapeutic intervention of interest by monitoring a population of subjects. This embodiment may also be used for selection of those patients more likely to be responsive to therapy.

[0224] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 1A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 1A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0225] The embodiments described above refer to the biomarkers of Table 1A. It will be appreciated, however, that the biomarkers of Table 1B, Table 1C, Table 2, Table 3, or Table 4 may be substituted for the biomarkers of Table 1A in any of the described embodiments.

B. Male Population

[0226] The invention also provides a method for predicting a male subject's response to therapeutic intervention comprising, (a) obtaining a physiological sample of the male subject; and (b) determining the extent of expression in said male subject of at least one biomarker from Table 5A or 16A, wherein the extent of expression of said at least one biomarker from Table 5A or 16A assists in predicting a male subject's response to said therapeutic intervention. Preferred biomarkers for use in this embodiment are those biomarkers shown to be responsive to the therapeutic intervention of interest by monitoring a population of male subjects. This embodiment may also be used for selection of those male patients more likely to be responsive to therapy.

[0227] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 5A or 16A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 5A or 16A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0228] The embodiments described above refer to the biomarkers of Table 5A or 16A. It will be appreciated, however, that the biomarkers of Table 5B, Table 5C, Table 6, Table 7, Table 8,

Table 16B, Table 16C, Table 17, Table 18, or Table 19 may be substituted for the biomarkers of Table 5A or 16A in any of the described embodiments.

C. Female Population

[0229] The invention also provides a method for predicting a female subject's response to therapeutic intervention comprising, (a) obtaining a physiological sample of the female subject; and (b) determining the extent of expression in said female subject of at least one biomarker from Table 9A or 20A, wherein the extent of expression of said at least one biomarker from Table 9A or 20A assists in predicting a female subject's response to said therapeutic intervention. Preferred biomarkers for use in this embodiment are those biomarkers shown to be responsive to the therapeutic intervention of interest by monitoring a population of female subjects. This embodiment may also be used for selection of those female patients more likely to be responsive to therapy.

[0230] In a preferred embodiment, the method comprises determining the extent of expression of a plurality of biomarkers from Table 9A or 20A. The plurality of biomarkers may comprise any of the combinations of biomarkers described above with respect to Table 9A or 20A. Indeed, it will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0231] The embodiments described above refer to the biomarkers of Table 9A or 20A. It will be appreciated, however, that the biomarkers of Table 9B, Table 9C, Table 10, Table 11, Table 12, Table 20B, Table 20C, Table 21, Table 22, or Table 23 may be substituted for the biomarkers of Table 9A or 20A in any of the described embodiments.

Methods of Designing Kits

A. General Population

[0232] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a subject comprising (a) selecting at least one biomarker from Table 1A; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0233] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a subject comprising (a) selecting at least one biomarker from Table 1B; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0234] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a subject comprising (a) selecting at least one biomarker from Table 1C; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0235] The invention also provides a method for designing a kit for diagnosing reactive airway disease in a subject comprising (a) selecting at least one biomarker from Table 2; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0236] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer in a subject comprising (a) selecting at least one biomarker from Table 3; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0237] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a subject comprising (a) selecting at least one biomarker from Table 4; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0238] In the above methods, steps (b) and (c) may alternatively be performed by (b) selecting detection agents for detecting said at least one biomarker, and (c) designing a kit comprising said detection agents for detecting at least one biomarker.

[0239] The invention also provides methods for designing kits comprising selecting at least one biomarker from more than one Table. For example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 2 and at least one biomarker from Table 3. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 2, at least one biomarker from Table 3, and at least one biomarker from Table 4. It will be understood that these methods also comprise steps (b) and (c) as previously described.

[0240] It will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

B. Male Population

[0241] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a male subject comprising (a) selecting at least one biomarker from Table 5A or

16A; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0242] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a male subject comprising (a) selecting at least one biomarker from Table 5B or 16B; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0243] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a male subject comprising (a) selecting at least one biomarker from Table 5C or 16C; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0244] The invention also provides a method for designing a kit for diagnosing reactive airway disease in a male subject comprising (a) selecting at least one biomarker from Table 6 or 17; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0245] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer in a male subject comprising (a) selecting at least one biomarker from Table 7 or 18; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0246] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a male subject comprising (a) selecting at least one biomarker from Table 8 or 19; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0247] In the above methods, steps (b) and (c) may alternatively be performed by (b) selecting detection agents for detecting said at least one biomarker, and (c) designing a kit comprising said detection agents for detecting at least one biomarker.

[0248] The invention also provides methods for designing kits comprising selecting at least one biomarker from more than one Table. For example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 6 and at least one biomarker from Table 7. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 6, at least one biomarker from Table 7, and at least one biomarker from Table 8. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 17 and at least one biomarker from Table 18. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 17, at least one biomarker from Table 18, and at least one biomarker from Table 19. It will be understood that these methods also comprise steps (b) and (c) as previously described.

[0249] It will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

C. Female Population

[0250] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a female subject comprising (a) selecting at least one biomarker from Table 9A or 20A; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0251] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a female subject comprising (a) selecting at least one biomarker from Table 9B or 20B; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0252] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer or reactive airway disease in a female subject comprising (a) selecting at least one biomarker from Table 9C or 20C; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0253] The invention also provides a method for designing a kit for diagnosing reactive airway disease in a female subject comprising (a) selecting at least one biomarker from Table 10 or 21; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0254] The invention also provides a method for designing a kit for diagnosing non-small cell lung cancer in a female subject comprising (a) selecting at least one biomarker from Table 11 or 22; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0255] The invention also provides a method for designing a kit for assisting in diagnosing a lung disease in a female subject comprising (a) selecting at least one biomarker from Table 12 or 23; (b) selecting a means for determining the extent of expression of said at least one biomarker; and (c) designing a kit comprising said means for determining the extent of expression.

[0256] In the above methods, steps (b) and (c) may alternatively be performed by (b) selecting detection agents for detecting said at least one biomarker, and (c) designing a kit comprising said detection agents for detecting at least one biomarker.

[0257] The invention also provides methods for designing kits comprising selecting at least one biomarker from more than one Table. For example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 10 and at least one biomarker from Table 11. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 10, at least one biomarker from Table 11, and at least one biomarker from Table 12. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 21 and at least one biomarker from Table 22. In another example, the invention provides a method for designing kit comprising selecting at least one biomarker from Table 21, at least one biomarker from Table 22, and at least one biomarker from Table 23. It will be understood that these methods also comprise steps (b) and (c) as previously described.

[0258] It will be appreciated that the plurality of biomarkers to be determined in these particular methods may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

Kits

[0259] The invention provides kits comprising means for determining the extent of expression of at least one of the biomarkers described herein. The invention also provides kits comprising detection agents for detecting at least one biomarker described herein.

[0260] The invention provides a kit comprising means for determining the extent of expression of at least one biomarker from Table 1A. The invention provides a kit comprising detection agents for detecting at least one biomarker from Table 1A.

[0261] The invention also provides a kit comprising means for determining the extent of expression of SEQ ID NO: 12. In one embodiment, the kit comprises means for determining the extent of expression of SEQ ID NO: 12 and any combination of SEQ ID NOS: 1-11 and 13-17.

[0262] The invention also provides a kit comprising, detection agents for detecting SEQ ID NO: 12. In one embodiment, the kit comprises detection agents for detecting SEQ ID NO: 12 and any combination of SEQ ID NOS: 1-11 and 13-17.

[0263] The invention also provides a kit comprising means for determining the extent of expression of at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17 and means for determining the extent of expression of at least one biomarker from Table 1A.

[0264] The invention also provides a kit comprising, detection agents for detecting at least one polypeptide selected from the group consisting of SEQ ID NOS: 1-17, and detection agents for detecting at least one biomarker from Table 1A.

[0265] The embodiments described above refer to the biomarkers of Table 1A. It will be appreciated, however, that the biomarkers of Table 1B, Table 1C, Table 2, Table 3, Table 4, Table 5A, Table 5B, Table 5C, Table 6, Table 7, Table 8, Table 9A, Table 9B, Table 9C, Table 10, Table 11, Table 12, Table 16A, Table 16B, Table 16C, Table 17, Table 18, Table 19, Table 20A, Table 20B, Table 20C, Table 21, Table 22, or Table 23 may be substituted for the biomarkers of Table 1A in any of the described kits.

[0266] The invention also provides a kit comprising, (a) first means for determining the extent of expression of at least one biomarker from Table 2; and (b) second means for determining the extent of expression of at least one biomarker from Table 3, wherein said at least one biomarker from Table 2 and Table 3 are not identical.

[0267] The invention also provides a kit comprising, (a) detection agents for detecting at least one biomarker from Table 2; and (b) detection agents for detecting at least one biomarker from Table 3, wherein said at least one biomarker from Table 2 and Table 3 are not identical.

[0268] The invention also provides a kit comprising, (a) first means for determining the extent of expression of at least one biomarker from Table 2; (b) second means for determining the extent of expression of at least one biomarker from Table 3; and (c) third means for determining the extent of expression of at least one biomarker from Table 4, wherein said at least one biomarker from Table 2, Table 3, and Table 4 are not identical.

[0269] The invention also provides a kit comprising, (a) detection agents for detecting at least one biomarker from Table 2; (b) detection agents for detecting at least one biomarker from

Table 3; and (c) detection agents for detecting at least one biomarker from Table 4, wherein said at least one biomarker from Table 2, Table 3, and Table 4 are not identical.

[0270] The embodiments described above refer to the biomarkers of Table 2, Table 3, and Table 4. It will be appreciated, however, that the biomarkers of Table 6, Table 7, Table 8, Table 17, Table 18, or Table 19 may be substituted for the biomarkers of Table 2, Table 3, and Table 4, respectively, in any of the described kits. Furthermore, it will be appreciated that the biomarkers of Table 10, Table 11, Table 12, Table 21, Table 22, or Table 23 may be substituted for the biomarkers of Table 2, Table 3, and Table 4, respectively, in any of the described kits. Even further, the skilled person will understand that the invention contemplates kits comprising means for detecting any particular combination of biomarkers described above for any method requiring detection of a particular plurality of biomarkers. It will also be appreciated that the plurality of biomarkers to be determined in these particular kits may be selected from the identified tables using the criteria discussed above in the section entitled "Selection of Biomarkers for Determination."

[0271] The following examples are provided to exemplify various modes of the invention disclosed herein, but they are not intended to limit the invention in any way.

Example 1

[0272] Human blood samples were collected from volunteers. Thirty samples were collected from individuals not known to have either non-small cell lung cancer or asthma. These thirty samples comprise, and are referred to herein as, the "normal population." Twenty-eight blood samples were collected from individuals known to have asthma and diagnosed as such by a physician. These twenty-eight samples comprise, and are referred to herein as, the "asthma population." Thirty blood samples were collected from individuals known to have non-small cell lung cancers and diagnosed as such by a physician. These thirty samples comprise, and are referred to herein as the "lung cancer population."

[0273] Research was performed to select biomarkers for which it was believed that altered expression levels would be associated with lung cancer or asthma. As used herein, "lung cancer" is meant to encompass those lung cancers which are known to be non-small celled lung cancers. The following fifty-nine biomarkers were selected to be tested: CD40, Hepatocyte Growth Factor ("HGF"), I-TAC ("CXCL11"; "chemokine (C-X-C motif) ligand 11," "interferon-inducible T-cell alpha chemoattractant"), Leptin ("LEP"), Matrix Metalloproteinase ("MMP") 1, MMP 2, MMP3, MMP 7, MMP 8, MMP 9, MMP 12, MMP 13, CD40 Soluble Ligand ("CD40 Ligand"), Epidermal Growth Factor ("EFG"), Eotaxin ("CCL11"), Fractalkine, Granulocyte Colony Stimulating Factor ("G-CSF"), Granulocyte Macrophage Colony Stimulating Factor

("GM-CSF"), Interferon γ ("IFN γ"), Interleukin ("IL") 1α, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IP-10, Monocyte Chemotactic Protein 1 ("MCP-1"), Macrophage Inflammatory Protein ("MIP") 1α, MIP-1β, Transforming Growth Factor α ("TGF α"), Tumor Necrosis Factor α ("TNF α"), Vascular Endothelial Growth Factor ("VEGF"), Insulin ("Ins"), C-peptide, Glucagon Like Protein-1/amyline ("GLP-1/amylin"), Amylin (total), Glucagon, Adiponectin, Plasminogen Activator Inhibitor 1 ("PAI-1"; "Serpin") (active/total), Resistin ("RETN"; "xcp1"), sFas, Soluble Fas Ligand ("sFasL"), Macrophage Migration Inhibitory Factor ("MIF"), sE-Selectin, Soluble Vascular Cell Adhesion Molecule ("sVCAM"), Soluble Intracellular Adhesion Molecule ("sICAM"), Myeloperoxidase ("MPO"), C-Reactive Protein ("CRP"), Serum Amyloid A ("SAA" ; "SAA1"), and Serum Amyloid P ("SAP").

[0274] Plasma specimens for each of the normal, asthma and lung cancer populations were screened for each of the fifty-nine biomarkers by subjecting the plasma specimens to analysis using Luminex's xMAP technology, a quantitative multiplexed immunoassay using automated bead-based technologies.

[0275] Several different assay kits were used with the Luminex xMAP technology to screen the biomarkers, namely Millipore's Human Cytokine/Chemokine (Cat# MPXHCYTO-60K, Human Endocrine (Cat# HENDO-65K), Human Serum Adipokines (Cat# HADKI-61K), Human Sepsis/Apoptosis (Cat# HSEP-63K), Human Cardiovascular Panel 1 (Cat# HCVD1-67AK) and Human Cardiovascular Panel 2 (HCVD2-67BK), R&D Systems, Inc.'s Human Fluorokine MAP Profiling Base Kit B (Cat# LUB00) and Human Fluorokine MAP MMP Profiling Base Kit (Cat# LMP000). The fluorescence intensity levels resulting from the multiplexed immunoassay were recorded for each of the fifty-nine biomarkers for each plasma specimen for each population. The recorded fluorescence intensity is proportional to the concentration of the corresponding biomarker in the sample, and to the extent of its expression in the individual. Averages, standard deviations, and relative standard deviations for fluorescence intensity level associated with each biomarker for each population were calculated. Figs. 1A through 1C show the average mean, standard deviation and relative standard deviation for each biomarker in the normal (NO), non-small cell lung cancer (LC), and asthma (AST) populations.

[0276] Student's t test was then used to characterize inter-pathology differences for each particular biomarker between each population. Mean fluorescence intensity measurements of each biomarker for the samples from normal patients were compared to those of the samples from patients suffering from lung cancer and also to those of samples derived from patients suffering from asthma. Fig. 1D shows the differences between the various population means for each marker. In addition, the mean fluorescence intensity measurements for the lung cancer patients were compared to the mean fluorescence intensity measurements for the asthma patients, and the significance was evaluated using the Student's t statistic.

[0277] Further analysis of the statistical differences for each biomarker between the normal, asthma and lung cancer populations was performed. To characterize the difference in mean expression levels for each biomarker between the populations, Student's t values were calculated using the t-test function available in the Microsoft EXCEL software package. The EXCEL t-test function was used to calculate the probability associated with the Student's t value under an assumption of equal variance using a two-tailed distribution.

[0278] The significance of the difference in expression levels between the populations was determined on the criteria that any Student's t value with an associated probability smaller than 0.05 was considered to be significant to indicate the presence of the given pathology, whether asthma or lung cancer. Using a criterion of 0.05 or less is generally accepted in the scientific community. Any Student's t value with an associated probability larger than 0.1 was considered to be insignificant to indicate the presence of the given pathology. Furthermore, any Student's t value with an associated probability between 0.051 and 0.1 was determined to be marginal.

[0279] Referring now to Fig. 1E, the Student's t values with an associated probability calculated comparing each biomarker for each population is shown. It should be noted that the Student's t values with an associated probability shown in Fig. 1E are calculated on the basis that each of the asthma, normal, and lung cancer populations has a single mean and a normal distribution.

[0280] The significance of the differences in biomarker expression levels were used to rank the relative importance of the biomarkers. Those biomarkers that were found to be most significantly different between pathologies were classed as relatively more important. The measurements of mean fluorescence intensity were examined, and data for all biomarkers having intensities that did not depart significantly from the average intensities of specimens in the other populations were excluded from further analysis. Those biomarkers having relatively low relative standard deviation were classed as more significant than those having relatively high standard deviation.

[0281] The direction of deviation, *i.e.* whether the average level of a particular marker increased or decreased in any pathology relative to any of the other pathologies, was not used to judge the relative significance of a particular marker. In this way, a group of biomarkers was assembled that showed high variability between pathologies, relatively low relative standard deviation and good instrumental detectability (defined as non-zero uncorrected mean fluorescence intensity). Those calculations were used to test the efficiency of the immunoassay and analyzed to determine the biomarkers which showed significant differences in expression levels between the expression levels of the normal population, as well as to determine reference ranges which are characteristic of and associated with the pathologies of lung cancer and/or asthma.

[0282] Still referring to Fig. 1E, the probabilities associated with the Student's t values were calculated to compare the asthma population to the normal population. Significant differences between the asthma population and the normal population were determined from the Student's t probability for the biomarkers sE-Selectin, EGF, Leptin, IL-5, PAI-1, Resistin, MMP-13, CD40 Ligand sVCAM-1, HGF, C-Peptide, sICAM-1, MMP-7, Adiponectin, GM-CSF and MIF. This determination was made on the basis that, when comparing the twenty-eight specimens from the asthma population with the thirty specimens from the normal population using the Student's t function described herein, the probabilities associated with the Student's t value for each of these biomarkers was smaller than 0.05. Difference was determined to be insignificant between the asthma population and the normal population for the biomarkers CRP, MMP-9, IL-4, IL-1α, SAA, IL-7 and IL-6, as the Student's t probability for each of these was significantly greater than 0.05.

[0283] As also shown in Fig. 1E, the probabilities associated with the Student's t values were calculated to compare the lung cancer population to the normal population. Significant difference between the lung cancer population and the normal population was determined from the Student's t probability for the biomarkers sE-Selectin, EGF, Leptin, IL-5, PAI-1, Resistin, CRP, MMP-9, IL-4, IL-1α, SAA, IL-7, CD40 Ligand, MMP-7 and MMP-12. Again, this determination was made on the basis that, when comparing the thirty specimens from the lung cancer population with the thirty specimens from the normal population using the Student's t function described herein, the Student's t probability for each of these biomarkers was smaller than 0.05. Difference was determined to be insignificant between the lung cancer population and the normal population for the biomarkers MMP-13, HGF, C-Peptide, sICAM, Adiponectin, GM-CSF, IL-17, TNF α, ITAC and MIF, as the Student's t probability for each of these biomarkers was significantly greater than 0.05.

[0284] Three biomarkers had probabilities associated with the Student's t values only slightly greater than 0.05 between the lung cancer population and the normal population. Specifically, when comparing the lung cancer population to the normal population, IL-6 had a Student's t probability of 0.076195528, sVCAM-1 had a Student's t probability of 0.08869949, and IL-15 had a Student's T probability of 0.086324372. These biomarkers are regarded as having insignificant difference between the lung cancer population and the normal population. However, due to the fact that the Student's t probability for these three biomarkers are close to 0.05, it is possible that each population may significantly vary between the normal and lung cancer populations.

[0285] Finally, as shown in Fig. 1E, further analysis was done by calculating the probabilities associated with the Student's t values to compare the lung cancer population to the asthma population. Significant difference between the lung cancer population and the asthma population was determined from the Student's t probability for the biomarkers sE-Selectin, EGF, Leptin, IL-5, PAI-1, Resistin, CRP, MMP-9, IL-4, IL-1α, SAA, IL-7, IL-6, MMP-13 sVCAM, HGF, C-Peptide, sICAM, Adiponectin, GM-CSF, IL-17, IL-15, TNF α and I-TAC. This determination was made on the basis that, when comparing the thirty specimens from the lung cancer population with the twenty-eight specimens from the asthma population using the Student's t function described herein, the Student's t probability for each of these biomarkers was smaller than 0.05. Difference was determined to be insignificant between the lung cancer population and the asthma population for the biomarkers CD40 Ligand, MMP-7, MMP-12 and MIF, as the Student's t probability for each of these biomarkers was significantly greater than 0.05.

Example 2

[0286] Human blood samples were collected from volunteers. One hundred forty-two samples were collected from individuals not known to have either non-small cell lung cancer or asthma. These samples comprise, and are referred to herein as, the "normal population." One hundred eight blood samples were collected from individuals known to have asthma and diagnosed as such by a physician. These samples comprise, and are referred to herein as, the "asthma population." One hundred forty-six blood samples were collected from individuals known to have non-small cell lung cancers and diagnosed as such by a physician. These comprise, and are referred to herein as the "lung cancer population."

[0287] The same methods described in Example 1 were performed. Figs. 2A-2E show the results obtained. These results provide guidance for selecting suitable biomarkers for the methods of this invention. In particular, the probability values for particular markers are useful in this regard.

[0288] Figure 2E shows the probability associated with the effectiveness of various biomarkers for discriminating between the physiological state of different populations. Probability values of 0.1 or less are highlighted on this table to identify biomarkers of interest. Biomarkers used in preferred methods of this invention will have probability values of 0.05 or less, more preferably 0.01, and even more preferably 0.001 or less.

Example 3

[0289] Human blood samples were collected from volunteers. Two hundred eighty eight samples were collected from individuals not known to have either non-small cell lung cancer or asthma. These samples comprise, and are referred to herein as, the "normal population." One hundred eighty blood samples were collected from individuals known to have asthma and diagnosed as such by a physician. These samples comprise, and are referred to herein as, the "asthma population." Three hundred sixty blood samples were collected from individuals known to have non-small cell lung cancers and diagnosed as such by a physician. These comprise, and are referred to herein as the "lung cancer population."

[0290] The same methods described in Example 1 were performed. A Panomics' Procarta Cytokine kit (Cat# PC1017) was also used. Antibodies for PAI-1 and Leptin were used from two different kits. Antibodies for PAI-1^A and Leptin^1 were produced by Millipore. Antibodies for PAI-1^B were produced by Panomics. Figs. 3A-3E show the results obtained. These results provide guidance for selecting suitable biomarkers for the methods of this invention. In particular, the probability values for particular markers are useful in this regard.

[0291] Figure 3E shows the probability associated with the effectiveness of various biomarkers for discriminating between the physiological state of different populations. Probability values of 0.1 or less are highlighted on this table to identify biomarkers of interest. Biomarkers used in preferred methods of this invention will have probability values of 0.05 or less, more preferably 0.01, and even more preferably 0.001 or less.

[0292] The data obtained was then segregated and analyzed by sex.

[0293] Figures 4A-4C show the average fluorescence intensity level of the biomarkers in the normal (NO), non-small cell lung cancer (LC), and asthma (AST) female population. FIG. 4D shows the percent change in the mean of each of the biomarkers in the AST v. NO female populations, LC v. NO female populations, and the AST v. LC female populations. FIG. 4E shows the probability associated with Student's t values calculated by comparing the mean fluorescence intensity measured for each biomarker, where the means to be compared are AST

v. NO female populations, LC v. NO female populations, and the AST v. LC female populations, respectively.

[0294] The same information with respect to the male population is shown in FIG. 5A-5E.

[0295] Next, the female and male population data was compared. FIG. 6A shows the percent change in the mean of each of the biomarkers in the AST male population compared to the AST female population, the LC male population compared to the LC female population, and the NO male population compared to the NO female population. FIG. 6B shows the probability associated with Student's t values calculated by comparing the mean fluorescence intensity measured for each biomarker in the male and female populations from Example 3, where the means to be compared are the AST male and female populations, LC male and female populations, and the NO male and female populations, respectively.

Example 4

[0296] The Kruskal-Wallis test is a well known, non-parametric statistical method. The data obtained from Example 3 was segregated by sex and analyzed using the Kruskal-Wallis (U test). Markers with probability values of 0.05 or less were considered significant. Markers showing marginally significant (probability between 0.051-0.10) and insignificant differences (probability above 0.10) were discarded. The results for the retained markers are shown in Figures 7-8.

[0297] Figure 7A shows the percent change in the mean concentration of each of the biomarkers in the LC v. NO female populations, AST v. NO female populations, and the AST v. LC female populations. The scalar sum (i.e., the sum of the absolute values of the percent change for all three comparisons) is also provided and was used to rank the biomarkers. FIG. 7B shows the probability associated with the Kruskal-Wallis test calculated by comparing the concentration measured for each biomarker, where the populations to be compared are AST v. NO female populations, LC v. NO female populations, and the AST v. LC female populations, respectively.

[0298] The same information with respect to the male population is shown in FIG. 8A and 8B.

[0299] The biomarkers showed unique gender- and disease- specific patterns. For unisex analysis of LC, 36 markers with an absolute change of at least 25% cutoff threshold and 32 markers with at least 50% cutoff were identified. For women, 32 markers with at least 25% cutoff and 30 with at least 50% cutoff were found. For men, 39 markers were found at least 25% cutoff and 37 at least 50% cutoff. Expression of four markers was unique for women with LC compared to NO: IL-8 and serum amyloid P (downregulated), serum amyloid A and C-reactive protein (all upregulated). Five markers were unique for men with LC compared to NO:

insulin (downregulated), matrix metalloproteinases-7 and -8, resistin and hepatocyte growth factor (all upregulated). Three markers showed opposite patterns of expression: (i) VEGF was downregulated in women and upregulated in men with LC compared to NO; (ii) Leptin was upregulated in women and downregulated in men; and (iii) and MIP-1a were upregulated in men and downregulated in women with LC versus NO.

[0300] The invention provides for various methods of gender-based identification of disease states. For example, the invention provides for methods of physiological characterization in a male subject comprising determining whether insulin is downregulated, and/or matrix metalloproteinases-7 and -8, resistin and hepatocyte growth factor are upregulated. Such patterns are indicative of disease. Assays within the contemplation of this invention include detecting abnormal up/down regulation of three, four, or five of these biomarkers in a male subject.

[0301] In another example, the invention provides for methods of physiological characterization in a female subject comprising determining whether IL-8 and/or serum amyloid P are downregulated, and/or serum amyloid A and C-reactive protein are upregulated. Such patterns are indicative of disease. Assays within the contemplation of this invention include detecting abnormal up/down regulation of three or four of these biomarkers in a female subject.

Example 5

[0302] Human blood samples were collected from volunteers. Thirty samples were collected from individuals not known to have either non-small cell lung cancer or asthma. The individuals known not to have either non-small cell lung cancer or asthma comprise, and are referred to herein as, the "normal population." Twenty-eight blood samples were collected from individuals known to have asthma and diagnosed as such by a physician. The individuals known to have asthma comprise, and are referred to herein as, the "asthma population." Thirty blood samples were collected from individuals known to have non-small cell lung cancers and diagnosed as such by a physician. The individuals known to have non-small cell lung cancer comprise, and are referred to herein as the "lung cancer population." Generally, as used herein, the term "lung cancer" or "lung cancers" is meant to refer to non-small cell lung cancers.

[0303] Eight to ten plasma specimens from each of the asthma population, normal population and lung cancer population were selected at random to be tested. Each plasma specimen from each population was subjected to a protease or digesting agent. Trypsin was used as the protease, and is desirable to be used as a protease because of its ability to make highly specific and highly predictable cleavages due to the fact that trypsin is known to cleave peptide chains at the carboxyl side of the lysine and arginine, except where a proline is present immediately following either the lysine or arginine. Although trypsin was used, it is possible to use other proteases or digesting agents. It is desirable to use a protease, or mixture of proteases, which cleave at least as specifically as trypsin.

[0304] The tryptic peptides, which are the peptides left by the trypsin after cleavage, were then separated from the insoluble matter by subjecting the specimens to a centrifugation and a capillary liquid chromatography, with an aqueous acetonitrile gradient with 0.1% formic acid using a 0.375 X 180 mm Supelcosil ABZ+ column on an Eksigent 2D capillary HPLC to effect chromatographic resolution of the generated tryptic peptides. This separation of the peptides is necessary because the electrospray ionization process is subject to ion co-suppression, wherein ions of a type having a higher proton affinity will suppress ion formation of ions having lower proton affinities if they are simultaneously eluting from the electrospray emitter, which in this case is co-terminal with the end of the HPLC column.

[0305] This methodology allows for the chromatographic separation of the large number of peptides produced in the tryptic digestions and helps to minimize co-suppression problems, thereby maximizing chances of the formation of pseudo-molecular ion co-suppression, thereby maximizing ion sampling. The tryptic peptides for each specimen were then subjected to an LC-ESIMS. The LC-ESIMS separated each peptide in each specimen in time by passing the peptides in each specimen through a column of solvent system consisting of water, acetonitrile and formic acid as described above.

[0306] The peptides were then sprayed with an electrospray ionization source to ionize the peptides and produce the peptide pseudo-molecular ions as described above. The peptides were passed through a mass analyzer in the LC-ESIMS where molecular masses were measured for each peptide pseudo-molecular ion. After passing through the LC-ESIMS, mass spectral readouts were produced for the peptides present in each sample from the mass spectral data, namely the intensities, the molecular weights and the time of elution from a chromatographic column of the peptides. The mass spectral readouts are generally graphic illustrations of the peptide pseudo-molecular ion signals recorded by the LC-ESIMS, wherein the x-axis is the measurement of mass to charge ratio, the y-axis is the intensity of the pseudo-molecular ion signal. These data are then processed by a software system that controls the LC-ESIMS and acquires and stores the resultant data.

[0307] Once the mass spectral data was obtained and placed on the mass spectral readouts, a comparative analysis was performed wherein the mass spectral readouts of each plasma specimen tested in the LC-ESIMS for each population was performed, both interpathologically and intrapathalogically. The mass spectral peaks were compared between each specimen tested in the normal population. The mass spectral peaks were then compared between each specimen tested in the asthma population and the lung cancer population. Once the intrapathological comparisons were performed, interpathological comparisons were performed wherein the mass spectral readouts for each specimen tested in the LC-ESIMS for the asthma population was compared against each specimen tested in the normal population. Likewise, the mass spectral readouts for each specimen tested in the LC-ESIMS for the lung cancer population was compared against each specimen tested in the normal population.

[0308] Peptides with mass spectral readouts that indicated the peptide intensities were inconsistently differentially expressed intrapathologically or were not substantially altered (less than 10 fold variance in intensity) when comparing the asthma population or lung cancer population to the normal population were determined to be insignificant and excluded. Generally, the exclusion criteria used involved comparing the peptide peak intensities for at least half of the identified characteristic peptides for a given protein across at least ten data sets derived from the analysis of individual patient plasma specimens from each pathology. If the intensity of the majority of peptide peaks derived from given protein were at least 10 fold higher in intensity for 80% of the plasma data sets, the protein was classed as differentially regulated between the two pathologic classes.

[0309] However, the identity of the proteins giving rise to the peptides that were observed to be differentially regulated were unknown and needed to be identified. To make the identification of the proteins, peptide pseudo-molecular ion signal intensities were compared across known databases which contain libraries of known proteins and peptides and suspected proteins and peptides.

[0310] The mass spectral readouts of the tryptic digests for each specimen from each of the normal, lung cancer and asthma population were inputted into a known search engine called MASCOT. MASCOT is a search engine known in the art which uses mass spectrometry data to identify proteins from four major sequencing databases, namely the MSDB, NCBInr, SwissProt and dbEST databases. These databases contain information on all proteins of known sequence and all putative proteins based on observation of characteristic protein transcription initiation regions derived from gene sequences. These databases are continually checked for accuracy and redundancy and are subject to continuous addition as new protein and gene sequences are identified and published in the scientific and patent literature.

[0311] Search criteria and parameters were inputted into the MASCOT program and the mass spectral data from the mass spectral readouts for each population were run through the MASCOT program. The mass spectral data entered into the MASCOT program were for the all specimens of each pathology. The MASCOT program then ran the mass spectral data for the peptides inputted against the sequencing databases, comparing the peak intensities and masses of each peptide to the masses and peak intensities of known peptides and proteins. MASCOT then produced a search result which returned a candidate list of possible protein identification matches, commonly known as "significant matches" for each sample that was analyzed.

[0312] Significant matches are determined by the MASCOT program by assigning a score called a "MOWSE score" for each specimen tested. The MOWSE score is an algorithm wherein the score is $-10*LOG_{10}(P)$, where P is the probability that the observed match is a random event, which correlates into a significance p value where p is less than 0.05, which is the generally accepted standard in the scientific community. MOWSE scores of approximately 55 to approximately 66 or greater are generally considered significant. The significance level varies somewhat due to specific search considerations and database parameters. The significant matches were returned for each peptide run, resulting in a candidate list of proteins.

[0313] Next, comparative analysis was performed using the same methods described in US 20090069189.

[0314] The data from the mass spectral readouts were cross checked with the significant matches to confirm the raw data, peak identities, charge multiplicities, isotope distribution and flanking charge states. A reverse search was then performed to add peptides to the candidate list which may have been missed by the automated search through the MASCOT program. The additional peptides were identified by selecting the "best match" meaning the single protein which substantially matched each parameter of the peptide compared, performing an *in silico* digest wherein the tryptic peptides and their respective molecular masses calculated based on the known amino acid or gene sequence of the protein. These predicted peptide masses were then searched against the raw mass spectral data and any peaks identified were examined and qualified as described above. Then, all of the peptides including those automatically identified by MASCOT and those identified by manual examination were entered into the mass list used by MASCOT. The refined match is then used to derive a refined MOWSE score.

[0315] As a result of the identification process, the protein Arginase-1 was determined to be significantly differentially expressed between the asthma population, lung cancer population and/or normal population. Other proteins identified using this method are BAC04615, Q6NSC8, CAF17350, Q6ZUD4, Q8N7P1, CAC69571, FERM domain containing protein 4, JCC1445 proteasome endopeptidase complex chain C2 long splice form, Syntaxin 11, AAK13083, and AAK130490. *See* US 20090069189.

[0316] Having identified a specific protein which is consistently differentially expressed in asthma and lung cancer patients, it is possible to diagnose these pathologies early in the progression of the diseases by subjecting proteins in a patient's plasma to tryptic digestion and analysis by the LC-ESIMS, obtaining the mass spectral data, and determining whether the mass spectral data includes peaks for one or more of Arginase-1, BAC04615, Q6NSC8, CAF17350, Q6ZUD4, Q8N7P1, CAC69571, FERM domain containing protein 4, JCC1445 proteasome endopeptidase complex chain C2 long splice form, Syntaxin 11, AAK13083, and AAK130490. The levels of any proteins found in the patient sample are then compared to the levels found in a normal population.

[0317] The amino acid sequence disclosed in SEQ ID NO: 1 is the primary amino acid sequence known as of the date of filing this application for the protein BAC04615. The amino acid sequence disclosed in SEQ ID NO: 2 is the primary amino acid sequence known as of the date of filing this application for the protein Q6NSC8. The amino acid sequence disclosed in SEQ ID NO: 3 is the primary amino acid sequence known as of the date of filing this application for the protein CAF17350. The amino acid sequence disclosed in SEQ ID NO: 4 is the primary amino acid sequence known as of the date of filing this application for the protein Q6ZUD4. The amino acid sequence disclosed in SEQ ID NO: 5 is the primary amino acid sequence known as of the date of filing this application for the protein FERM domain containing protein 4. The amino acid sequence disclosed in SEQ ID NO: 6 is the primary amino acid sequence known as of the date of filing this application for the protein AAK13083. The amino acid sequence disclosed in SEQ ID NO: 7 is the primary amino acid sequence known as of the date of filing this application for the protein Q8N7P1. The amino acid sequence disclosed in SEQ ID NO: 8 is the primary amino acid sequence known as of the date of filing this application for the protein CAC69571. The amino acid sequence disclosed in SEQ ID NO: 9 is the primary amino acid sequence known as of the date of filing this application for the protein JCC1445 proteasome endopetidase complex chain C2 long splice. The amino acid sequence disclosed in SEQ ID NO: 10 is the primary amino acid sequence known as of the date of filing this application for the protein Syntaxin 11. The amino acid sequence disclosed in SEQ ID NO: 11 is the primary amino acid sequence known as of the date of filing this application for the protein AAK13049. The amino acid sequence disclosed in SEQ ID NO: 12 is the primary amino acid sequence known as of the date of filing this application for the protein Arginase-1.

Example 6

[0318] Selected tissue specimens from asthma patients was subjected to the same methods described in Example 5. *See also* Application No. 61/176,437.

[0319] As a result of the identification process, the following proteins were determined to be significantly differentially expressed in the asthma patient:

Accession Number	Gene or Protein	Suggested Function From Literature	Mass	Mowse Score	SEQ ID NO:
Q6ZR64 (Human)	FLJ46603	hypothetical protein HBV preS1-transactivated protein 1	23397	51	13
Q8WUX6 (Human)	AAH19232	expressed in lung tissue	12347	49	14
Q5YA4	CCDC52 protein fragment	potential role in regulation of RhoA GTPase	11748	51	15
Q5T2Z1 (Human)	DDA3	activated by p53	25035	56	16
OSHU7C	cytochrome c oxidase chain VIIc precursor	terminal component of the mitochondrial respiratory chain complex; conversion of redox energy to ATP	7241	46	17

[0320] Having identified five specific proteins which are consistently differentially expressed in asthma patients, it is possible to diagnose these pathologies early in the progression of the diseases by subjecting proteins in a patient's tissue specimen to tryptic digestion and analysis by the LC-ESIMS, obtaining the mass spectral data, and determining whether the mass spectral data includes peaks for one or more of SEQ ID NOS: 13-17. The levels of any proteins found in the patient sample are then compared to the levels found in a normal population.

Example 7

Diagnostic Test for Non-Small Cell Lung Cancer

[0321] A sample of a biological fluid is obtained from a patient for whom diagnostic information is desired. The sample is preferably blood serum or plasma. The concentration in the sample of seven (7) of the following 14 biomarkers is determined: IL-13, I-TAC, MCP-1, MMP-1, MPO, HGF, Eotaxin, MMP-9, MMP-7, IP-10, SAA, Resistin, IL-5, and sVACM-1. The measured concentration from the sample for each biomarker is compared to the range of concentrations of that marker found in the same fluid in normal human individuals, a population of individuals diagnosed with asthma, and a population of individuals diagnosed with NSCLC. Deviation from the normal range is indicative of lung disease, and deviation from the range for the population of individuals with asthma is indicative of NSCLC. Tests on a patient using biomarkers from the same set of 14 may be used in analogous procedures for diagnosis of asthma or other reactive airway diseases.

Example 8

Monitoring Therapy for Non-Small Cell Lung Cancer

[0322] A pretreatment sample of a biological fluid is obtained from a patient who has been diagnosed with NSCLS before any treatment for the disease. The sample is preferably blood serum or plasma. The concentration in the sample of eight (8) of the following 24 biomarkers is determined: IL-13, EGF, I-TAC, MMP-1, IL-12 (p70), Eotaxin, MMP-8, MCP-1, MPO, IP-10, SAA, HGF, MMP-9, MMP-12, Amylin (Total), MMP-7, IL-6, MIL-1β, Adiponectin, IL-10, IL-5, IL-4, SE-selectin, and MIP-1α. The measured concentration from the sample for each biomarker may be compared to the range of concentrations of that marker found in the same fluid in normal human individuals. After the pretreatment sample has been taken the patient undergoes therapeutic intervention comprising surgery followed by irradiation. Samples of the same fluid are taken after surgery, but before irradiation. Additional samples are taken after each irradiation session. The concentration in each sample of the same eight (8) biomarkers is determined. Changes in the level of expression of each biomarker are noted and compared with other symptoms of progression of the disease.

Example 9

Selection of Predictive Biomarkers

[0323] A pretreatment sample of a biological fluid is obtained from a population of patients who have been diagnosed with NSCLS before any treatment for the disease. The sample is preferably blood serum or plasma. The concentration in the sample of the following 24 biomarkers is determined: IL-13, EGF, I-TAC, MMP-1, IL-12 (p70), Eotaxin, MMP-8, MCP-1, MPO, IP-10, SAA, HGF, MMP-9, MMP-12, Amylin (Total), MMP-7, IL-6, MIL-1β, Adiponectin, IL-10, IL-5, IL-4, SE-selectin, and MIP-1α. The measured concentration from the sample for each biomarker is compared to the range of concentrations of that marker found in the same fluid in normal human individuals. After the pretreatment sample has been taken each patient undergoes therapeutic intervention comprising surgery followed by irradiation. Samples of the same fluid are taken after surgery, but before irradiation. Additional samples are taken after each irradiation session. The concentration in each sample of the 24 biomarkers is determined. Changes in the level of expression of each biomarker are noted and compared with other symptoms of progression of the disease. All biomarkers whose level changes after therapy are identified.

Example 10

Selection of Susceptible Patients

[0324] A sample of a biological fluid is obtained from a patient who has been diagnosed with NSCLS. The sample is preferably blood serum or plasma. The concentration in the sample of each of the biomarkers identified in the previous example is determined, and patients for whom the highest number of biomarkers show values deviating from normal are selected for treatment.

Example 11

Diagnostic Test for Non-Small Cell Lung Cancer In Male Subject

[0325] A sample of a biological fluid is obtained from a male patient for whom diagnostic information is desired. The sample is preferably blood serum or plasma. The concentration in the sample of seven (7) of the following 14 biomarkers is determined: I-TAC, MPO, HGF, MMP-1, MMP-8, Eotaxin, IL-8, MMP-7, IP-10, sVACM-1, IL-10, Adiponectin, SAP, and IFN-γ. The measured concentration from the sample for each biomarker is compared to the range of concentrations of that marker found in the same fluid in normal human male individuals, a population of male individuals diagnosed with asthma, and a population of male individuals diagnosed with NSCLC. Deviation from the normal range is indicative of lung disease, and deviation from the range for the population of individuals with asthma is indicative of NSCLC. Tests on a patient using biomarkers from the same set of 14 may be used in analogous procedures for diagnosis of asthma or other reactive airway diseases.

Example 12

Alternative Test for Non-small Cell Lung Cancer in a Male Subject

[0326] Many, if not all, of the biomarkers identified in Tables 1-15 participate in communications pathways of the sort described above. Some of the biomarkers are related to each other as first order interactors. Selection of markers for use in a diagnostic or prognostic assay may be facilitated using known relationships between particular biomarkers and their first order interactors. The known communication relationships between the biomarkers listed on Table 16B can be seen in Figure 9, generated by the Ariadne system. Figure 9 shows that first order interactors of HGF (Hepatocyte Growth Factor) include sFasL (soluble Fas ligand), PAI-1 (serpin Plasminogen Activator Inhibitor 1) (active/total), Ins (Insulin; which also includes C-peptide), EGF (Epidermal Growth Factor), MPO (Myeloperoxidase), and MIF (Migration Inhibitory Factor). Other interactors (not first order) include RETN (resistin, xcp1), SAA1 (Serum Amyloid A, SAA), CCL11 (Eotaxin), LEP (Leptin) and CXCL11 (Chemokine (C-X-C motif) ligand 11, Interferon-inducible T-cell alpha chemoattractant (I-TAC) or Interferon-gamma-inducible protein 9 (IP-9)). In addition, Figure 9 shows that two biomarkers MMP1 and MMP-8 (Matrix Metalloproteinases 1 and 8) are not on a communication pathway with HGF.

[0327] One way to maximize the information collected by measuring a selection of biomarkers, is to select a plurality of biomarkers such that biomarkers which are <u>not</u> in the same communication pathway are included in the collection. Using the list of biomarkers in Table 16B, it appears that if the levels of at least HGF or another biomarker that is a first order interactor with HGF, and MMP-8 are abnormal in a male subject, the likelihood that the subject has lung cancer is much higher. If the level of MMP-1 is also abnormal, then the likelihood is even higher. Thus, one method according to the present invention for diagnosing lung cancer in a male subject would be to determine the level of at least HGF or another biomarker that is a first order interactor with HGF, and MMP-8, and the levels compared to the range expected for a normal population to see of the levels of these biomarkers is abnormal. In a preferred mode, the diagnostic method would also include determining whether the level of MMP-1 was normal. More preferable, one or more of CXCL11, LEP, SAA1 and/or RETN would also be determined, and the levels compared to the range expected for a population of normal individuals. The more of these biomarkers which are present at an abnormal level, the more likely that the subject has lung cancer.

<u>Example 13</u>

Monitoring Therapy for Non-Small Cell Lung Cancer In A Male

[0328] A pretreatment sample of a biological fluid is obtained from a male patient who has been diagnosed with NSCLS before any treatment for the disease. The sample is preferably blood serum or plasma. The concentration in the sample of eight (8) of the following 24 biomarkers is determined: IL-13, I-TAC, EGF, MPO, HGF, MMP-1, MMP-8, MIF, Eotaxin, IL-12 (p70), MCP-1, MMP-9, SAA, IP-10, Amylin (Total), MMP-7, Resistin, IL-6, MIP-1β, TNF-α, IL-8, IL-5, CRP, and IL-10. The measured concentration from the sample for each biomarker may be compared to the range of concentrations of that marker found in the same fluid in normal human individuals. After the pretreatment sample has been taken the patient undergoes therapeutic intervention comprising surgery followed by irradiation. Samples of the same fluid are taken after surgery, but before irradiation. Additional samples are taken after each irradiation session. The concentration in each sample of the same eight (8) biomarkers is determined. Changes in the level of expression of each biomarker are noted and compared with other symptoms of progression of the disease.

Example 14

Selection of Predictive Biomarkers

[0329] A pretreatment sample of a biological fluid is obtained from a population of male patients who have been diagnosed with NSCLS before any treatment for the disease. The sample is preferably blood serum or plasma. The concentration in the sample of the following 24 biomarkers is determined: IL-13, I-TAC, EGF, MPO, HGF, MMP-1, MMP-8, MIF, Eotaxin, IL-12 (p70), MCP-1, MMP-9, SAA, IP-10, Amylin (Total), MMP-7, Resistin, IL-6, MIP-1β, TNF-α, IL-8, IL-5, CRP, and IL-10. The measured concentration from the sample for each biomarker is compared to the range of concentrations of that marker found in the same fluid in normal human individuals. After the pretreatment sample has been taken each patient undergoes therapeutic intervention comprising surgery followed by irradiation. Samples of the same fluid are taken after surgery, but before irradiation. Additional samples are taken after each irradiation session. The concentration in each sample of the 24 biomarkers is determined. Changes in the level of expression of each biomarker are noted and compared with other symptoms of progression of the disease. All biomarkers whose level changes after therapy are identified.

Example 15

Selection of Susceptible Patients

[0330] A sample of a biological fluid is obtained from a male patient who has been diagnosed with NSCLS. The sample is preferably blood serum or plasma. The concentration in the sample of each of the biomarkers identified in the previous example is determined, and patients for whom the highest number of biomarkers show values deviating from normal are selected for treatment.

CLAIMS:

1. A method of physiological characterization of a subject comprising determining the extent of expression of a set of at least six biomarkers, wherein the set comprises Matrix Metallopeptidase 7 ("MMP-7"), Interleukin-5 ("IL-5"), Interleukin-10 ("IL-10"), Leptin, Macrophage Migration Inhibitory Factor ("MIF"), and Serum Amyloid A ("SAA"), in a biological fluid sample,

wherein the biological fluid sample is blood, plasma, serum, or a combination thereof; and

wherein the extent of expression of the biomarkers is indicative of non-small cell lung cancer in the subject.

2. The method of claim 1, wherein the subject is a mammal.

3. The method of claim 2, wherein the mammal is a human.

4. The method of any one of claims 1-3, wherein the subject is female.

5. The method of any one of claims 1-3, wherein the subject is male.

6. The method of any one of claims 1-5, wherein the set of at least six biomarkers further comprises Monocyte Chemoattractant Protein-1 ("MCP-1") and Soluble Intercellular Adhesion Molecule-1 ("sICAM-1").

7. The method of any one of claims 1-6, wherein determining the extent of expression comprises performing a quantitative multiplex immunoassay.

FIGURE 1A

FLUORESCENCE INTENSITY LEVEL IN THE NORMAL POPULATION							
Biomarker	**Ave**	**S.D.**	**R.S.D.**	**Biomarker**	**Ave**	**S.D.**	**R.S.D.**
sE-Selectin	-3754.00	35.15	-0.94	**MMP-3**	15660.06	5918.30	37.79
EGF	5015.80	4447.17	88.66	**IP-10**	3408.61	4279.11	125.54
IL-5	-293.76	1201.87	-409.13	**IL-10**	401.93	816.03	203.03
PAI-1 (total)	4650.05	1273.31	27.38	**MMP-8**	2673.57	1392.34	52.08
Resistin	3138.02	2234.38	71.20	**MMP-2**	24052.74	928.10	3.86
Leptin	8089.08	9137.49	112.96	**G-CSF**	-17.85	1164.65	-6525.86
sVCAM-1	1017.74	609.37	59.87	**sFasL**	59.40	29.11	49.00
MMP-13	0.30	6.35	2141.82	**IL-8**	7726.40	6653.62	86.12
SAA	1541.92	4224.24	273.96	**TGF-ALPHA**	2521.01	2820.52	111.88
sICAM-1	-3488.15	1784.70	-51.16	**IFN-gamma**	347.30	1150.62	331.31
CD40 Ligand	161.20	192.05	119.14	**MPO**	1960.51	4609.11	235.10
IL-7	-553.29	2222.61	-401.71	**MIP-1alpha**	2579.96	3201.87	124.11
C-Peptide	8734.89	8388.22	96.03	**IL-1ra**	828.88	1771.02	213.66
HGF	650.01	413.44	63.61	**VEGF**	4791.40	5321.58	111.07
CRP	10243.98	8699.54	84.92	**IL-13**	664.75	2013.87	302.95
IL-1alpha	6574.13	9870.69	150.14	**Insulin**	1485.90	3380.72	227.52
MMP-7	309.12	88.76	28.71	**IL-12(p70)**	1174.46	4080.10	347.40
IL-4	2261.07	2731.85	120.82	**IL-1B**	778.53	2976.43	382.32
Adiponectin	24525.83	1290.90	5.26	**GLP-1 (Active)**	708.76	2492.64	351.69
MMP-9	28540.58	803.64	2.82	**FRACTALKINE**	916.75	2024.48	220.83
GM-CSF	103.94	973.06	936.21	**IL-2**	534.85	1262.10	235.97
MMP-12	-2.29	2.48	-108.31	**EOTAXIN**	6342.86	6613.72	104.27
IL-15	231.98	679.37	292.85	**MIP-1beta**	1513.50	3551.71	234.67
IL-17	1680.73	4225.28	251.40	**sFas**	181.43	63.29	34.88
IL-12(p40), free	171.55	1091.36	636.16	**Amylin (Active)**	1447.02	4589.31	317.16
MIF	97.55	71.64	73.44	**MMP-1**	6010.22	4006.18	66.66
TNF-alpha	1185.89	3586.20	302.41	**Glucagon**	1869.83	4635.13	247.89
I-TAC	19.16	34.72	181.17	**MCP-1**	27869.05	965.91	3.47
IL-6	3557.94	5666.10	159.25	**SAP**	24732.42	803.37	3.25

FIGURE 1B

FLUORESCENCE INTENSITY LEVEL IN THE LUNG CANCER POPULATION

Biomarker	Ave	S.D.	R.S.D.	Biomarker	Ave	S.D.	R.S.D.
sE-Selectin	-3710.76	26.27	-0.71	MMP-3	15470.68	5795.86	37.46
EGF	12471.39	9397.19	75.35	IP-10	5641.16	6148.52	108.99
IL-5	-947.96	859.85	-90.70	IL-10	1053.38	2430.64	230.75
PAI-1 (total)	3776.85	1098.08	29.07	MMP-8	2845.20	1305.16	45.87
Resistin	2084.83	853.26	40.93	MMP-2	23768.35	1272.05	5.35
Leptin	24.05	8538.62	35503.61	G-CSF	-209.22	896.64	-428.57
sVCAM-1	1364.62	912.59	66.88	sFasL	487.48	2434.79	499.46
MMP-13	-1.10	3.81	-345.47	IL-8	7258.97	8912.30	122.78
SAA	7712.92	10706.73	138.82	TGF-ALPHA	1859.04	4739.54	254.95
sICAM-1	-2484.13	3305.66	-133.07	IFN-gamma	248.91	1141.12	458.45
CD40 Ligand	539.63	495.14	91.76	MPO	2899.33	5179.23	178.64
IL-7	-1723.03	869.90	-50.49	MIP-1alpha	4869.60	7669.88	157.51
C-Peptide	10380.92	8201.68	79.01	IL-1ra	298.27	535.27	179.46
HGF	560.93	421.36	75.12	VEGF	7222.50	6074.27	84.10
CRP	20810.52	6683.21	32.11	IL-13	373.67	713.36	190.90
IL-1alpha	404.89	5992.97	1480.15	Insulin	1758.20	3177.46	180.72
MMP-7	583.63	674.48	115.57	IL-12(p70)	208.66	701.87	336.37
IL-4	422.10	1652.03	391.38	IL-1B	1278.97	3946.61	308.58
Adiponectin	24462.63	1616.93	6.61	GLP-1 (Active)	229.15	437.88	191.09
MMP-9	27269.90	1233.41	4.52	FRACTALKINE	279.20	747.82	267.85
GM-CSF	-188.48	771.98	-409.58	IL-2	178.31	421.41	236.34
MMP-12	-0.65	2.25	-345.94	EOTAXIN	6982.76	6113.93	87.56
IL-15	19.63	139.23	709.13	MIP-1beta	924.40	2438.49	263.79
IL-17	265.67	812.81	305.94	sFas	214.14	140.95	65.82
IL-12(p40), free	-115.91	847.03	-730.77	Amylin (Active)	889.30	1721.49	193.58
MIF	331.60	1019.01	307.30	MMP-1	7517.19	6016.10	80.03
TNF-alpha	379.20	644.19	169.88	Glucagon	2319.96	3302.68	142.36
I-TAC	7.90	24.12	305.44	MCP-1	27622.58	1394.00	5.05
IL-6	1581.59	3261.99	206.25	SAP	24537.83	742.40	3.03

FIGURE 1C

FLUORESCENCE INTENSITY LEVEL IN THE ASTHMA POPULATION

Biomarker	Ave	S.D.	R.S.D.	Biomarker	Ave	S.D.	R.S.D.
sE-Selectin	106.77	49.48	46.35	MMP-3	12561.00	6374.02	50.74
EGF	1891.44	2231.32	117.97	IP-10	2936.55	4067.24	138.50
IL-5	1652.42	2751.18	166.49	IL-10	230.17	601.55	261.35
PAI-1 (total)	-2816.18	1591.94	-56.53	MMP-8	2134.40	1521.23	71.27
Resistin	1113.54	1337.70	120.13	MMP-2	24772.42	2488.88	10.05
Leptin	17523.57	8823.12	50.35	G-CSF	164.26	1467.85	893.59
sVCAM-1	3784.43	856.94	22.64	sFasL	49.41	80.27	162.46
MMP-13	3.94	4.58	116.10	IL-8	5297.59	6465.20	122.04
SAA	1415.16	1503.12	106.22	TGF-ALPHA	3565.40	4963.06	139.20
sICAM-1	5039.72	2494.41	49.49	IFN-gamma	66.13	170.57	257.94
CD40 Ligand	703.27	459.33	65.31	MPO	2477.35	3110.70	125.57
IL-7	-634.33	1674.74	-264.02	MIP-1alpha	3104.09	3288.61	105.94
C-Peptide	21354.00	5055.31	23.67	IL-1ra	573.51	893.00	155.71
HGF	904.47	455.18	50.33	VEGF	5570.03	4663.60	83.73
CRP	12052.08	8985.63	74.56	IL-13	541.10	816.76	150.94
IL-1alpha	5722.73	9910.91	173.19	Insulin	2948.06	4584.49	155.51
MMP-7	385.99	137.37	35.59	IL-12(p70)	444.58	651.36	146.51
IL-4	2366.17	4127.72	174.45	IL-1B	166.73	367.22	220.25
Adiponectin	21241.91	3183.07	14.98	GLP-1 (Active)	273.07	539.24	197.47
MMP-9	28559.72	916.63	3.21	FRACTALKINE	318.34	752.30	236.32
GM-CSF	574.11	1081.15	188.32	IL-2	386.20	561.42	145.37
MMP-12	-0.88	3.26	-372.85	EOTAXIN	6985.59	4047.59	57.94
IL-15	193.73	294.67	152.10	MIP--beta	550.53	1038.97	188.72
IL-17	1267.20	2096.39	165.44	sFas	238.89	184.67	77.30
IL-12(p40), free	361.39	899.35	248.86	Amylin (Active)	995.40	2621.90	263.40
MIF	143.50	79.27	55.24	MMP-1	6968.82	5642.31	80.97
TNF-alpha	917.00	878.85	95.84	Glucagon	1598.53	3655.30	228.67
I-TAC	20.77	12.07	58.12	MCP-1	27601.89	2359.19	8.55
IL-6	4559.95	6199.94	135.97	SAP	24394.42	1810.34	7.42

FIGURE 1D

PERCENT CHANGE IN MEAN FLUORESCENCE INTENSITY							
Biomarker	**LC vs NO**	**AST vs NO**	**LC vs AST**	**Biomarker**	**LC vs NO**	**AST vs NO**	**LC vs AST**
sE-Selectin	-1.15	-102.84	**-3600.09**	**MMP-3**	-1.21	-19.79	**-51.45**
EGF	148.64	-62.29	**1255.02**	**IP-10**	65.50	-13.85	**709.83**
IL-5	222.70	**-662.50**	-104.99	**IL-10**	**162.08**	-42.73	-10.72
PAI-1 (total)	-18.78	-160.56	**-222.72**	**MMP-8**	6.42	-20.17	**122.89**
Resistin	-33.56	-64.51	**186.59**	**MMP-2**	-1.18	2.99	**-7.54**
Leptin	-99.70	116.63	**-129.48**	**G-CSF**	**1072.30**	-1020.42	293.76
sVCAM-1	34.08	**271.85**	-65.45	**sFasL**	**720.72**	-16.82	-14.38
MMP-13	-471.91	**1229.05**	-8.70	**IL-8**	-6.05	-31.44	**339.52**
SAA	400.22	-8.22	**524.17**	**TGF-ALPHA**	-26.26	41.43	**300.77**
sICAM-1	-28.78	**-244.48**	-215.09	**IFN-gamma**	-28.33	-80.96	**232.69**
CD40 Ligand	**234.76**	336.27	-60.21	**MPO**	47.89	26.36	**-218.24**
IL-7	**211.41**	14.65	70.89	**MIP-1alpha**	88.75	20.32	**147.13**
C-Peptide	18.84	**144.47**	-58.39	**IL-1ra**	-64.02	-30.81	**137.89**
HGF	-13.70	39.15	**-129.39**	**VEGF**	50.74	16.25	**115.00**
CRP	103.15	17.65	**117.23**	**IL-13**	-43.79	-18.60	**-101.85**
IL-1alpha	**-93.84**	-12.95	-27.14	**Insulin**	18.33	**98.40**	-82.50
MMP-7	**88.80**	24.87	-27.90	**IL-12(p70)**	**-82.23**	-62.15	-46.85
IL-4	**-81.33**	4.65	-65.13	**IL-1B**	64.28	**-78.58**	10.66
Adiponectin	-0.26	**-13.39**	12.60	**GLP-1 (Active)**	-67.67	-61.47	**70.47**
MMP-9	**-4.45**	0.07	-3.87	**FRACTALKINE**	**-69.54**	-65.28	-43.21
GM-CSF	-281.34	**452.37**	-140.55	**IL-2**	**-66.66**	-27.79	14.84
MMP-12	-71.66	-61.85	**-100.00**	**EOTAXIN**	10.09	10.13	**-64.76**
IL-15	**-91.54**	-16.49	-8.38	**MIP-1beta**	-38.92	**-63.63**	7.17
IL-17	-84.19	-24.60	**-94.81**	**sFas**	18.03	31.67	**-54.25**
IL-12(p40), free	-167.56	110.66	**-297.38**	**Amylin (Active)**	**-38.54**	-31.21	18.55
MIF	239.94	47.11	**267.31**	**MMP-1**	**25.07**	15.95	-23.61
TNF-alpha	**-68.02**	-22.67	-7.11	**Glucagon**	24.07	-14.51	**-24.79**
I-TAC	**-58.79**	8.37	-38.37	**MCP-1**	-0.88	**-0.96**	-0.24
IL-6	**-55.55**	28.16	-54.85	**SAP**	-0.79	-1.37	-2.51

FIGURE 1E

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T VALUE

Biomarker	LC vs NO	AST vs NO	LC vs AST	Biomarker	LC vs NO	AST vs NO	LC vs AST
sE-Selectin	0.000	0.000	0.000	MMP-3	0.901	0.060	0.074
EGF	0.000	0.001	0.000	IP-10	0.108	0.669	0.055
IL-5	0.018	0.001	0.000	IL-10	0.169	0.368	0.087
PAI-1 (total)	0.006	0.000	0.000	MMP-8	0.624	0.164	0.061
Resistin	0.019	0.000	0.002	MMP-2	0.327	0.145	0.056
Leptin	0.001	0.000	0.000	G-CSF	0.479	0.602	0.244
sVCAM-1	0.089	0.000	0.000	sFasL	0.340	0.526	0.346
MMP-13	0.305	0.016	0.000	IL-8	0.819	0.165	0.344
SAA	0.005	0.881	0.003	TGF-ALPHA	0.514	0.325	0.186
sICAM-1	0.149	0.000	0.000	IFN-gamma	0.741	0.206	0.405
CD40 Ligand	0.000	0.000	0.198	MPO	0.461	0.621	0.711
IL-7	0.009	0.877	0.003	MIP-1alpha	0.137	0.541	0.265
C-Peptide	0.467	0.000	0.000	IL-1ra	0.122	0.496	0.157
HGF	0.412	0.030	0.004	VEGF	0.105	0.557	0.253
CRP	0.000	0.440	0.000	IL-13	0.459	0.764	0.408
IL-1alpha	0.005	0.744	0.016	Insulin	0.761	0.170	0.283
MMP-7	0.031	0.014	0.134	IL-12(p70)	0.206	0.354	0.191
IL-4	0.003	0.909	0.021	IL-1B	0.581	0.285	0.143
Adiponectin	0.868	0.000	0.000	GLP-1 (Active)	0.357	0.369	0.751
MMP-9	0.000	0.933	0.000	FRACTALKINE	0.111	0.147	0.843
GM-CSF	0.202	0.087	0.003	IL-2	0.148	0.569	0.115
MMP-12	0.009	0.067	0.760	EOTAXIN	0.699	0.660	0.998
IL-15	0.099	0.785	0.005	MIP-1beta	0.457	0.173	0.456
IL-17	0.077	0.642	0.018	sFas	0.251	0.114	0.567
IL-12(p40), free	0.259	0.475	0.042	Amylin (Active)	0.575	0.650	0.866
MIF	0.215	0.024	0.335	MMP-1	0.258	0.456	0.722
TNF-alpha	0.230	0.701	0.010	Glucagon	0.690	0.806	0.462
I-TAC	0.150	0.818	0.014	MCP-1	0.429	0.570	0.967
IL-6	0.103	0.523	0.025	SAP	0.334	0.357	0.691

FIGURE 2A

FLUORESCENCE INTENSITY LEVEL IN THE NORMAL POPULATION			
Biomarker	**Ave**	**S.D.**	**R.S.D.**
Adiponectin	**2339.70**	1601.88	68.46
Resistin	**114.63**	91.26	79.61
PAI-1	**366.00**	424.13	115.88
SE-selectin	**63.11**	49.58	78.55
sVCAM-1	**1634.10**	408.80	25.02
sICAM-1	**3541.68**	1752.76	49.49
MPO	**717.62**	1645.83	229.35
CRP	**8839.73**	6391.84	72.31
SAA	**1970.35**	3668.84	186.20
SAP	**2160.88**	597.89	27.67
Leptin	**2638.03**	3025.87	114.70
GLP-1	**57.77**	100.58	174.10
Amylin (Total)	**120.65**	256.04	212.21
C-Peptide	**5015.95**	2022.03	40.31
Insulin	**322.77**	436.71	135.30
Sfas	**49.72**	183.17	368.37
sFSl	**13.17**	12.32	93.54
MIF	**61.95**	113.33	182.95
IL-1β	**23.50**	11.80	50.24
IL-2	**13.13**	55.39	421.85
IL-1ra	**22.32**	24.11	108.02
IL-4	**112.05**	66.15	59.03
IL-5	**17.40**	19.03	109.38
IL-6	**36.69**	50.08	136.52
IL-7	**14.32**	5.44	37.95
TGF-α	**32.10**	8.07	25.13
Fractalkine	**12.69**	3.15	24.80
IL-8	**280.43**	703.76	250.96
IL-10	**12.55**	3.87	30.82
IL-15	**24.21**	4.43	18.28
IL-17	**36.44**	13.75	37.74
IL-1α	**83.74**	148.45	177.27
IFN-γ	**21.75**	5.92	27.23
G-CSF	**23.75**	4.58	19.27
GM-CSF	**27.98**	14.45	51.65
TNF-α	**39.67**	17.41	43.88
MCP-1	**49.72**	34.31	69.02
IL-12 (p40), free	**24.17**	7.86	32.54
MIP-1α	**39.78**	27.14	68.22
MIP-1β	**20.27**	9.77	48.19
VEGF	**41.55**	13.17	31.71

FIGURE 2B

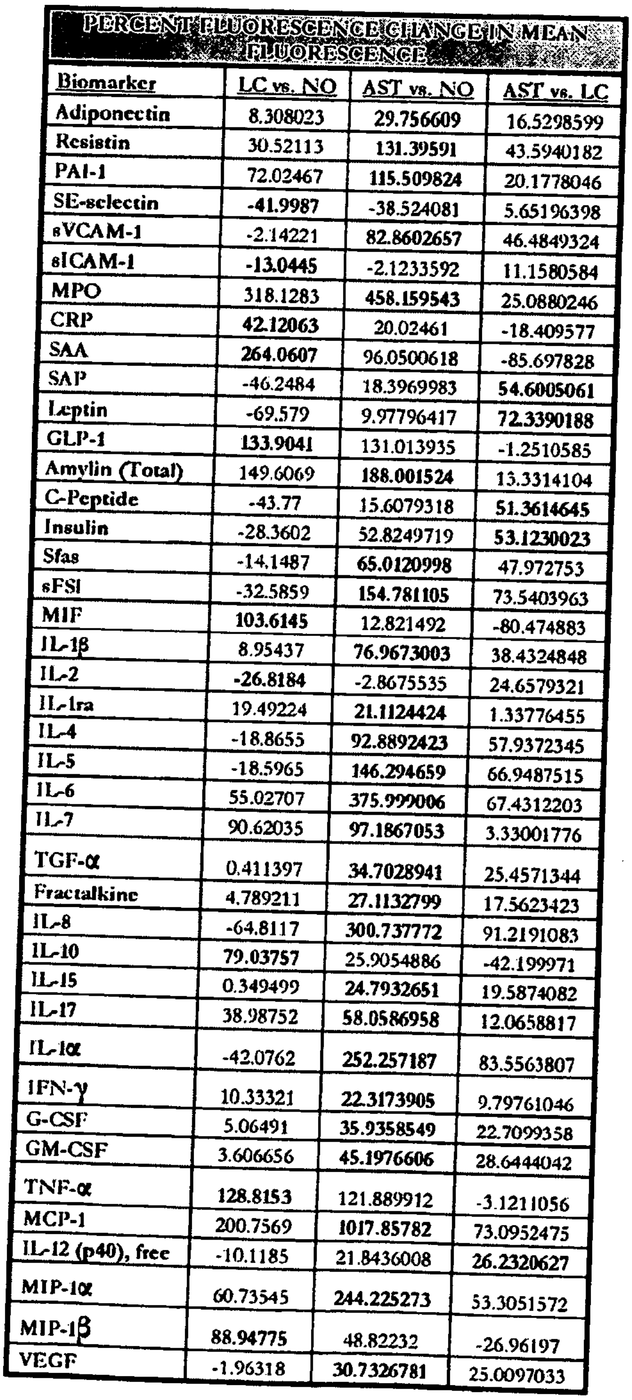
FLUORESCENCE INTENSITY LEVEL IN THE LUNG CANCER POPULATION			
Biomarker	**Ave**	**S.D.**	**R.S.D.**
Adiponectin	**2534.09**	1827.31	72.11
Resistin	**149.61**	142.26	95.09
PAI-1	**629.60**	307.11	48.78
SE-selectin	**36.61**	58.58	160.04
sVCAM-1	**1599.09**	586.93	36.70
sICAM-1	**3079.69**	1607.64	52.20
MPO	**3000.57**	2145.32	71.50
CRP	**12563.08**	6483.62	51.61
SAA	**7173.28**	7659.62	106.78
SAP	**1161.51**	897.26	77.25
Leptin	**802.52**	1210.97	150.90
GLP-1	**135.12**	727.35	538.29
Amylin (Total)	**301.16**	1096.20	363.99
C-Peptide	**2820.47**	1931.78	68.49
Insulin	**231.23**	704.98	304.88
Sfas	**42.69**	32.34	75.77
sFSI	**8.88**	5.26	59.19
MIF	**126.13**	225.30	178.62
IL-1β	**25.60**	3.06	11.94
IL-2	**9.61**	3.44	35.78
IL-1ra	**26.67**	6.50	24.39
IL-4	**90.91**	45.70	50.27
IL-5	**14.17**	4.90	34.60
IL-6	**56.88**	201.09	353.57
IL-7	**27.30**	115.37	422.52
TGF-α	**32.23**	8.82	27.38
Fractalkine	**13.29**	3.05	22.97
IL-8	**98.68**	120.57	122.19
IL-10	**22.46**	74.90	333.47
IL-15	**24.30**	2.82	11.61
IL-17	**50.64**	39.88	78.76
IL-1α	**48.51**	23.72	48.91
IFN-γ	**23.99**	6.93	28.87
G-CSF	**24.96**	16.23	65.03
GM-CSF	**28.98**	3.60	12.43
TNF-α	**90.78**	565.51	622.96
MCP-1	**149.53**	230.71	154.29
IL-12 (p40), free	**21.72**	8.15	37.51
MIP-1α	**63.94**	150.25	235.00
MIP-1β	**38.30**	127.37	332.54
VEGF	**40.73**	22.15	54.38

FIGURE 2C

FLUORESCENCE INTENSITY LEVEL IN THE LUNG CANCER POPULATION

Biomarker	Ave	S.D.	R.S.D.
Adiponectin	3035.92	2008.84	66.17
Resistin	265.24	189.24	71.35
PAI-1	788.76	267.12	33.87
SE-selectin	38.80	30.29	78.07
sVCAM-1	2988.12	948.06	31.73
sICAM-1	3466.48	1326.54	38.27
MPO	4005.47	2572.12	64.22
CRP	10609.85	6186.73	58.31
SAA	3862.88	4256.70	110.19
SAP	2558.42	654.11	25.57
Leptin	2901.26	3238.21	111.61
GLP-1	133.45	627.72	470.36
Amylin (Total)	347.48	1257.27	361.82
C-Peptide	5798.84	2767.02	47.72
Insulin	493.28	1468.20	297.64
Sfas	82.05	41.68	50.80
sFSl	33.56	50.68	151.01
MIF	69.89	48.66	69.62
IL-1β	41.58	151.82	365.11
IL-2	12.75	3.63	28.46
IL-1ra	27.03	5.07	18.78
IL-4	216.14	368.04	170.28
IL-5	42.86	89.64	209.14
IL-6	174.63	694.85	397.90
IL-7	28.25	15.68	55.53
TGF-α	43.24	13.20	30.54
Fractalkine	16.13	3.54	21.96
IL-8	1123.79	2876.50	255.96
IL-10	15.80	3.81	24.10
IL-15	30.22	8.18	27.08
IL-17	57.59	27.56	47.86
IL-1α	294.98	854.12	289.55
IFN-γ	26.60	5.96	22.40
G-CSF	32.29	14.01	43.40
GM-CSF	40.62	33.59	82.70
TNF-α	88.03	350.87	398.57
MCP-1	555.77	2390.21	430.08
IL-12 (p40), free	29.45	8.77	29.77
MIP-1α	136.92	834.62	609.56
MIP-1β	30.17	41.55	137.72
VEGF	54.31	19.95	36.73

FIGURE 2D



PERCENT FLUORESCENCE CHANGE IN MEAN FLUORESCENCE

Biomarker	LC vs. NO	AST vs. NO	AST vs. LC
Adiponectin	8.308023	**29.756609**	16.5298599
Resistin	30.52113	**131.39591**	43.5940182
PAI-1	72.02467	**115.509824**	20.1778046
SE-selectin	**-41.9987**	-38.524081	5.65196398
sVCAM-1	-2.14221	**82.8602657**	46.4849324
sICAM-1	**-13.0445**	-2.1233592	11.1580584
MPO	318.1283	**458.159543**	25.0880246
CRP	**42.12063**	20.02461	-18.409577
SAA	**264.0607**	96.0500618	-85.697828
SAP	-46.2484	18.3969983	**54.6005061**
Leptin	-69.579	9.97796417	**72.3390188**
GLP-1	**133.9041**	131.013935	-1.2510585
Amylin (Total)	149.6069	**188.001524**	13.3314104
C-Peptide	-43.77	15.6079318	**51.3614645**
Insulin	-28.3602	52.8249719	**53.1230023**
Sfas	-14.1487	**65.0120998**	47.972753
sFSI	-32.5859	**154.781105**	73.5403963
MIF	**103.6145**	12.821492	-80.474883
IL-1β	8.95437	**76.9673003**	38.4324848
IL-2	**-26.8184**	-2.8675535	24.6579321
IL-1ra	19.49224	**21.1124424**	1.33776455
IL-4	-18.8655	**92.8892423**	57.9372345
IL-5	-18.5965	**146.294659**	66.9487515
IL-6	55.02707	**375.999006**	67.4312203
IL-7	90.62035	**97.1867053**	3.33001776
TGF-α	0.411397	**34.7028941**	25.4571344
Fractalkine	4.789211	**27.1132799**	17.5623423
IL-8	-64.8117	**300.737772**	91.2191083
IL-10	**79.03757**	25.9054886	-42.199971
IL-15	0.349499	**24.7932651**	19.5874082
IL-17	38.98752	**58.0586958**	12.0658817
IL-1α	-42.0762	**252.257187**	83.5563807
IFN-γ	10.33321	**22.3173905**	9.79761046
G-CSF	5.06491	**35.9358549**	22.7099358
GM-CSF	3.606656	**45.1976606**	28.6444042
TNF-α	**128.8153**	121.889912	-3.1211056
MCP-1	200.7569	**1017.85782**	73.0952475
IL-12 (p40), free	-10.1185	21.8436008	**26.2320627**
MIP-1α	60.73545	**244.225273**	53.3051572
MIP-1β	**88.94775**	48.82232	-26.96197
VEGF	-1.96318	**30.7326781**	25.0097033

FIGURE 2E

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T VALUE

Biomarker	T LC vs. NO	T AST vs. NO	T AST vs. LC
Adiponectin	0.306	0.001	0.039
Resistin	0.007	0.000	0.000
PAI-1	0.000	0.000	0.000
SE-selectin	0.000	0.000	0.723
sVCAM-1	0.526	0.000	0.000
sICAM-1	0.014	0.700	0.043
MPO	0.000	0.000	0.001
CRP	0.000	0.022	0.016
SAA	0.000	0.000	0.000
SAP	0.000	0.000	0.000
Leptin	0.000	0.486	0.000
GLP-1	0.157	0.112	0.985
Amylin (Total)	0.032	0.019	0.755
C-Peptide	0.000	0.006	0.000
Insulin	0.151	0.145	0.061
Sfas	0.647	0.072	0.000
sFSl	0.000	0.000	0.000
MIF	0.001	0.490	0.011
IL-1β	0.037	0.111	0.204
IL-2	0.444	0.944	0.000
IL-1ra	0.035	0.046	0.632
IL-4	0.001	0.000	0.000
IL-5	0.046	0.000	0.000
IL-6	0.193	0.008	0.053
IL-7	0.130	0.000	0.933
TGF-α	0.888	0.000	0.000
Fractalkine	0.079	0.000	0.000
IL-8	0.002	0.000	0.000
IL-10	0.075	0.000	0.357
IL-15	0.841	0.000	0.000
IL-17	0.000	0.000	0.121
IL-1α	0.005	0.001	0.001
IFN-γ	0.002	0.000	0.002
G-CSF	0.341	0.000	0.000
GM-CSF	0.411	0.000	0.000
TNF-α	0.224	0.064	0.965
MCP-1	0.000	0.005	0.042
IL-12 (p40), free	0.006	0.000	0.000
MIP-1α	0.034	0.117	0.302
MIP-1β	0.058	0.002	0.524
VEGF	0.679	0.000	0.000

FIGURE 3A

FLUORESCENCE INTENSITY LEVEL IN THE NORMAL POPULATION

Biomarker	Average	S.D.	R.S.D.	Biomarker	Average	S.D.	R.S.D.
Adiponectin	2259.55	1504.98	66.61	IL-17	37.60	13.52	35.95
Resistin	124.37	90.22	72.55	IL-1α	95.36	185.32	194.33
PAI-1^{A}	335.10	371.63	110.90	IFN-γ	23.30	9.28	39.81
SE-selectin	60.34	47.58	78.85	G-CSF	23.84	4.12	17.29
sVCAM-1	1753.31	513.04	29.26	GM-CSF	28.60	12.57	43.94
sICAM-1	3295.75	1574.35	47.77	TNF-α	43.52	15.71	36.10
MPO	592.73	1503.40	253.64	MCP-1	46.39	29.47	63.51
CRP	8384.00	6292.94	75.06	IL-12 (p40)	24.93	7.26	29.11
SAA	2202.71	4017.36	182.38	MIP-1α	41.04	27.61	67.27
SAP	2200.71	570.75	25.93	MIP-1β	21.28	9.86	46.35
Leptin^{1}	2204.67	2771.37	125.70	VEGF	40.80	11.45	28.07
GLP-1	56.21	102.46	182.27	IL12 (p70)	7.66	36.69	479.00
Amylin (Total)	142.47	339.44	238.25	IL-13	11.41	57.04	499.86
C-Peptide	4748.58	1971.09	41.51	MMP-2	155.73	439.49	282.21
Insulin	278.12	424.50	152.63	MMP-1	103.52	179.41	173.31
Sfas	58.81	148.38	252.31	MMP-3	6668.81	2442.77	36.63
sFSI	15.13	16.89	111.59	Eotaxin	196.78	208.46	105.94
MIF	52.08	112.39	215.81	Leptin^{2}	3767.80	3885.08	103.11
IL-1β	23.71	9.52	40.16	IP-10	543.43	820.15	150.92
IL-2	12.26	44.02	358.99	MMP-9	1090.51	1599.16	146.64
IL-1ra	23.11	19.35	83.77	MMP-13	9.95	35.67	358.67
IL-4	114.39	72.33	63.23	PAI-1^{B}	38.94	98.35	252.58
IL-5	18.37	18.35	99.90	I-TAC	30.16	154.83	513.40
IL-6	40.99	56.56	137.98	MMP-12	12.41	27.69	223.09
IL-7	15.82	6.61	41.79	HGF	206.22	619.73	300.52
TGF-α	31.46	8.00	25.44	MMP-7	1004.07	577.62	57.53
Fractalkine	13.36	3.35	25.09	EGF	30.91	49.02	158.60
IL-8	239.47	629.22	262.76	sCD40 ligand	155.50	434.04	279.12
IL-10	13.06	3.63	27.82	MMP-8	70.26	318.85	453.80
IL-15	24.53	4.27	17.42				

FIGURE 3B

FLUORESCENCE INTENSITY LEVEL IN THE LUNG CANCER POPULATION

Biomarker	Average	S.D.	R.S.D.	Biomarker	Average	S.D.	R.S.D.
Adiponectin	3563.76	2288.94	64.23	IL-17	46.36	29.90	64.51
Resistin	191.29	136.17	71.19	IL-1α	122.68	394.79	321.80
PAI-1^{A}	787.03	389.14	49.44	IFN-γ	28.31	9.99	35.29
SE-selectin	41.71	45.85	109.94	G-CSF	29.55	19.35	65.51
sVCAM-1	1422.20	548.56	38.57	GM-CSF	33.26	10.06	30.23
sICAM-1	3121.73	1440.47	46.14	TNF-α	75.33	359.82	477.69
MPO	2188.38	1952.85	89.24	MCP-1	183.83	210.11	114.29
CRP	12951.79	5490.37	42.39	IL-12 (p40)	26.01	8.07	31.01
SAA	7383.07	6685.22	90.55	MIP-1α	53.41	97.52	182.57
SAP	1663.82	907.73	54.56	MIP-1β	33.74	81.49	241.55
Leptin^{1}	1441.00	1806.76	125.38	VEGF	43.93	17.22	39.19
GLP-1	130.29	700.99	538.03	IL-12(p70)	46.82	92.35	197.24
Amylin (Total)	337.43	1028.44	304.79	IL-13	401.00	520.85	129.89
C-Peptide	3431.59	2603.38	75.87	MMP-2	165.31	320.62	193.95
Insulin	373.06	777.90	208.52	MMP-1	744.40	766.38	102.95
Sfas	68.60	41.26	60.15	MMP-3	8137.21	3111.85	38.24
sFSl	18.15	58.41	321.91	Eotaxin	818.65	576.64	70.44
MIF	76.00	155.45	204.54	Leptin^{2}	3214.92	3305.20	102.81
IL-1β	27.08	4.32	15.94	IP-10	1846.73	1381.81	74.82
IL-2	11.66	5.89	50.57	MMP-9	3141.11	2616.52	83.30
IL-1ra	29.38	9.12	31.04	MMP-13	21.06	189.11	898.16
IL-4	154.43	214.60	138.96	PAI-1^{B}	268.11	201.13	75.02
IL-5	26.12	43.27	165.65	I-TAC	429.48	315.35	73.43
IL-6	74.67	185.61	248.58	MMP-12	32.74	168.74	515.37
IL-7	23.67	73.58	310.88	HGF	678.43	787.38	116.06
TGF-α	37.56	12.62	33.59	MMP-7	2070.90	1166.98	56.35
Fractalkine	15.27	4.37	28.58	EGF	455.13	462.40	101.60
IL-8	168.26	286.40	170.21	sCD40 ligand	120.25	367.11	305.30
IL-10	20.28	47.88	236.16	MMP-8	284.02	494.63	174.15
IL-15	28.90	7.04	24.37				

FIGURE 3C

FLUORESCENCE INTENSITY LEVEL IN THE ASTHMA POPULATION							
Biomarker	**Average**	**S.D**	**R.S.D.**	**Biomarker**	**Average**	**S.D**	**R.S.D.**
Adiponectin	2963.37	1855.21	62.60	**IL-17**	48.31	24.48	50.68
Resistin	290.12	347.80	119.88	**IL-1α**	250.35	734.45	293.38
PAI-1[A]	791.20	259.58	32.81	**IFN-γ**	27.57	6.28	22.79
SE-selectin	37.42	26.25	70.14	**G-CSF**	27.42	12.61	45.98
sVCAM-1	2580.30	1000.17	38.76	**GM-CSF**	35.60	26.94	75.67
sICAM-1	3202.40	1322.66	41.30	**TNF-α**	69.25	272.27	393.15
MPO	3799.80	2433.44	64.04	**MCP-1**	436.56	1969.47	451.13
CRP	10529.32	5894.42	55.98	**IL-12 (p40)**	26.81	8.15	30.41
SAA	3637.04	3965.44	109.03	**MIP-1α**	97.63	647.11	662.84
SAP	2336.72	646.89	27.68	**MIP-1β**	26.41	32.51	123.10
Leptin[1]	3009.58	2925.20	97.20	**VEGF**	46.98	18.08	38.48
GLP-1	106.06	513.75	484.39	**IL12 (p70)**	32.48	54.69	168.40
Amylin (Total)	274.00	1007.69	367.77	**IL-13**	297.79	362.17	121.62
C-Peptide	5509.05	2653.46	48.17	**MMP-2**	184.87	248.32	134.32
Insulin	407.57	1178.79	289.22	**MMP-1**	192.84	280.81	145.61
Sfas	76.93	40.19	52.24	**MMP-3**	6150.32	2828.47	45.99
sFSI	31.20	48.27	154.71	**Eotaxin**	652.21	484.14	74.23
MIF	61.77	80.34	130.06	**Leptin**[2]	4322.85	3756.93	86.91
IL-1β	34.22	117.74	344.13	**IP-10**	1575.99	1241.02	78.75
IL-2	12.75	3.38	26.52	**MMP-9**	4097.20	2679.44	65.40
IL-1ra	26.25	5.19	19.76	**MMP-13**	9.37	38.54	411.12
IL-4	190.65	318.74	167.19	**PAI-1**[B]	461.47	358.67	77.72
IL-5	36.72	75.10	204.50	**I-TAC**	783.04	637.12	81.36
IL-6	132.01	548.69	415.63	**MMP-12**	20.79	42.60	204.89
IL-7	23.30	13.78	59.16	**HGF**	1381.06	921.98	66.76
TGF-α	38.36	16.97	44.25	**MMP-7**	487.07	493.70	101.36
Fractalkine	15.87	3.41	21.51	**EGF**	419.64	417.55	99.50
IL-8	829.39	2332.42	281.22	**sCD40 ligand**	175.32	259.04	147.75
IL-10	15.64	4.29	27.42	**MMP-8**	140.35	889.83	634.00
IL-15	27.01	7.96	29.47				

FIGURE 3D

PERCENT CHANGE IN MEAN FLUORESCENCE INTENSITY							
Biomarker	AST vs. NO	LC vs. NO	AST vs. LC	Biomarker	AST vs. NO	LC vs. NO	AST vs. LC
Adiponectin	31.15	57.72	-20.26	IL-17	28.48	23.29	4.04
Resistin	133.28	53.81	34.07	IL-1α	162.52	28.65	51.00
PAI-1^A	136.11	134.86	0.53	IFN-γ	18.31	21.46	-2.67
SE-selectin	-37.99	-30.88	-11.46	G-CSF	15.02	23.95	-7.76
sVCAM-1	47.17	-18.89	44.88	GM-CSF	24.45	16.28	6.57
sICAM-1	-2.83	-5.28	2.52	TNF-α	59.14	73.10	-8.77
MPO	541.07	269.21	42.41	MCP-1	841.00	296.25	57.89
CRP	25.59	54.48	-23.01	IL-12 (p40)	7.53	4.30	3.00
SAA	65.12	235.18	-103.00	MIP-1α	137.86	30.14	45.29
SAP	6.18	-24.40	28.80	MIP-1β	24.09	58.53	-27.75
Leptin^1	36.51	-34.64	52.12	VEGF	15.13	7.67	6.48
GLP-1	88.68	131.77	-22.84	IL12 (p70)	324.00	511.21	-44.15
Amylin (Total)	92.32	136.84	-23.15	IL-13	2509.42	3413.77	-34.66
C-Peptide	16.01	-27.73	37.71	MMP-2	18.71	6.15	10.58
Insulin	46.55	34.14	8.47	MMP-1	86.29	619.10	-286.01
Sfas	30.81	16.64	10.83	MMP-3	-7.77	22.02	-32.31
sFSI	106.17	19.89	41.85	Eotaxin	231.44	316.02	-25.52
MIF	18.62	45.93	-23.03	Leptin^2	14.73	-14.67	25.63
IL-1β	44.28	14.18	20.86	IP-10	190.01	239.83	-17.18
IL-2	3.97	-4.95	8.58	MMP-9	275.71	188.04	23.34
IL-1ra	13.62	27.16	-11.92	MMP-13	-5.75	111.71	-124.61
IL-4	66.67	35.00	19.00	PAI-1^B	1085.19	588.59	41.90
IL-5	99.90	42.20	28.87	I-TAC	2496.41	1324.07	45.15
IL-6	222.04	82.15	43.44	MMP-12	67.51	163.78	-57.47
IL-7	47.25	49.61	-1.60	HGF	569.70	228.98	50.88
TGF-α	21.95	19.41	2.08	MMP-7	-51.49	106.25	-325.18
Fractalkine	18.84	14.34	3.78	EGF	1257.75	1372.57	-8.46
IL-8	246.35	-29.74	79.71	sCD40 ligand	12.74	-22.67	31.41
IL-10	19.70	55.21	-29.66	MMP-8	99.75	304.23	-102.36
IL-15	10.11	17.84	-7.02				

FIGURE 3E

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T VALUE

Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC	Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC
Adiponectin	0.000	0.000	0.002	IL-17	0.000	0.000	0.449
Resistin	0.000	0.000	0.000	IL-1α	0.001	0.279	0.009
PAI-1^A	0.000	0.000	0.896	IFN-γ	0.000	0.000	0.367
SE-selectin	0.000	0.000	0.246	G-CSF	0.000	0.000	0.181
sVCAM-1	0.000	0.000	0.000	GM-CSF	0.000	0.000	0.146
sICAM-1	0.508	0.143	0.529	TNF-α	0.110	0.134	0.842
MPO	0.000	0.000	0.000	MCP-1	0.001	0.000	0.016
CRP	0.000	0.000	0.000	IL-12 (p40)	0.010	0.079	0.277
SAA	0.000	0.000	0.000	MIP-1α	0.139	0.037	0.205
SAP	0.018	0.000	0.000	MIP-1β	0.013	0.010	0.246
Leptin^1	0.003	0.000	0.000	VEGF	0.000	0.008	0.057
GLP-1	0.111	0.076	0.681	IL12 (p70)	0.000	0.000	0.055
Amylin (Total)	0.042	0.002	0.497	IL-13	0.000	0.000	0.017
C-Peptide	0.000	0.000	0.000	MMP-2	0.417	0.749	0.473
Insulin	0.090	0.063	0.685	MMP-1	0.000	0.000	0.000
Sfas	0.110	0.232	0.026	MMP-3	0.036	0.000	0.000
sFSI	0.000	0.397	0.010	Eotaxin	0.000	0.000	0.001
MIF	0.314	0.029	0.250	Leptin^2	0.129	0.051	0.000
IL-1β	0.133	0.000	0.251	IP-10	0.000	0.000	0.027
IL-2	0.882	0.796	0.021	MMP-9	0.000	0.000	0.000
IL-1ra	0.033	0.000	0.000	MMP-13	0.870	0.326	0.413
IL-4	0.000	0.003	0.119	PAI-1^B	0.000	0.000	0.000
IL-5	0.000	0.005	0.038	I-TAC	0.000	0.000	0.000
IL-6	0.005	0.003	0.074	MMP-12	0.010	0.044	0.350
IL-7	0.000	0.072	0.946	HGF	0.000	0.000	0.000
TGF-α	0.000	0.000	0.539	MMP-7	0.000	0.000	0.000
Fractalkine	0.000	0.000	0.107	EGF	0.000	0.000	0.386
IL-8	0.000	0.056	0.000	sCD40 ligand	0.580	0.263	0.072
IL-10	0.000	0.011	0.195	MMP-8	0.224	0.000	0.016
IL-15	0.000	0.000	0.005				

FIGURE 4A

FLUORESCENCE INTENSITY IN THE NORMAL FEMALE POPULATION							
Biomarker	**Average**	**S.D.**	**R.S.D.**	**Biomarker**	**Average**	**S.D.**	**R.S.D.**
Adiponectin	2116.17	1225.14	57.89	**IL-17**	41.58	12.84	30.88
Resistin	158.01	95.09	60.18	**IL-1α**	83.31	155.36	186.48
PAI-1^{A}	499.52	438.77	87.84	**IFN-γ**	26.73	11.79	44.13
SE-selectin	75.37	56.07	74.40	**G-CSF**	26.24	3.43	13.09
sVCAM-1	1658.49	448.12	27.02	**GM-CSF**	30.93	4.43	14.32
sICAM-1	3520.37	1512.81	42.97	**TNF-α**	54.88	13.94	25.40
MPO	1269.64	2084.74	164.20	**MCP-1**	59.33	39.47	66.53
CRP	7532.14	5561.16	73.83	**IL-12 (p40)**	27.09	6.13	22.64
SAA	1773.59	3197.93	180.31	**MIP-1α**	50.13	39.77	79.34
SAP	2238.83	555.16	24.80	**MIP-1β**	26.92	11.02	40.96
Leptin^{1}	860.83	1311.22	152.32	**VEGF**	43.79	9.56	21.83
GLP-1	54.12	99.58	184.01	**IL-12(p70)**	7.87	11.21	142.41
Amylin (Total)	161.50	469.30	290.59	**IL-13**	27.17	74.25	273.33
C-Peptide	4048.15	1725.03	42.61	**MMP-2**	132.15	148.40	112.30
Insulin	287.45	463.63	161.29	**MMP-1**	182.66	242.24	132.62
Sfas	63.98	42.36	66.21	**MMP-3**	7467.26	2419.60	32.40
sFSl	13.35	10.94	81.94	**Eotaxin**	289.67	253.39	87.48
MIF	105.73	157.68	149.14	**Leptin^{2}**	2169.69	2831.50	130.50
IL-1β	26.63	13.82	51.90	**IP-10**	452.58	410.35	90.67
IL-2	11.04	2.32	21.05	**MMP-9**	1713.45	2162.04	126.18
IL-1ra	24.48	3.85	15.71	**MMP-13**	14.07	51.00	362.52
IL-4	105.32	60.09	57.05	**PAI-1^{B}**	66.22	113.55	171.47
IL-5	18.93	19.27	101.80	**I-TAC**	92.89	207.98	223.91
IL-6	43.44	57.12	131.50	**MMP-12**	15.55	36.33	233.59
IL-7	17.80	6.28	35.26	**HGF**	538.75	846.22	157.07
TGF-α	34.76	7.54	21.70	**MMP-7**	1163.17	547.69	47.09
Fractalkine	14.96	2.95	19.71	**EGF**	48.56	64.54	132.92
IL-8	401.98	915.65	227.79	**sCD40 ligand**	139.86	213.56	152.69
IL-10	14.76	3.07	20.81	**MMP-8**	222.46	446.02	200.50
IL-15	26.97	3.76	13.93				

FIGURE 4B

FLUORESCENCE INTENSITY IN THE FEMALE POPULATION WITH LUNG CANCER							
Biomarker	**Average**	**S.D.**	**R.S.D.**	**Biomarker**	**Average**	**S.D.**	**R.S.D.**
Adiponectin	4041.07	2456.96	60.80	**IL-17**	45.15	20.95	46.40
Resistin	165.14	104.34	63.18	**IL-1α**	101.95	454.84	446.12
PAI-1^A	680.06	281.74	41.43	**IFN-γ**	29.47	10.86	36.86
SE-selectin	39.28	24.76	63.02	**G-CSF**	28.03	7.27	25.95
sVCAM-1	1486.20	580.24	39.04	**GM-CSF**	33.00	6.80	20.61
sICAM-1	3129.94	1388.46	44.36	**TNF-α**	113.63	639.16	562.50
MPO	2236.00	2021.46	90.41	**MCP-1**	175.20	224.24	127.99
CRP	12373.56	6171.04	49.87	**IL-12 (p40)**	25.63	9.73	37.96
SAA	6407.43	6756.26	105.44	**MIP-1α**	67.31	169.37	251.61
SAP	1610.90	912.77	56.66	**MIP-1β**	41.90	143.28	341.91
Leptin^1	1836.07	2086.83	113.66	**VEGF**	41.74	11.81	28.30
GLP-1	112.59	751.23	667.22	**IL-12(p70)**	58.24	142.89	245.35
Amylin (Total)	252.51	919.35	364.09	**IL-13**	410.97	510.37	124.18
C-Peptide	3270.49	2492.50	76.21	**MMP-2**	139.64	205.55	147.20
Insulin	338.74	801.56	236.63	**MMP-1**	602.07	546.07	90.70
Sfas	64.72	36.09	55.76	**MMP-3**	7690.73	3309.64	43.03
sFSl	13.51	21.88	161.99	**Eotaxin**	814.32	573.07	70.37
MIF	55.10	97.46	176.88	**Leptin^2**	3933.88	3864.56	98.24
IL-1β	27.70	4.44	16.03	**IP-10**	1631.25	1066.13	65.36
IL-2	12.03	5.77	47.98	**MMP-9**	3423.98	2497.43	72.94
IL-1ra	29.50	8.33	28.23	**MMP-13**	11.66	33.41	286.54
IL-4	130.02	173.21	133.21	**PAI-1^B**	244.76	206.20	84.24
IL-5	22.15	44.14	199.26	**I-TAC**	387.53	314.80	81.23
IL-6	70.54	257.76	365.41	**MMP-12**	24.93	36.33	145.74
IL-7	31.77	130.07	409.42	**HGF**	537.31	620.75	115.53
TGF-α	36.97	12.35	33.42	**MMP-7**	2122.99	1114.85	52.51
Fractalkine	15.30	4.82	31.48	**EGF**	422.32	483.79	114.56
IL-8	126.83	168.86	133.13	**sCD40 ligand**	99.65	261.62	262.55
IL-10	27.03	84.44	312.39	**MMP-8**	247.64	364.87	147.34
IL-15	28.91	6.61	22.86				

FIGURE 4C

FLUORESCENCE INTENSITY LEVEL IN THE FEMALE POPULATION WITH ASTHMA							
Biomarker	**Average**	**S.D.**	**R.S.D.**	**Biomarker**	**Average**	**S.D.**	**R.S.D.**
Adiponectin	2944.64	1614.58	54.83	**IL-17**	43.39	21.49	49.53
Resistin	332.29	416.68	125.40	**IL-1α**	164.86	441.25	267.64
PAI-1^{A}	841.92	266.60	31.67	**IFN-γ**	27.88	6.03	21.64
SE-selectin	36.85	25.26	68.56	**G-CSF**	21.06	4.35	20.67
sVCAM-1	2435.16	971.17	39.88	**GM-CSF**	28.29	5.43	19.20
sICAM-1	3084.39	1425.90	46.23	**TNF-α**	43.88	8.91	20.30
MPO	4056.85	2406.72	59.32	**MCP-1**	337.96	1126.34	333.28
CRP	11504.22	6148.46	53.45	**IL-12 (p40)**	23.53	5.49	23.34
SAA	3764.05	3808.32	101.18	**MIP-1α**	47.70	29.11	61.04
SAP	2216.83	576.86	26.02	**MIP-1β**	22.11	6.60	29.87
Leptin^{1}	4148.56	3054.11	73.62	**VEGF**	40.46	9.69	23.95
GLP-1	153.82	647.05	420.66	**IL-12(p70)**	30.32	53.08	175.08
Amylin (Total)	377.66	1265.49	335.09	**IL-13**	307.87	373.66	121.37
C-Peptide	5897.41	2865.27	48.59	**MMP-2**	222.30	297.87	134.00
Insulin	521.74	1464.79	280.75	**MMP-1**	239.58	326.58	136.31
Sfas	88.02	45.72	51.94	**MMP-3**	6158.26	2312.49	37.55
sFSl	26.67	43.10	161.59	**Eotaxin**	676.90	441.51	65.23
MIF	50.56	92.56	183.07	**Leptin^{2}**	5870.26	3702.57	63.07
IL-1β	23.81	4.81	20.19	**IP-10**	1698.22	1212.85	71.42
IL-2	12.46	3.21	25.76	**MMP-9**	4522.52	2746.21	60.72
IL-1ra	26.42	5.68	21.51	**MMP-13**	11.59	48.42	417.84
IL-4	147.54	195.73	132.66	**PAI-1^{B}**	512.32	379.29	74.03
IL-5	27.72	42.32	152.66	**I-TAC**	890.86	677.56	76.06
IL-6	69.69	142.44	204.39	**MMP-12**	24.82	51.96	209.35
IL-7	18.13	7.94	43.80	**HGF**	1549.14	858.93	55.45
TGF-α	32.08	15.94	49.69	**MMP-7**	517.14	462.20	89.38
Fractalkine	15.25	2.85	18.70	**EGF**	405.07	357.97	88.37
IL-8	704.24	1718.95	244.09	**sCD40 ligand**	213.58	312.95	146.52
IL-10	14.95	4.15	27.78	**MMP-8**	192.74	1107.98	574.85
IL-15	22.54	4.34	19.27				

FIGURE 4D

PERCENT CHANGE IN MEAN FLUORESCENCE INTENSITY IN THE FEMALE POPULATION

Biomarker	AST vs. NO	LC vs. NO	AST vs. LC	Biomarker	AST vs. NO	LC vs. NO	AST vs. LC
Adiponectin	39.15	90.96	-37.23	IL-17	4.37	8.59	-4.04
Resistin	110.29	4.51	50.30	IL-1α	97.89	22.38	38.16
PAI-1^{A}	68.55	36.14	19.23	IFN-γ	4.32	10.26	-5.70
SE-selectin	-51.10	-47.88	-6.59	G-CSF	-19.75	6.81	-33.10
sVCAM-1	46.83	-10.39	38.97	GM-CSF	-8.54	6.71	-16.67
sICAM-1	-12.38	-11.09	-1.48	TNF-α	-20.04	107.06	-158.93
MPO	219.53	76.11	44.88	MCP-1	469.66	195.31	48.16
CRP	52.74	64.28	-7.56	IL-12 (p40)	-13.17	-5.40	-8.95
SAA	112.23	261.27	-70.23	MIP-1α	-4.86	34.27	-41.12
SAP	-0.98	-28.05	27.33	MIP-1β	-17.86	55.68	-89.54
Leptin^{1}	381.92	113.29	55.74	VEGF	-7.61	-4.67	-3.18
GLP-1	184.22	108.05	26.80	IL-12(p70)	285.01	639.61	-92.10
Amylin (Total)	133.85	56.35	33.14	IL-13	1033.27	1412.81	-33.49
C-Peptide	45.68	-19.21	44.54	MMP-2	68.22	5.67	37.18
Insulin	81.51	17.85	35.07	MMP-1	31.16	229.61	-151.30
Sfas	37.56	1.15	26.47	MMP-3	-17.53	2.99	-24.88
sFSl	99.73	1.15	49.36	Eotaxin	133.68	181.12	-20.30
MIF	-52.18	-47.89	-8.98	Leptin^{2}	170.56	81.31	32.99
IL-1β	-10.60	3.99	-16.33	IP-10	275.23	260.44	3.94
IL-2	12.92	8.99	3.48	MMP-9	163.94	99.83	24.29
IL-1ra	7.92	20.47	-11.63	MMP-13	-17.64	-17.11	-0.64
IL-4	40.08	23.45	11.87	PAI-1^{B}	673.63	269.60	52.23
IL-5	46.45	17.02	20.10	I-TAC	859.07	317.20	56.50
IL-6	60.44	62.39	-1.22	MMP-12	59.60	60.26	-0.42
IL-7	1.86	78.45	-75.19	HGF	187.55	-0.27	65.32
TGF-α	-7.71	6.37	-15.26	MMP-7	-55.54	82.52	-310.52
Fractalkine	1.99	2.30	-0.31	EGF	734.23	769.75	-4.26
IL-8	73.19	-68.45	81.99	sCD40 ligand	52.72	-28.75	53.35
IL-10	1.34	83.17	-80.75	MMP-8	-13.36	11.32	-28.48
IL-15	-16.41	7.20	-28.25				

FIGURE 4E

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T VALUE FOR FEMALE POPULATIONS

Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC	Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC
Adiponectin	0.000	0.000	0.000	IL-17	0.429	0.113	0.768
Resistin	0.000	0.583	0.000	IL-1α	0.057	0.670	0.010
PAI-1^A	0.000	0.000	0.078	IFN-γ	0.354	0.065	0.054
SE-selectin	0.000	0.000	0.179	G-CSF	0.000	0.016	0.150
sVCAM-1	0.000	0.011	0.000	GM-CSF	0.000	0.006	0.253
sICAM-1	0.025	0.040	0.658	TNF-α	0.000	0.311	0.451
MPO	0.000	0.000	0.000	MCP-1	0.007	0.000	0.148
CRP	0.000	0.000	0.000	IL-12 (p40)	0.000	0.166	0.553
SAA	0.000	0.000	0.000	MIP-1α	0.596	0.277	0.208
SAP	0.767	0.000	0.000	MIP-1β	0.000	0.251	0.098
Leptin^1	0.000	0.000	0.000	VEGF	0.009	0.144	0.163
GLP-1	0.094	0.395	0.248	IL-12(p70)	0.000	0.000	0.142
Amylin (Total)	0.080	0.335	0.147	IL-13	0.000	0.000	0.075
C-Peptide	0.000	0.006	0.000	MMP-2	0.003	0.747	0.564
Insulin	0.095	0.545	0.608	MMP-1	0.129	0.000	0.000
Sfas	0.000	0.886	0.797	MMP-3	0.000	0.553	0.000
sFSl	0.001	0.945	0.017	Eotaxin	0.000	0.000	0.007
MIF	0.001	0.004	0.303	Leptin^2	0.000	0.000	0.021
IL-1β	0.041	0.433	0.278	IP-10	0.000	0.000	0.001
IL-2	0.000	0.081	0.136	MMP-9	0.000	0.000	0.003
IL-1ra	0.002	0.000	0.000	MMP-13	0.704	0.671	0.416
IL-4	0.024	0.140	0.147	PAI-1^B	0.000	0.000	0.000
IL-5	0.039	0.463	0.094	I-TAC	0.000	0.000	0.000
IL-6	0.082	0.259	0.046	MMP-12	0.113	0.049	0.380
IL-7	0.723	0.237	0.798	HGF	0.000	0.988	0.000
TGF-α	0.097	0.096	0.724	MMP-7	0.000	0.000	0.000
Fractalkine	0.435	0.505	0.631	EGF	0.000	0.000	0.690
IL-8	0.091	0.002	0.001	sCD40 ligand	0.035	0.196	0.106
IL-10	0.677	0.110	0.000	MMP-8	0.785	0.637	0.000
IL-15	0.000	0.006	0.001				

FIGURE 5A

FLUORESCENCE INTENSITY IN THE NORMAL MALE POPULATION							
Biomarker	**Average**	**S.D.**	**R.S.D.**	**Biomarker**	**Average**	**S.D.**	**R.S.D.**
Adiponectin	2375.01	1677.13	70.62	**IL-17**	34.68	13.33	38.44
Resistin	99.39	78.12	78.60	**IL-1α**	104.49	205.17	196.36
PAI-1^{A}	213.37	254.21	119.14	**IFN-γ**	20.78	5.73	27.58
SE-selectin	49.28	36.75	74.57	**G-CSF**	22.05	3.68	16.68
sVCAM-1	1822.54	548.47	30.09	**GM-CSF**	26.91	15.97	59.33
sICAM-1	3128.49	1607.19	51.37	**TNF-α**	35.13	11.03	31.38
MPO	95.40	396.70	415.85	**MCP-1**	36.83	12.40	33.66
CRP	8947.58	6700.43	74.89	**IL-12 (p40)**	23.35	7.64	32.74
SAA	2444.04	4420.98	180.89	**MIP-1α**	34.36	7.76	22.58
SAP	2170.66	583.20	26.87	**MIP-1β**	17.13	6.29	36.73
Leptin^{1}	3159.85	3108.95	98.39	**VEGF**	38.60	12.27	31.79
GLP-1	57.97	105.08	181.26	**IL-12(p70)**	7.53	47.57	631.42
Amylin (Total)	127.91	196.50	153.63	**IL-13**	-0.07	36.12	-48855.51
C-Peptide	5243.25	1975.58	37.68	**MMP-2**	173.98	566.49	325.61
Insulin	269.95	395.42	146.48	**MMP-1**	45.54	70.31	154.37
Sfas	54.83	192.79	351.58	**MMP-3**	6075.64	2300.75	37.87
sFSl	16.47	20.16	122.40	**Eotaxin**	127.35	132.34	103.92
MIF	12.66	6.60	52.09	**Leptin^{2}**	4899.58	4110.65	83.90
IL-1β	21.56	2.60	12.05	**IP-10**	612.33	1020.53	166.66
IL-2	13.17	58.19	441.69	**MMP-9**	635.66	724.02	113.90
IL-1ra	22.06	25.34	114.88	**MMP-13**	6.92	16.91	244.36
IL-4	121.09	79.91	65.99	**PAI-1^{B}**	13.51	73.47	543.75
IL-5	17.97	17.75	98.78	**I-TAC**	-15.62	70.30	-450.19
IL-6	-39.17	-56.42	144.04	**MMP-12**	-10.06	18.84	187.30
IL-7	14.36	6.51	45.31	**HGF**	-38.01	32.84	-86.39
TGF-α	29.04	7.49	25.78	**MMP-7**	892.29	570.49	63.94
Fractalkine	12.16	3.14	25.82	**EGF**	17.96	27.01	150.43
IL-8	120.31	200.43	166.59	**sCD40 ligand**	167.64	543.74	324.35
IL-10	11.81	3.52	29.85	**MMP-8**	-41.37	35.20	-85.08
IL-15	22.73	3.72	16.36				

FIGURE 5B

FLUORESCENCE INTENSITY LEVEL IN THE MALE LUNG CANCER POPULATION							
Biomarker	**Average**	**S.D.**	**R.S.D.**	**Biomarker**	**Average**	**S.D.**	**R.S.D.**
Adiponectin	3308.09	2112.68	63.86	**IL-17**	46.93	33.34	71.03
Resistin	203.24	147.53	72.59	**IL-1α**	132.63	364.95	275.17
PAI-1^{A}	837.56	421.88	50.37	**IFN-γ**	27.78	9.55	34.39
SE-selectin	42.79	52.96	123.79	**G-CSF**	30.21	22.90	75.83
sVCAM-1	1392.31	532.93	38.28	**GM-CSF**	33.37	11.28	33.81
sICAM-1	3120.40	1469.20	47.08	**TNF-α**	57.62	17.36	30.13
MPO	2172.28	1925.67	88.65	**MCP-1**	187.93	203.98	108.54
CRP	13265.74	5095.50	38.41	**IL-12 (p40)**	26.17	7.19	27.49
SAA	7854.70	6623.56	84.33	**MIP-1α**	47.00	23.96	50.98
SAP	1689.28	907.93	53.75	**MIP-1β**	30.00	14.79	49.29
Leptin^{1}	1241.43	1616.24	130.19	**VEGF**	44.89	19.17	42.71
GLP-1	138.75	679.26	489.56	**IL-12(p70)**	41.57	54.79	131.80
Amylin (Total)	377.72	1076.85	285.09	**IL-13**	397.79	527.21	132.54
C-Peptide	3518.32	2653.51	75.42	**MMP-2**	177.06	362.25	204.59
Insulin	388.05	769.26	198.24	**MMP-1**	811.18	843.83	104.03
Sfas	70.28	43.45	61.83	**MMP-3**	8324.20	2990.06	35.92
sFSI	20.35	69.16	339.84	**Eotaxin**	815.97	575.92	70.58
MIF	85.95	175.61	204.33	**Leptin**^{2}	2860.57	2948.31	103.07
IL-1β	26.79	4.24	15.84	**IP-10**	1949.78	1499.56	76.91
IL-2	11.49	5.97	51.93	**MMP-9**	3017.93	2667.24	88.38
IL-1ra	29.34	9.50	32.36	**MMP-13**	25.44	228.13	896.89
IL-4	165.98	231.18	139.28	**PAI-1**^{B}	279.67	198.30	70.91
IL-5	28.01	42.91	153.18	**I-TAC**	450.09	314.46	69.87
IL-6	76.81	141.04	183.63	**MMP-12**	36.42	203.07	557.58
IL-7	19.93	9.24	46.38	**HGF**	746.89	847.21	113.43
TGF-α	37.83	12.78	33.77	**MMP-7**	2049.58	1193.44	58.23
Fractalkine	15.26	4.16	27.25	**EGF**	471.66	452.79	96.00
IL-8	187.89	325.97	173.49	**sCD40 ligand**	129.51	407.77	314.85
IL-10	17.14	6.35	37.07	**MMP-8**	300.58	545.34	181.43
IL-15	28.87	7.24	25.09				

FIGURE 5C

FLUORESCENCE INTENSITY LEVEL IN THE MALE ASTHMA POPULATION

Biomarker	Average	S.D.	R.S.D.	Biomarker	Average	S.D.	R.S.D.
Adiponectin	2947.72	2190.78	74.32	**IL-17**	56.69	27.10	47.81
Resistin	221.87	167.29	75.40	**IL-1α**	396.04	1049.31	264.95
PAI-1^{A}	711.75	224.74	31.58	**IFN-γ**	27.11	6.72	24.79
SE-selectin	38.26	28.16	73.59	**G-CSF**	38.02	14.71	38.68
sVCAM-1	2829.19	1012.66	35.79	**GM-CSF**	47.83	40.94	85.59
sICAM-1	3394.91	1123.56	33.10	**TNF-α**	111.92	444.94	397.55
MPO	3403.65	2428.77	71.36	**MCP-1**	606.76	2887.62	475.91
CRP	8963.97	5125.42	57.18	**IL-12 (p40)**	32.29	8.99	27.84
SAA	3443.76	4262.10	123.76	**MIP-1α**	182.01	1059.60	582.15
SAP	2535.86	714.51	28.18	**MIP-1β**	33.66	52.04	154.58
Leptin^{1}	1141.66	1299.27	113.81	**VEGF**	57.87	23.15	40.00
GLP-1	27.56	38.25	138.81	**IL-12(p70)**	36.55	57.75	158.03
Amylin (Total)	104.12	110.92	106.53	**IL-13**	284.25	346.16	121.78
C-Peptide	4893.34	2138.06	43.69	**MMP-2**	124.45	107.75	86.58
Insulin	222.02	317.93	143.20	**MMP-1**	117.09	156.76	133.88
Sfas	58.05	16.92	29.15	**MMP-3**	6119.28	3561.09	58.19
sFSl	38.99	55.65	142.74	**Eotaxin**	610.56	552.32	90.46
MIF	80.03	50.32	62.87	**Leptin^{2}**	1764.32	2112.24	119.72
IL-1β	51.76	192.52	371.97	**IP-10**	1300.30	1119.80	86.12
IL-2	13.27	3.63	27.33	**MMP-9**	3405.05	2443.42	71.76
IL-1ra	26.01	4.29	16.50	**MMP-13**	5.81	7.96	136.94
IL-4	263.83	449.92	170.54	**PAI-1^{B}**	381.16	307.44	80.66
IL-5	52.10	109.08	209.37	**I-TAC**	610.84	524.00	85.78
IL-6	237.79	874.22	367.65	**MMP-12**	14.41	17.40	120.78
IL-7	32.02	16.91	52.81	**HGF**	1109.68	967.32	87.17
TGF-α	48.84	13.25	27.13	**MMP-7**	437.39	545.63	124.75
Fractalkine	16.93	4.02	23.75	**EGF**	447.62	505.26	112.88
IL-8	1050.01	3114.51	296.62	**sCD40 ligand**	111.41	103.15	92.58
IL-10	16.83	4.30	25.53	**MMP-8**	55.11	265.37	481.49
IL-15	34.44	7.08	20.56				

FIGURE 5D

PERCENT CHANGE IN MEAN FLUORESCENCE INTENSITY IN THE MALE POPULATION

Biomarker	AST vs. NO	LC vs. NO	AST vs. LC	Biomarker	AST vs. NO	LC vs. NO	AST vs. LC
Adiponectin	24.11	39.29	-12.23	IL-17	63.48	35.35	17.21
Resistin	123.24	104.49	8.40	IL-1α	279.04	26.93	66.51
PAI-1^A	233.58	292.55	-17.68	IFN-γ	30.48	33.66	-2.44
SE-selectin	-22.36	-13.18	-11.82	G-CSF	72.41	36.96	20.56
sVCAM-1	55.23	-23.61	50.79	GM-CSF	77.75	24.02	30.22
sICAM-1	8.52	-0.26	8.09	TNF-α	218.55	64.00	48.52
MPO	3467.91	2177.11	36.18	MCP-1	1547.33	410.23	69.03
CRP	0.18	48.26	-47.99	IL-12 (p40)	38.29	12.09	18.95
SAA	40.90	221.38	-128.09	MIP-1α	-429.75	36.79	74.18
SAP	16.82	-22.18	33.38	MIP-1β	96.57	75.16	10.89
Leptin^1	-63.87	-60.71	-8.74	VEGF	49.91	16.29	22.42
GLP-1	-52.47	139.34	-403.53	IL-12(p70)	385.11	451.75	-13.74
Amylin (Total)	-18.59	195.31	-262.76	IL-13	-384539.50	-538089.98	-39.94
C-Peptide	-6.67	-32.90	28.10	MMP-2	-28.47	1.77	-42.28
Insulin	-17.76	-18.75	-74.78	MMP-1	157.09	1681.05	-592.77
Sfas	5.87	28.17	-21.07	MMP-3	0.72	37.01	-36.03
sFSI	136.67	23.54	47.80	Eotaxin	379.44	540.73	-33.64
MIF	532.08	578.78	-7.39	Leptin^2	-63.99	-41.62	-62.13
IL-1β	140.10	24.29	48.24	IP-10	112.35	218.42	-49.95
IL-2	0.73	-12.80	13.44	MMP-9	435.68	374.77	11.37
IL-1ra	17.94	33.04	-12.81	MMP-13	-16.01	267.66	-337.75
IL-4	117.87	37.07	37.09	PAI-1^B	2721.13	1969.94	26.63
IL-5	189.94	55.89	46.23	I-TAC	-4011.55	-2982.15	26.32
IL-6	507.08	96.10	67.70	MMP-12	43.25	262.07	-152.76
IL-7	122.93	38.77	37.75	HGF	-3019.71	-2065.16	32.69
TGF-α	68.18	30.25	22.55	MMP-7	-50.98	129.70	-368.60
Fractalkine	39.18	25.49	9.83	EGF	2392.73	2526.60	-5.37
IL-8	772.73	56.17	82.11	sCD40 ligand	-33.54	-22.74	-16.25
IL-10	42.52	45.16	-1.85	MMP-8	-233.23	-826.58	-445.37
IL-15	51.52	27.02	16.17				

FIGURE 5E

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T-VALUE FOR MALE POPULATIONS

Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC	Biomarker	T AST vs. NO	T LC vs. NO	T AST vs. LC
Adiponectin	0.033	0.000	0.000	IL-17	0.000	0.000	0.256
Resistin	0.000	0.000	0.157	IL-1α	0.001	0.369	0.035
PAI-1^	0.000	0.000	0.000	IFN-γ	0.000	0.000	0.042
SE-selectin	0.029	0.172	0.206	G-CSF	0.000	0.000	0.381
sVCAM-1	0.000	0.000	0.000	GM-CSF	0.000	0.000	0.112
sICAM-1	0.217	0.958	0.454	TNF-α	0.027	0.000	0.390
MPO	0.000	0.000	0.016	MCP-1	0.012	0.000	0.213
CRP	0.986	0.000	0.000	IL-12 (p40)	0.000	0.000	0.302
SAA	0.116	0.000	0.000	MIP-1α	0.074	0.000	0.174
SAP	0.000	0.000	0.000	MIP-1β	0.000	0.000	0.157
Leptin[1]	0.000	0.000	0.695	VEGF	0.000	0.000	0.519
GLP-1	0.022	0.131	0.056	IL-12(p70)	0.000	0.000	0.207
Amylin (Total)	0.353	0.003	0.003	IL-13	0.000	0.000	0.079
C-Peptide	0.234	0.000	0.003	MMP-2	0.479	0.946	0.626
Insulin	0.378	0.070	0.012	MMP-1	0.000	0.000	0.000
Sfas	0.892	0.227	0.000	MMP-3	0.912	0.000	0.000
sFSI	0.000	0.484	0.149	Eotaxin	0.000	0.000	0.002
MIF	0.000	0.000	0.795	Leptin[2]	0.000	0.000	0.147
IL-1β	0.044	0.000	0.199	IP-10	0.000	0.000	0.000
IL-2	0.989	0.653	0.371	MMP-9	0.000	0.000	0.530
IL-1ra	0.206	0.000	0.000	MMP-13	0.608	0.299	0.309
IL-4	0.000	0.017	0.317	PAI-1[B]	0.000	0.000	0.002
IL-5	0.000	0.005	0.211	I-TAC	0.000	0.000	0.008
IL-6	0.004	0.001	0.086	MMP-12	0.105	0.097	0.229
IL-7	0.000	0.000	0.114	HGF	0.000	0.000	0.000
TGF-α	0.000	0.000	0.547	MMP-7	0.000	0.000	0.000
Fractalkine	0.000	0.000	0.869	EGF	0.000	0.000	0.615
IL-8	0.000	0.018	0.019	sCD40 ligand	0.402	0.418	0.155
IL-10	0.000	0.000	0.001	MMP-8	0.000	0.000	0.007
IL-15	0.000	0.000	0.057				

FIGURE 6A

COMPARISON OF PERCENT CHANGE IN MEAN FLUORESCENCE INTENSITY IN MALE AND FEMALE POPULATIONS

Biomarker	AST men vs. women	LC men vs. women	NO men vs. women	Biomarker	AST men vs. women	LC men vs. women	NO men vs. women
Adiponectin	0.10	-18.14	-10.90	IL-17	30.64	3.96	19.90
Resistin	-33.23	23.07	58.99	IL-1α	140.22	30.08	-20.27
PAI-1^A	-15.46	23.16	134.12	IFN-γ	-2.75	-5.75	28.62
SE-selectin	3.83	8.93	52.93	G-CSF	80.57	7.77	18.99
sVCAM-1	16.18	-6.32	-9.00	GM-CSF	69.08	1.12	14.94
sICAM-1	10.07	-0.30	12.53	TNF-α	155.04	-49.29	56.20
MPO	-16.10	-2.85	1230.91	MCP-1	79.54	7.27	61.07
CRP	-22.08	7.21	-15.82	IL-12 (p40)	37.24	2.10	16.05
SAA	-8.51	22.59	-27.43	MIP-1α	281.59	-30.18	45.91
SAP	14.39	4.87	3.14	MIP-1β	52.27	-28.41	57.17
Leptin^1	-72.48	-32.39	-72.76	VEGF	43.04	7.55	13.44
GLP-1	-82.09	23.23	-6.65	IL-12(p70)	20.55	-28.63	4.52
Amylin (Total)	-72.43	49.59	26.27	IL-13	-7.67	-3.21	-36841.43
C-Peptide	-17.03	7.58	-22.79	MMP-2	-44.02	26.80	-24.04
Insulin	-57.45	14.56	6.48	MMP-1	-51.13	34.73	301.06
Sfas	-34.05	8.59	16.69	MMP-3	-0.63	8.24	22.90
sFSl	46.15	50.64	-18.92	Eotaxin	-9.80	0.20	127.46
MIF	58.30	55.98	735.03	Leptin^2	-69.94	-27.28	-55.72
IL-1β	117.39	-3.26	23.55	IP-10	-23.43	19.53	-26.09
IL-2	6.47	-4.51	-16.22	MMP-9	-24.71	-11.86	169.56
IL-1ra	-1.56	-0.52	11.01	MMP-13	-49.86	118.12	103.36
IL-4	78.82	27.65	-13.02	PAI-1^B	-25.60	14.26	390.15
IL-5	87.95	26.47	5.34	I-TAC	-31.43	16.14	-694.81
IL-6	241.21	8.89	10.90	MMP-12	-41.95	46.11	54.62
IL-7	76.56	-37.27	23.96	HGF	-28.37	39.00	-1517.50
TGF-α	52.28	2.33	19.67	MMP-7	-15.42	-3.46	30.36
Fractalkine	10.96	-0.26	22.99	EGF	10.50	11.68	170.40
IL-8	49.10	48.14	234.11	sCD40 ligand	-47.84	29.97	-16.57
IL-10	12.51	-36.60	25.00	MMP-8	-71.41	21.38	-637.74
IL-15	52.75	-0.16	18.67				

FIGURE 6B

SIGNIFICANCE OF MALE AND FEMALE POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF STUDENT'S T VALUE

Biomarker	T AST men vs. women	T LC men vs. women	T NO men vs. women	Biomarker	T AST men vs. women	T LC men vs. women	T NO men vs. women
Adiponectin	0.991	0.004	0.150	IL-17	0.000	0.599	0.000
Resistin	0.040	0.014	0.000	IL-1α	0.042	0.495	0.340
PAI-1^A	0.001	0.000	0.000	IFN-γ	0.431	0.135	0.000
SE-selectin	0.729	0.501	0.000	G-CSF	0.000	0.322	0.000
sVCAM-1	0.011	0.132	0.007	GM-CSF	0.000	0.746	0.007
sICAM-1	0.130	0.954	0.037	TNF-α	0.107	0.171	0.000
MPO	0.082	0.774	0.000	MCP-1	0.380	0.594	0.000
CRP	0.005	0.150	0.059	IL-12 (p40)	0.000	0.556	0.000
SAA	0.603	0.056	0.156	MIP-1α	0.181	0.066	0.000
SAP	0.001	0.448	0.319	MIP-1β	0.021	0.199	0.000
Leptin[1]	0.000	0.003	0.000	VEGF	0.000	0.107	0.000
GLP-1	0.113	0.743	0.754	IL-12(p70)	0.463	0.112	0.938
Amylin (Total)	0.080	0.284	0.409	IL-13	0.675	0.824	0.000
C-Peptide	0.014	0.402	0.000	MMP-2	0.010	0.305	0.427
Insulin	0.101	0.577	0.731	MMP-1	0.005	0.016	0.000
Sfas	0.000	0.235	0.607	MMP-3	0.929	0.072	0.000
sFSI	0.100	0.303	0.123	Eotaxin	0.378	0.980	0.000
MIF	0.017	0.080	0.000	Leptin[2]	0.000	0.004	0.000
IL-1β	0.126	0.065	0.000	IP-10	0.030	0.042	0.103
IL-2	0.124	0.418	0.686	MMP-9	0.007	0.172	0.000
IL-1ra	0.610	0.883	0.295	MMP-13	0.334	0.522	0.094
IL-4	0.018	0.140	0.068	PAI-1^B	0.018	0.126	0.000
IL-5	0.036	0.233	0.663	I-TAC	0.004	0.080	0.000
IL-6	0.048	0.766	0.529	MMP-12	0.114	0.549	0.097
IL-7	0.000	0.157	0.000	HGF	0.002	0.019	0.000
TGF-α	0.000	0.549	0.000	MMP-7	0.298	0.580	0.000
Fractalkine	0.001	0.936	0.000	EGF	0.512	0.348	0.000
IL-8	0.340	0.060	0.000	sCD40 ligand	0.010	0.474	0.593
IL-10	0.004	0.069	0.000	MMP-8	0.319	0.346	0.000
IL-15	0.000	0.954	0.000				

Figure 7A

PERCENT CHANGE IN MEAN CONCENTRATION IN THE FEMALE POPULATION									
Biomarker	LC vs. NO	AST vs. NO	AST vs. LC	Scalar Sum	Biomarker	LC vs. NO	AST vs. NO	AST vs. LC	Scalar Sum
I-TAC	232.54	808.32	63.39	1104.24	MMP-1	211.09	-0.40	-212.35	423.84
PAI-1	236.98	331.62	21.93	590.52	Fractalkine	-19.66	-80.00	-301.66	401.32
MMP-7	84.44	-63.12	-400.06	547.62	IL-1α	71.64	260.31	52.36	384.32
MMP-3	296.73	24.09	-219.71	540.53	CRP	212.05	85.24	-68.45	365.74
IL-8	-82.05	205.35	94.12	381.53	MIP-1β	-94.59	-97.96	-164.40	356.96
MPO	146.60	208.72	20.12	375.44	IP-10	137.95	151.54	5.40	294.88
Leptin	66.76	199.02	44.23	310.02	IL-1ra	-99.93	-99.96	-67.00	266.89
sFSl	-11.14	188.58	69.21	268.93	MIP-1α	-85.14	-90.57	-57.53	233.24
HGF	-25.19	161.40	71.38	257.97	VEGF	-97.45	-97.90	-21.17	216.52
Resistin	5.74	110.38	49.74	165.86	IFN-γ	69.14	100.91	15.81	185.87
C-Peptide	-34.06	58.58	58.42	151.06	Adiponectin	90.42	79.52	-6.07	176.01
MMP-13	-33.46	-57.79	-57.65	148.91	Eotaxin	79.43	65.79	-8.23	153.45
SAP	-37.39	-58.12	-49.49	145.01	IL-6	31.65	52.43	13.63	97.71
sVCAM-1	-14.50	30.75	34.61	79.87	MMP-12	49.48	5.90	-41.14	96.52
MMP-8	-6.98	-3.33	3.78	14.08	sICAM-1	19.75	-13.48	-38.41	71.64
IL-10	607.39	-88.77	-6198.42	6894.58	MIF	-33.32	-32.99	0.49	66.79
MMP-9	1467.27	3385.22	55.03	4907.52	Sfas	22.05	18.36	-3.12	43.53
G-CSF	793.53	263.78	-145.62	1202.93	IL-12 (p40)	75.26	219.85	45.21	340.31
EGF	627.08	408.97	-42.85	1078.90	IL-4	-37.26	26.12	50.25	113.63
MCP-1	477.55	324.18	-36.16	837.89	Insulin	-42.14	-18.85	28.70	89.70
SAA	453.90	100.01	-176.94	730.85					

Figure 7B

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF KRUKSAL-WALLIS FOR FEMALE POPULATIONS							
Biomarker	**LC vs. NO**	**AST vs. NO**	**AST vs. LC**	**Biomarker**	**LC vs. NO**	**AST vs. NO**	**AST vs. LC**
I-TAC	1.31E-19	2.50E-30	1.19E-11	**MMP-1**	1.81E-13	0.143	7.91E-13
PAI-1	1.01E-17	1.64E-21	0.030	**Fractalkine**	2.82E-49	0.391	0.008
MMP-7	1.04E-12	9.55E-23	2.83E-29	**IL-1α**	0.218	1.67E-05	5.81E-04
MMP-3	0.041	9.37E-07	2.16E-06	**CRP**	8.76E-47	1.78E-07	0.253
IL-8	4.82E-32	0.004	0.004	**MIP-1β**	0.027	0.812	0.004
MPO	5.92E-36	3.36E-14	0.004	**IP-10**	1.05E-19	4.53E-20	0.754
Leptin	1.48E-15	1.02E-15	4.03E-09	**IL-1ra**	2.09E-55	1.35E-09	0.332
sFSI	7.36E-35	1.77E-11	1.23E-19	**MIP-1α**	0.004	1.29E-04	0.238
HGF	0.001	7.52E-15	1.52E-19	**VEGF**	1.43E-05	6.72E-04	0.314
Resistin	8.18E-14	1.19E-12	3.57E-10	**IFN-γ**	3.77E-05	3.77E-05	0.638
C-Peptide	7.34E-40	0.004	4.76E-10	**Adiponectin**	2.00E-42	1.08E-11	0.316
MMP-13	0.024	9.50E-08	1.11E-05	**Eotaxin**	3.41E-09	1.59E-09	0.313
SAP	8.57E-42	6.46E-07	0.031	**IL-6**	1.94E-32	0.009	0.225
sVCAM-1	6.40E-03	5.43E-07	2.43E-13	**MMP-12**	2.05E-05	0.528	4.44E-05
MMP-8	0.015	2.79E-04	1.12E-11	**sICAM-1**	5.58E-44	0.009	0.184
IL-10	3.35E-38	0.251	8.52E-06	**MIF**	1.16E-46	9.67E-04	0.109
MMP-9	2.57E-13	2.35E-13	0.704	**Sfas**	8.55E-28	1.32E-07	0.708
G-CSF	2.31E-05	3.38E-04	0.363	**IL-12 (p40)**	0.19	0.077	0.002
EGF	1.43E-24	2.10E-24	0.298	**IL-4**	0.252	0.003	0.071
MCP-1	5.11E-19	6.55E-22	0.361	**Insulin**	1.73E-25	0.753	0.145
SAA	3.15E-40	4.84E-14	0.093				

Figure 8A

PERCENT CHANGE IN MEAN CONCENTRATION IN THE MALE POPULATION

Biomarker	LC vs. NO	AST vs. NO	AST vs. LC	Scalar Sum	Biomarker	LC vs. NO	AST vs. NO	AST vs. LC	Scalar Sum
HGF	31570.67	70709.80	55.27	102335.75	MIP-1α	167.51	556.86	59.27	783.64
MMP-8	24387.41	3673.65	-548.91	28609.97	MMP-13	253.63	75.96	-100.97	430.56
I-TAC	5284.92	20195.93	73.47	25554.31	G-CSF	218.18	11.35	-185.74	415.28
EGF	3182.66	3895.15	17.83	7095.65	IFN-γ	66.22	-57.48	-290.89	414.58
PAI-1	1766.61	2974.80	39.29	4780.71	MMP-7	161.73	-3.44	-171.06	336.23
MMP-1	2657.85	496.02	-362.71	3516.58	IP-10	165.05	158.57	-2.51	326.13
MPO	858.94	1804.89	49.66	2713.49	CRP	81.49	-35.15	-179.84	296.48
MIF	687.69	491.62	-33.14	1212.44	Insulin	-24.90	-56.95	-74.46	156.32
Eotaxin	365.15	522.18	25.24	912.57	VEGF	72.02	51.88	-13.26	137.15
MMP-12	399.62	158.80	-93.05	651.48	SAP	-39.69	-53.58	-29.91	123.18
SAA	49.02	-62.23	-294.54	405.78	Adiponectin	43.04	-9.06	-57.29	109.39
Resistin	90.46	29.15	-47.46	167.07	sVCAM-1	-15.22	16.68	27.34	59.24
sFSI	-0.87	78.86	44.58	124.31	Sfas	13.76	6.21	-7.11	27.08
Leptin	-51.27	-49.51	3.49	104.26	IL-1ra	24.34	-42.18	-115.05	181.57
C-Peptide	-7.14	29.77	28.45	65.36	IL-12 (p40)	-72.67	4.72	73.90	151.30
MMP-9	10857.07	1705.79	-506.77	13069.63	MIP-1β	4.75	64.53	36.34	105.61
MCP-1	235.25	829.77	63.94	1128.96	sICAM-1	9.18	-31.45	-59.29	99.93
MMP-3	515.02	216.75	-94.17	825.93					

Figure 8B

SIGNIFICANCE OF POPULATION DIFFERENCES EXPRESSED AS PROBABILITY OF KRUKSAL-WALLIS MEANS VALUE FOR MALE POPULATIONS

Biomarker	LC vs. NO	AST vs. NO	AST vs. LC	Biomarker	LC vs. NO	AST vs. NO	AST vs. LC
HGF	5.68718E-58	1.25E-44	1.44E-10	MIP-1α	1.23E-09	9.06E-04	0.582
MMP-8	7.13597E-36	0.003	4.08E-13	MMP-13	4.07E-12	0.002	0.377
I-TAC	1.904E-63	1.22E-44	3.22E-08	G-CSF	0.119	0.002	6.22E-05
EGF	4.54971E-61	3.00E-31	0.006	IFN-γ	0.992	0.014	0.018
PAI-1	9.65123E-52	3.91E-32	0.032	MMP-7	2.98033E-28	0.213	4.52E-18
MMP-1	2.591E-52	1.34E-09	2.27E-16	IP-10	1.04E-31	1.87E-18	0.725
MPO	3.30E-47	3.03E-32	4.17E-11	CRP	1.04E-11	0.758	1.87E-9
MIF	3.05E-27	2.20E-29	0.006	Insulin	4.18E-04	3.54E-05	0.219
Eotaxin	2.54E-43	5.55E-28	0.001	VEGF	6.046E-13	2.61E-09	0.699
MMP-12	7.21087E-32	1.39E-04	0.002	SAP	3.20E-07	1.22E-05	0.739
SAA	4.51E-19	0.002	1.23E-06	Adiponectin	6.95E-11	0.285	4.39E-08
Resistin	5.26E-16	0.011	3.40E-04	sVCAM-1	0.674	9.79E-10	1.45E-15
sFSl	3.39E-21	0.019	3.88E-17	Sfas	2.65E-15	4.94E-08	0.245
Leptin	1.08E-10	0.013	8.32E-04	IL-1ra	6.40E-09	0.088	0.009
C-Peptide	9.13E-11	1.72E-02	1.07E-08	IL-12 (p40)	0.001	0.335	0.180
MMP-9	6.48556E-33	4.61E-20	0.759	MIP-1β	3.79E-05	0.066	0.434
MCP-1	2.78E-39	3.91E-21	0.186	sICAM-1	0.723	2.17E-05	0.218
MMP-3	5.09E-15	2.29E-05	0.170				

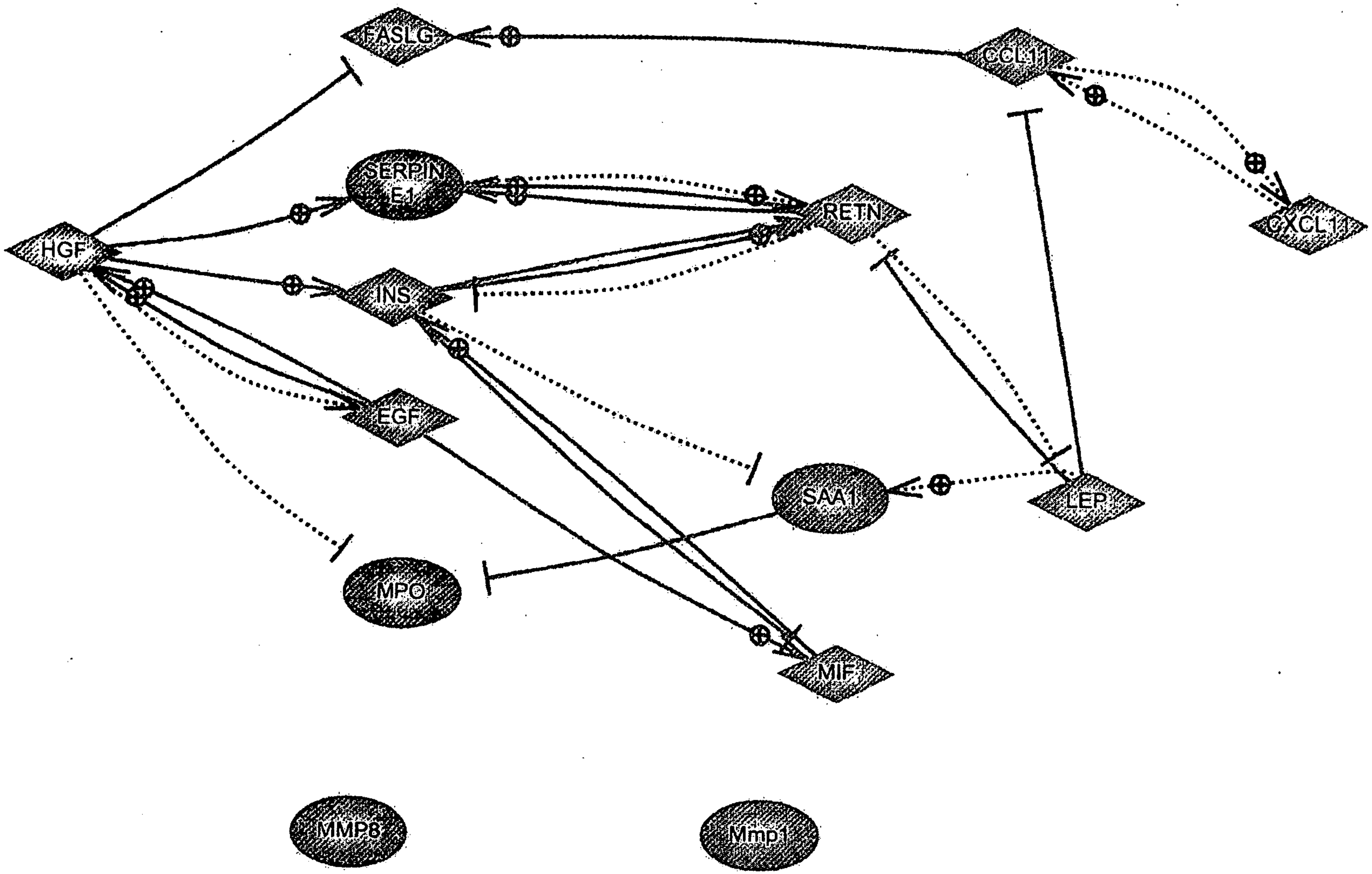

FASLG
CCL11
CXCL1
SERPIN E1
RETN
HGF
INS
EGF
SAA1
LEP
MPO
MIF
MMP8
Mmp1

Figure 9