megakaryocytes, resulting in an increased level of IFN-I regulated proteins in platelets. The expressions of genes from megakaryocytes are thus correlated to SLE or vascular disease. No disclosure is made that RNA/DNA from diseased cells may be taken up by platelets.

The present invention aims to overcome the problem of the prior art that not all diseased tissues or disease types (e.g. tumours) result in circulating disease cells (e.g. circulating tumour cells). The present invention also aims to overcome the problem that protein markers for detecting diseases such as cancer are difficult to detect. Further, the present invention aims to provide methods that do not require biopsies, and allow extensive monitoring of patients.

SUMMARY OF THE INVENTION

The present invention in a first aspect provides a method of analysing a blood sample of a subject for the presence of a disease marker, wherein said disease is an autoimmune disease, a cardiovascular disease and/or a neurological disorder, said method comprising the steps of

a) extracting nucleic acid from anucleated blood cells preferably thrombocytes, in said blood sample to provide an anucleated blood cell-extracted nucleic acid fraction, wherein said anucleated blood cell-extracted nucleic acid fraction is not megakaryocyte derived nucleic acid or megakaryocyte derived RNA, and

b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a disease marker,

wherein said disease marker is a disease-specific mutation in a gene of a nucleated cell of said subject, wherein said nucleated cell is not a megakaryocyte, or

wherein said disease marker is a disease-specific expression profile of genes of a nucleated cell of said subject, wherein said nucleated cell is not a megakaryocyte.

Also disclosed is a method of analysing a blood sample of a subject for the presence of a disease marker, said method comprising the steps of a) extracting nucleic acid from anucleated blood cells, preferably thrombocytes, in said blood sample to provide an anucleated blood cell-extracted nucleic acid fraction, and b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a disease marker, wherein said disease marker is a disease-specific mutation in a gene of a nucleated cell of said subject, or wherein said disease marker is a disease-specific expression profile of genes of a nucleated cell of said subject.

It was surprisingly found that nucleic acids from nucleated cells are present in anucleated blood cells such as thrombocytes. It may be that nucleated cells excrete nucleic acids into the blood stream and these excreted nucleic acids are then taken up from the blood stream by anucleated cells such as thrombocytes or that in some other way of transport nucleic acids from nucleated cells are transferred to anucleated blood cells. The inventors realized for the first time that disease markers may be used on the nucleic acids extracted from anucleated blood cells to identify diseases from nucleated cells.

Superficial siderosis; Sydenham's chorea; Syncope; Synesthesia; Syringomyelia; Tarsal tunnel syndrome; Tardive dyskinesia; Tarlov cyst; Tay-Sachs disease; Temporal arteritis; Tetanus; Tethered spinal cord syndrome; Thomsen disease; Thoracic outlet syndrome; Tic Douloureux; Todd's paralysis; Tourette syndrome; Toxic encephalopathy; Transient ischemic attack; Transmissible spongiform encephalopathies; Transverse myelitis; Traumatic brain injury; Tremor; Trigeminal neuralgia; Tropical spastic paraparesis; Trypanosomiasis; Tuberous sclerosis; Von Hippel-Lindau disease; Viliuisk Encephalomyelitis ; Wallenberg's syndrome; Werdnig-Hoffman disease; West syndrome; Whiplash; Williams syndrome; Wilson's disease; and Zellweger syndrome.

In other preferred embodiments of aspects of the invention the cardiovascular disease is selected from the group consisting of Aneurysm; Angina; Atherosclerosis; Cerebrovascular Accident (Stroke); Cerebrovascular disease; Congestive Heart Failure; Coronary Artery Disease; Myocardial infarction (Heart Attack); and Peripheral vascular disease.

In other preferred embodiments of aspects of the invention the cardiovascular disease is not systemic lupus erythematosus.

In another aspect, the present invention provides a method of diagnosing disease in a subject using the method of analysing a blood sample according to the invention. Hence, in another preferred embodiment of a method of the invention, said method of analysing a blood sample according to the invention is part of a method of diagnosing disease in a subject, and wherein the presence of said disease marker in said anucleated blood cell-extracted nucleic acid fraction is indicative of said subject suffering from said disease.

In another aspect, the present invention provides a method for determining the stage of disease or the efficacy of a disease treatment in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a disease marker using the method as defined herein at a first time point to thereby provide a first value for the level of said disease marker in said subject,

- analysing a blood sample of said subject for the presence of a disease marker using the method as defined herein at a second time point to thereby provide a second value for the level of said disease marker in said subject, wherein said subject has been subjected to a disease treatment between said first and second time point, and

- comparing said first and second value to determine the efficacy of said disease treatment in said subject.

Also disclosed is a method for determining the efficacy of a disease treatment in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a disease marker using the method of analysing a blood sample according to the invention at a first time point to thereby provide a first value for the level of said disease marker in said subject;
- analysing a blood sample of said subject for the presence of a disease marker using the method of analysing a blood sample according to the invention at a second time point that is earlier or later, preferably later, than said first time point, to thereby provide a second value for the level of said disease marker in said subject, wherein said subject has been subjected to a disease treatment between said first and second time point, and
- comparing said first and second value to determine the efficacy of said disease treatment in said subject.

The skilled artisan will understand that treatment prior to the first time point and subsequent measurements at a second, later, time point without any disease treatment having occurred between said time points, is included in aspects of the invention for determining the efficacy of a disease treatment.

In another aspect, the present invention provides a method for determining the stage of a disease in a subject, comprising the steps of:
- analysing a blood sample of a subject for the presence of a disease marker using the method as defined herein to thereby provide a test value for the level of said disease marker in said subject,
- providing a reference value for the level of said disease marker wherein said reference value is correlated to a particular stage of disease, and
- comparing said test and reference value to determine the stage of disease in said subject.

Also disclosed is a method for determining the stage of disease. In order to determine the stage of disease, it is beneficial to correlate disease marker values as determined by methods of this invention to disease stages. A single measurement of the disease marker may than be compared to one or more reference values to obtain an indication of the stage of the disease.

Also disclosed is a method for determining the stage of disease in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a disease marker using the method of analysing a blood sample of a subject for the presence of a disease marker according to the present invention to thereby provide a test value for the level of said disease marker in said subject,

- providing a reference value for the level of said disease marker wherein said reference value is correlated to a particular stage of disease, and
- comparing said test and reference value to determine the stage of disease in said subject.

In yet another aspect, the present invention provides a kit of parts adapted for performing the method recited herein, the kit comprising a packaging material which comprises:

- a container for holding anucleated blood cells separated from a blood sample of a subject;
- an agent for extracting nucleic acids from said anucleated blood cells;
- an agent for selectively amplifying from said nucleic acids extracted from said anucleated blood cells a disease-specific mutation in a gene of a nucleated cell of said subject by reverse transcriptase polymerase chain reaction amplification, and
- a printed or electronic instruction for performing the method recited herein,

the kit further comprising;

- a reference for said disease marker, wherein said reference is indicative for the presence or absence of said disease marker in said anucleated blood cells-extracted nucleic acid fraction, wherein said gene is not a gene from a megakaryocyte and wherein the disease marker is not derived from a megakaryocyte, wherein said instruction is selected from an instruction for bead-based anucleated blood cells isolation, an instruction for FACS sorting of anucleated blood cells, an instruction for anucleated blood cell recovery by centrifugation, or negative selection of non-anucleated blood cell components.

Also disclosed is a kit of parts adapted for performing a method of the invention as described herein above, the kit comprising a packaging material which comprises at least one of:

- a container for holding anucleated blood cells, preferably thrombocytes, separated from a blood sample;
- an agent for extracting nucleic acids from said anucleated blood cells;

- an agent for selectively amplifying from said nucleic acids extracted from said anucleated blood cells a disease-specific marker as described herein above, such as a disease-specific mutation in a gene of a nucleated cell of a subject or a disease-specific expression profile of nucleic acid from a nucleated cell of said subject, for instance by reverse transcriptase polymerase chain reaction amplification, and

- a printed or electronic instruction for performing a method of the invention as described herein above,

the kit further comprising:

- a reference for said disease marker, wherein said reference is indicative for the presence or absence of said disease marker in said anucleated blood cells-extracted nucleic acid fraction.

In a preferred embodiment of a kit according to the present invention said reference is a reference value for the level of nucleic acids comprising said disease-specific mutation in anucleated blood cells in a healthy control subject or in a control subject suffering from disease, or wherein said reference is a reference expression profile, for instance for a plurality of mRNAs in anucleated blood cells from a healthy control subject or from a control subject suffering from disease.

In another preferred embodiment of a kit according to the present invention said agent or instruction is selected from a particle or fluorescent marker-labeled anti-anucleated blood cell antibody (preferably a fluorescent marker-labeled anti-thrombocyte antibody), an instruction for bead-based anucleated blood cells isolation (preferably thrombocyte isolation), an instruction for FACS sorting of anucleated blood cells (preferably of thrombocytes), an instruction for anucleated blood cell (preferably thrombocyte) recovery by centrifugation, or negative selection of non-anucleated blood cell components (preferably non-thrombocyte components).

In yet another aspect, the present invention provides a device for diagnosing disease, the device comprising a support and at least one agent for specifically determining a level and/or activity of at least one nucleic acid mutant in an anucleated blood cell sample of the subject attached to said support, and

a computer-readable medium having computer-executable instructions for performing the method recited herein, wherein said disease is not a disease associated with pathological megakaryocyte function and wherein said nucleic acid mutant does not comprise megakaryocyte derived nucleic acid, wherein said at least one agent is an oligonucleotide probe or sequencing primer, comprising a lateral flow device, a dipstick or a cartridge for performing a nucleic acid hybridization reaction between:

- an anucleated blood cells-extracted nucleic acid and at least one nucleic acid mutation-specific amplification primer or oligonucleotide probe, wherein said nucleic acid mutation-specific amplification primer or oligonucleotide probe is specific for a disease-specific mutation, or

- an anucleated blood cells-extracted nucleic acid and a plurality of gene-specific amplification primers or oligonucleotide probes for providing an disease-specific gene expression profile.

Also disclosed is a device for diagnosing disease, the device comprising a support and at least one agent for specifically determining a level and/or activity of at least one nucleic acid mutant in a anucleated blood cells sample of the subject, said agent being attached to said support, and a computer-readable medium having computer-executable instructions for performing a method of the invention as described herein above.

In a preferred embodiment of a device according to the present invention, said at least one agent is an oligonucleotide probe or sequencing primer.

In a preferred embodiment of a device according to the present invention, the device comprises a lateral flow device, a dipstick or a cartridge for performing a nucleic acid hybridization reaction between an anucleated blood cells-extracted nucleic acid and at least one nucleic acid mutation-specific amplification primer or oligonucleotide probe, or between an anucleated blood cells-extracted nucleic acid and a plurality of gene-specific amplification primers or oligonucleotide probes for providing an disease-specific gene expression profile.

DESCRIPTION OF THE DRAWINGS