

FEVER TRIAGE

FIELD OF THE INVENTION

The present invention relates to methods for distinguishing between different diseases or conditions in subjects with fever, or for determining the presence of different diseases or conditions in subjects with fever. The invention also relates to kits that may be used in practicing such methods. The invention relates to methods of treatment and medical uses of therapeutic agents based upon the methods of the invention. The invention further relates to methods of identifying genes that may be used in ratio-based biomarkers, and to methods of identifying origin-proxy reference genes.

BACKGROUND

A fever may be characterised as a temporary increase in body temperature, typically as a response to an underlying illness, infection, or other medical condition. Triage for individuals with fever can be a challenging task due to the myriad of potential underlying conditions that could lead to an elevated body temperature. The symptom of fever is non-specific, meaning it can be associated with a wide range of illnesses, infections, and non-infectious factors. This complexity makes it difficult for healthcare professionals to immediately pinpoint the precise cause of the fever.

Gene expression profiling using transcriptome analysis has become a valuable tool in understanding the immune response and identifying potential biomarkers for various diseases, including those associated with fever. Currently, there are various blood transcriptome gene panels which are available to triage subjects with fever into various classifications e.g., bacterial, or viral disease.

However, there are multiple disadvantages to these panels.

The majority of these gene panels use non-CoDa analysis which commonly generates a long list of differential expression genes, for example in the 10's or 100's. These long lists are therefore challenging for use in clinical applications due to their complexity.

There are a select few gene panels which utilise less than 10 genes to differentiate between different diseases or conditions e.g., viral, and bacterial infection. However, these panels also have disadvantages. Firstly, there is little overlap of the selected genes in panels. In clinical

practice, a lack of consensus on a set of commonly agreed-upon genes can make it difficult to rely on these panels for accurate and consistent identification and differentiation between different diseases.

The second problem is that the classification decision boundary between case and control groups is linear in these panels because only a simple ratio was taken and usually between an activated gene and another suppressed gene. Linear decision boundaries are generally less flexible in capturing complex and non-linear relationships between gene expression patterns and disease states. In many cases, diseases and conditions exhibit intricate molecular signatures that are not adequately represented by a linear model. This can lead to reduced discriminative power, making it challenging to accurately distinguish between case and control groups.

Therefore, there is currently no simple and consistently accurate way to triage subjects with fever.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a method for distinguishing between non-interferon activating disease and interferon activating disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
 - providing a first ratio-based biomarker (RBB) which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and

distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison.

According to a second aspect of the invention, there is provided a method for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between systemic fungal infection and the group of diseases consisting of: bacterial infection and Kawasaki disease on the basis of this comparison.

Optionally, the subject may have been distinguished as having non-interferon activating disease by a method of the first aspect of the invention.

According to a third aspect of the invention, there is provided a method for distinguishing between bacterial infection and Kawasaki disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison.

Optionally, the subject may have been distinguished as having bacterial infection or Kawasaki disease by a method of the second aspect of the invention.

According to a fourth aspect of the invention, there is provided a method for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE) in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison.

Optionally, the subject may have been distinguished as having an interferon-activated disease by a method of the first aspect of the invention.

According to a fifth aspect of the invention, there is provided a method for distinguishing between SLE and viral disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between SLE and viral disease on the basis of this comparison.

Optionally, the subject may have been distinguished as having SLE or a viral disease by a method of the fourth aspect of the invention.

According to a sixth aspect of the invention, there is provided a method for distinguishing between viral disease and active TB in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 16 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
distinguishing between viral disease and active TB on the basis of this comparison.

Optionally, the subject may have been distinguished as having a viral disease or active TB by a method of the fourth aspect of the invention.

According to a seventh aspect of the invention, there is provided a method for distinguishing between SLE, viral disease, and active TB in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 16 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB, second RBB and third RBB; and
distinguishing between SLE, viral disease, and active TB on the basis of this comparison.

Optionally, the subject may have been distinguished as having SLE, a viral disease, or active TB by a method of the fourth, fifth or sixth aspects of the invention.

According to an eighth aspect of the invention, there is provided a method of determining the presence of Kawasaki disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 17 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and determining the presence of Kawasaki disease on the basis of this comparison.

Optionally, the subject may have been distinguished as potentially having Kawasaki disease by a method of the first aspect of the invention (indicating that they have non-interferon activating disease), or by a method of the second or third aspects of the invention.

According to a ninth aspect of the invention, there is provided a method of determining the presence of viral disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 18 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and determining the presence of viral disease on the basis of this comparison.

Optionally, the subject may have been distinguished as potentially having a viral disease by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth, fifth, sixth, or seventh aspects of the invention.

According to a tenth aspect of the invention, there is provided a method of determining the presence of TB in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 19 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and determining the presence of active TB on the basis of this comparison.

Optionally, the subject may have been distinguished as potentially having active TB by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth, sixth, or seventh aspects of the invention.

According to an eleventh aspect of the invention, there is provided a method of indicating the cause of a subject's fever, the method comprising:

(a) analysing gene expression in a sample representative of gene expression in the subject with fever, and, based on this analysis:

(b) providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3; and

providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;

comparing the first RBB and second RBB; and

distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison; wherein

if non-interferon activating disease is distinguished further step (c) and optionally a still further step (d) is performed based on the analysis in (a), and

if interferon activating disease is distinguished further step (e) and optionally a still further step (f) is performed based on the analysis in (a);

(c) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3, and

providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;

comparing the third RBB and fourth RBB; and

distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease on the basis of this comparison; wherein

if systemic fungal disease is distinguished, the method indicates systemic fungal disease is the cause of the subject's fever; and

if the group of diseases consisting of: bacterial disease and Kawasaki disease is distinguished, a further step (d) is performed;

(d) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3; and

providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;

comparing the fifth RBB and sixth RBB; and

distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison; wherein

if bacterial disease is distinguished, the method indicates bacterial disease is the cause of the subject's fever; and

if Kawasaki disease is distinguished, the method indicates Kawasaki disease is the cause of the subject's fever;

(e) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3; and

providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;

comparing the third RBB and fourth RBB; and

distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison; wherein

if active TB is distinguished, the method indicates active TB is the cause of the subject's fever; and

if the group of diseases consisting of: viral disease and SLE is distinguished, a further step (f) is performed;

(f) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3; and

providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;

comparing the fifth RBB and sixth RBB; and

distinguishing between SLE and viral disease on the basis of this comparison; wherein

if SLE is distinguished, the method indicates SLE is the cause of the subject's fever;

and

if viral disease is distinguished, the method indicates viral disease is the cause of the subject's fever.

Steps of analysing gene expression in a sample, such as in the context of the methods of the first to eleventh aspect of the invention, may be practiced other than on the body of the subject, for example *in vitro*.

According to a twelfth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2.

According to a thirteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9.

According to a fourteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11.

According to a fifteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13.

According to a sixteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15.

According to a seventeenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 16.

According to an eighteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and
- iv. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 16.

According to a nineteenth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 17; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reference standard indicating the reference level of the corresponding biomarker.

According to a twentieth aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 18; and

- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reference standard indicating the reference level of the corresponding biomarker.

According to a twenty-first aspect of the invention there is provided a kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 19; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reference standard indicating the reference level of the corresponding biomarker.

It will be appreciated that methods in accordance with the first to eleventh aspects of the invention may be practice using appropriate kits in accordance with twelfth to twenty-first aspects of the invention.

Kits in accordance with any of the twelfth to twenty-first aspects of the invention may, for the sake of brevity, be referred to as “kits of the invention.” Similarly, methods in accordance with any of the first to eleventh aspects of the invention may be referred to as “a method of the invention.” Methods in accordance with either the forty-eighth or forty-ninth aspects of the invention may, for brevity, be referred to as a “marker identification method of the invention.”

According to a forty-eighth aspect of the invention there is provided a method of identifying genes for use in an RBB for determining presence of a disease, the method comprising:

- obtaining a first set of data representative of gene expression in a sample of patients with the disease;
- obtaining a second set of data representative of gene expression in a sample of controls without the disease;
- performing an unweighted LRA analysis in respect of the first and second sets of data to generate an LRA biplot;
- identifying a first group of genes closest to the origin in the LRA biplot; and
- identifying a second group of genes furthest from the first group of genes in the LRA biplot, wherein
- the first group of genes provides the denominator of the RBB and the second group of genes provides the numerator of the RBB.

According to a forty-ninth aspect of the invention there is provided a method of identifying an origin-proxy reference gene in respect of a state of interest, the method comprising:

- obtaining a first set of data representative of gene expression in a sample of patients with the state of interest;
- obtaining a second set of data representative of gene expression in a sample of controls without the state of interest;
- performing an unweighted LRA analysis in respect of the first and second sets of data to generate an LRA biplot; and
- identifying the group of genes closest to the origin in the LRA biplot as origin-proxy reference genes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 – Schematic illustrating an overview of the workflow utilised to determine RBBs.

Figure 2 – A hierarchical classification flowchart used to triage patients with fever into various aetiologies, according to two main groups; Group 1 (G1) non-interferon activating diseases and Group 2 (G2) interferon activating diseases by using Group 1 specific RBBs and Group 2 specific RBBs.

Figure 3 - LRA biplots of a list of granulocyte genes in 4 different KD datasets. **A** – LRA biplot of a list of granulocyte genes in GSE73461. **B** – LRA biplot of a list of granulocyte genes in GSE68004. **C** – LRA biplot of a list of granulocyte genes in GSE73463. **D** – LRA biplot of a list of granulocyte genes in GSE63881. **E** – LRA biplot of a list of granulocyte genes in GSE63881. Two genes, CTSS and PSAP, are shown by arrows. **F**- Plot showing the correlation between an RBB containing CD14 and gene expression of LYZ. **G** - Plot showing the correlation between an RBB containing CTSS and gene expression of LYZ. **H** - Plot showing the correlation between an RBB containing CD14 and gene expression of VNN1. **I** - Plot showing the correlation between an RBB containing PSAP and gene expression of VNN1.

Figure 4 - PCA biplot of ALRs using SAT1 as OP reference gene. Data from GSE73461 using KD as an example of group 1 non-interferon activating disease.

Figure 5 – **A** - PCA biplot of ALRs using SAT1 as the OP-reference gene. Data from GSE73461 using KD as an example of group 1 non-interferon activating disease. **B** - PCA biplot of ALRs using SAT1 as the OP reference gene using dataset GSE68004. **C** and **D** - Comparable PCA biplots of ALRs when using other OP reference genes (NCF1 and SRGN).

Figure 6 - Box plots and ROC of RBBs using 8 predictor genes and SAT1 as the denominator of dataset GSE73461. **A** - Box plots and ROC of FCER1A/SAT1. **B** - Box plots and ROC of CCR3/SAT1. **C** - Box plots and ROC of HDC/SAT1. **D** - Box plots and ROC of ANXA3/SAT1. **E** - Box plots and ROC of IL1R2/SAT1. **F** - Box plots and ROC of MMP9/SAT1. **G** - Box plots and ROC of ALPL/SAT1. **H** - Box plots and ROC of ARG1/SAT1.

Figure 7 - MoM distribution of a positive predictor gene RBB (IL1R2/SAT1) of control and KD are shown in box plot for 4 datasets (GSE63881, GSE68004, GSE73461, GSE73463).

Figure 8 – **A** - Box plot distribution of RBB (using SRGC) to calculate RBB for the positive predictor gene ANXA3. **B** – Classification performance of ANXA3/SRGN. **C** - Box plot distribution of RBB (using NCF) to calculate RBB for the positive predictor gene ANXA3. **D** - Classification performance of ANXA3/NCF1.

Figure 9 - 2-Dimensional (2-D) plots showing 2 RBBs for the classification of KD. MoM of log (ANXA3/SAT1), a positive predictor RBB is plotted against MoM of log (FCER1A/SAT1), a negative predictor RBB.

Figure 10 - PCA biplot of ALRs for viral disease (VD) and controls. The PCA has been rotated to maximise the separation of VD and control along PC1 in this biplot. SAT1 is used as the OP reference gene. Each ALR label represents the apex of that ALR loading vector in the biplot. An example vector of the RBB (RSAD2/SAT1 which largely overlap with IFIT1/SAT1 vector) is shown as the arrow-line in the biplot. As labels of some vectors overlap, they are enlarged for display in the inset at the top right corner.

Figure 11 - Box plots and ROC of RBBs using 7 different predictor genes and NCF1 as denominator of dataset GSE73461. **A** - Box plots and ROC of PI3/NCF1. **B** - Box plots and ROC of ADGRE3/NCF1. **C** - Box plots and ROC of HERC5/NCF1. **D** - Box plots and ROC of IFIT1/NCF1. **E** - Box plots and ROC of RSAD2/NCF1. **F** - Box plots and ROC of IFIT3/NCF1.

Figure 12 - Box plots and ROC of RBBs using 7 different predictor genes and SAT1 as denominator of dataset GSE73461. **A** - Box plots and ROC of PI3/SAT1. **B** - Box plots and ROC of ADGRE3/SAT1. **C** - Box plots and ROC of HERC5/SAT1. **D** - Box plots and ROC of IFIT1/SAT1. **E** - Box plots and ROC of RSAD2/SAT1. **F** - Box plots and ROC of IFIT3/SAT1.

Figure 13 - 2D plots using only samples of 2 disease groups in GSE68004 and GSE73461 (KD and VD). RBBs using SAT1 as OP reference genes are used to delineate KD vs VD in two sets of 2D plots. **A** is ANXA3/SAT1 vs RSAD2/SAT1 and for classification, a linear boundary is used. **B** is an example using ANXA31/SAT1 vs LY6E/SAT1 illustrating that, for classification, a rectangular boundary is used.

Figure 14 - Classification by 2 granulocyte RBBs of febrile patients into group 1 or group 2 diseases using pooled samples of datasets in Table 21. **A** - shows 2D plot of 2 RBBs, MoM of log (RSAD2/SAT1) vs MoM of log (ANXA3 / SAT1). Various classification boundaries can be used, for example, a linear boundary (dash line) or alternatively, regional boundary (rectangle box) or a combination of them. **B** - shows the classification performance of using a naïve Bayes classification

Figure 15 - Classification performance of using 4 RBBs (that were derived from granulocytes and monocyte gene lists) of febrile patients into group 1 or group 2 diseases using pooled samples of datasets in Table 21. Monocyte RBBs used in **15A** and **15B** show a plot or confusion matrix for IFI27/PSAP and VNN1/PSAP, while that in **15C** and **15D** show a plot or confusion matrix for IFI44L/PSAP and VNN1/PSAP.

Figure 16 - 2D plots of RSAD2/SAT1 vs a negative predictor RBB. **A** – 2D plot of RSAD2/SAT1 vs CCR3/SAT1. **B** – Confusion matrix of RSAD2/SAT1 vs CCR3/SAT1. **C** – 2D plot of RSAD2/SAT1 vs CCN3/SAT1. **D** – Confusion matrix of RSAD2/SAT1 vs CCN3/SAT1

Figure 17 - 2D plots of RBBs that can differentiate patients with active TB from viral disease within the Group 2 diseases using GSE100150 dataset. These RBBs are (A) ANKRD22/SAT1, (B) SERPING1/SAT1, (C) BATF2/SAT1.

Figure 18 – **A** – 3D plot using ANKRD22/NCF1 (as x axis), FCER1A/NCF1 (as y-axis) and RSAD2/NCF1 (as z-axis) to differentiate between samples of the Group 2 Interferon activating diseases (NCF1 was used as the OP reference gene). Viral disease can be separated from SLE and Tuberculosis. **B** - ROC curves of classification of the 3 diseases by a naïve Bayesian method. **C** - the confusion matrix when using 3 RBBs, ANKRD22/NCF1, FCER1A/NCF1 and RSAD2/NCF1.

Figure 19 – **A** - 2D plot of MoM log (ANXA3 / SAT1) vs MoM (CEACAM8 / SAT1). **B** - the confusion matrix when using ANXA3 / SAT1 and CEACAM8 / SAT1. **C** - 2D plot of MoM log

(ANXA3 / SAT1) vs MoM (CAMP / SAT1). D - the confusion matrix when using ANXA3 / SAT1 and CAMP/ SAT1. E - 2D plot of MoM log (IFITM3 / SAT1) vs MoM (CEACAM8 / SAT1).

Figure 20 – A - t-SNE plot using multiple RBBs to differentiate between group 1 and group 2 diseases. **B** – UMAP plot using multiple RBBs to differentiate between various diseases within group 2 (interferon activating diseases) - VD, SLE and TB.

Figure 21 – Schematic of a computer system Client-server collaboration to determine the post-test odd.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, fever caused by a wide range of different underlying diseases or conditions exhibits substantially the same clinical symptoms, in terms of the subject's elevated temperature. This similarity of symptoms, caused by markedly different underlying causes, poses great difficulties for those seeking to determine the reason for a subject's fever, and so a suitable course of treatment. The various methods, kits, methods of treatment and medical uses of the invention address the long-standing need to improve identification and differentiation between diseases or conditions causing fever and clinical care in respect of patients with fever.

The invention will now be further described with reference to the abbreviations and definitions set out below.

Abbreviations

2D – 2-dimensional

3D – 3-dimensional

ALR - Additive log ratios

AUC – Area under the curve

cDNA – Complementary DNA

CoDa - Compositional data analysis

DEG – Differentially expressed genes

DNA - Deoxyribonucleic acid

ELISA - Enzyme-linked immunosorbent assay

HPA – Human Protein Atlas

KD – Kawasaki Disease

LAMP - Loop-mediated isothermal amplification

LDA - Linear Discriminant Analysis
LR – Likelihood ratios
LRA - Log-ratio analysis
MoM - Multiple of control group median
OP – Origin Proxy
PBMC - Peripheral blood mononuclear cells
PCA - Principal component analysis
PCR - Polymerase chain reaction
RBB – Ratio based biomarker
RNA - Ribonucleic acid
SLE - Systemic lupus erythematosus
TB - Tuberculosis
TPM – Transcript per million
t-SNE - t-Distributed Stochastic Neighbour Embedding
UMAP - Uniform Manifold Approximation and Projection
VD – Viral disease
WB – Whole blood

Distinguishing between diseases or conditions or determining the presence of a disease or condition

The first to seventh aspects of the present invention relate to methods for distinguishing between diseases or conditions in a subject with fever. The eighth to tenth aspects of the invention relate to methods for determining the presence of a disease or condition in a subject with fever.

For the purposes of the present invention both methods for distinguishing and methods for determining may be taken as providing methods that facilitate or enable attribution of the cause of the subject's fever. As explained further below, such methods may be used for purposes that are not diagnostic in their character.

In the case of a method for “distinguishing” (of the first to seventh aspects of the invention), the cause of the fever may be attributed to one of two or more alternative causes. These causes may be categories of diseases (such as non-interferon activating diseases or interferon activating diseases referred to in the first aspect of the invention, or the bacterial infections referred to in the third aspect of the invention), groups of diseases (such as the group of diseases consisting of: viral disease and SLE, referred to in the fourth aspect of the

invention), or specific diseases (such as SLE or TB, as considered in the seventh aspect of the invention).

In the case of a method for “determining” (of the eighth to tenth aspects of the invention), the method enables attribution of whether or not the fever is caused by a specific disease or condition. For example, such a method may allow attribution of whether or not a fever is caused by Kawasaki disease. Alternatively, such a method may allow attribution of whether or not a fever is caused by a viral disease. Still further, such a method may allow attribution of whether or not a fever is caused by active TB.

Methods of distinguishing between diseases or conditions, or of determining the presence of a disease or condition, may be useful in arriving at a clinical decision in respect of the subject with fever. Such methods may be suitable for contributing to a clinical decision (which is to say, the method of the invention is not the only method taken into consideration when arriving at a clinical decision), though the methods themselves may be non-diagnostic in character.

Alternatively, such methods may be suitable for enabling a clinical decision to be taken (which is to say, the method of the invention may be the only method taken into consideration when arriving at a clinical decision, though the results of the method of the invention may be confirmed by other means as required).

Merely by way of example, a suitable clinical decision may comprise indicating that a particular disease or condition, or group of diseases or conditions, is likely to be the cause of a subject's fever. In an embodiment, a suitable clinical decision may comprise identifying a particular disease or condition, or group of diseases or conditions, as the cause of the subject's fever.

Alternatively, a suitable clinical decision may comprise indicating that a particular disease or condition, or group of diseases or conditions, is unlikely to be the cause of a subject's fever. In an embodiment, a suitable clinical decision may comprise eliminating a particular disease or condition, or group of diseases or conditions, as the cause of the subject's fever.

The methods of the invention may be useful in contributing to a clinical decision in respect of treatment, or management of a subject's disease or condition. The methods of the invention may allow a clinical decision to be made in respect of the diagnosis, treatment, or management of a subject's disease or condition.

A method of the invention may contribute to the selection of a suitable empirical treatment regimen before a definitive diagnosis is established in respect a subject's fever. It will be recognised that definitive diagnosis often requires lengthy laboratory procedures. If treatment is deferred until these have been completed, an opportunity for early clinical intervention may be lost, which may have adverse impact upon the subject. In contrast, a method of the invention may allow rapid triage of a subject with fever, and thus selection of a suitable treatment regimen in respect of a subject's fever to be made, even before a diagnosis has been reached. This avoids unnecessary delay and allows appropriate and focused management of the subject's illness to start at the earliest possible timepoint. A selection of a treatment regimen contributed to by a method of the invention, or made on the basis of such a method, may be subject to confirmation by alternative means or methods, as considered further below.

A method of the invention used in the selection of a treatment regimen, such as an empirical treatment regimen, may further comprise a step of providing the selected treatment regimen to the subject. Furthermore, the invention provides medicaments for use in regimens for the treatment of fever, wherein the medicament and/or the treatment regimen has been selected by a method in accordance with the present invention.

A method of the invention may contribute to the selection of a suitable plan for the management of a subject's fever. In a suitable embodiment, a method of the invention may allow selection of a suitable plan for the management of a subject's fever to be made. A plan for management of a subject's fever contributed to by a method of the invention, or made on the basis of such a method, may be subject to confirmation by alternative means or methods, as considered further below.

In suitable embodiment, a method of the invention may further comprise an additional confirmation step. As used herein, a further confirmatory step refers to an additional test, procedure, or clinical assessment performed after application of at least one aspect of a method of the invention to provide additional evidence or certainty regarding the initial finding(s) of the method of the invention. A confirmation step may employ any suitable method which can be used to confirm the initial finding(s) of the method of the invention. Merely by way of example, a suitable confirmation step may comprise performing confirmatory laboratory cultures. A suitable confirmation step may comprise performing confirmatory imaging scans. A suitable confirmation step may comprise confirmatory genetic sequencing.

Diseases or conditions

The first to eleventh aspects of the invention provide methods for distinguishing between various diseases and conditions or determining the presence of a disease or condition. In order to triage fever patients, various causes of febrile illness are divided into two main groups based on the characteristic RBBs deranged in each of the diseases as revealed by this invention. These diseases and conditions are as discussed below.

Non-interferon activating disease

Non-interferon activating disease (also referred to as “group 1” or “G1” in figures and tables) refers to a group of diseases or conditions that encompasses infectious diseases due to recognised pathogens such as bacterial infection and systemic fungal infection and non-infectious diseases without a well-characterised pathogen such as Kawasaki disease. These diseases are characterised in that they do not cause a predominant activation of the interferon pathways. For the purposes of the present invention, a suitable non-interferon activating disease may be selected from the group consisting of: a bacterial infection, Kawasaki disease; and a systemic fungal infection.

Thus, in a suitable embodiment, the non-interferon activating disease is a bacterial infection. Alternatively, in a suitable embodiment the non-interferon activating disease is Kawasaki disease. In a further suitable embodiment, the non-interferon activating disease is a systemic fungal infection.

For the purposes of the present invention, a finding that a subject is “distinguished as having a non-interferon activating disease” may be taken as identifying that the subject’s fever is caused by a non-interferon activating disease. In particular, such a finding may be taken as identifying that the subject’s fever is caused by a bacterial infection, by Kawasaki disease, or by a systemic fungal infection.

The methods and kits of the invention may be used in selecting a regimen for treatment of a non-interferon activating disease. The methods and kits of the invention may be used in selecting a plan for management of a non-interferon activating disease.

Kawasaki disease

Kawasaki disease (KD) is a serious autoimmune disease which primarily affects children aged 5 and under. KD is an acute, medium- and small-sized vasculitis characterized by

inflammation of the blood vessels throughout the body, particularly those in the coronary arteries.

The exact cause of Kawasaki disease is unknown, but it is believed to involve an abnormal immune response triggered by an infection or other environmental factors.

KD is particularly prevalent in Japan with an annual incidence of 120-180 cases per 100,000 in children less than 4 years of age. In the United States, the incidence of Kawasaki disease has been best estimated as 9-20 per 100,000 in children less than 5 years of age.

For the purposes of the present invention, a finding that a subject is “distinguished as having Kawasaki disease” may be taken as identifying that the subject’s fever is caused by Kawasaki disease.

The methods and kits of the invention may be used in selecting a regimen for treatment of Kawasaki disease. The methods and kits of the invention may be used in selecting a plan for management of Kawasaki diseases.

Bacterial infection

As described herein, bacterial infection refers to any infection caused by the presence of bacteria. In particular, a suitable bacterial infection may be caused by pyogenic bacteria, infection with which involves the production of pus.

Suitably, a bacterial infection may be an infection with bacteria independently selected from the group consisting of: *Staphylococcus aureus*; *Streptococcus pyogenes*; and *Pseudomonas aeruginosa*.

For the purposes of the present invention, a finding that a subject is “distinguished as having bacterial infection” may be taken as identifying that the subject’s fever is caused by a bacterial infection.

The methods and kits of the invention may be used in selecting a regimen for treatment of a bacterial infection. The methods and kits of the invention may be used in selecting a plan for management of a bacterial infection.

Systemic fungal infection

Systemic fungal infection, also known as systemic mycosis, refers to an infection caused by fungi that spreads throughout the body and affects multiple organ systems. Unlike localized fungal infections that primarily affect the skin, nails, or mucous membranes, systemic fungal infections are characterized by the invasion of fungi into the bloodstream and dissemination to various organs.

In a suitable embodiment, the systemic fungal infection is Candidemia.

For the purposes of the present invention, a finding that a subject is “distinguished as having systemic fungal infection” may be taken as identifying that the subject’s fever is caused by systemic fungal infection.

The methods and kits of the invention may be used in selecting a regimen for treatment of a systemic fungal infection. The methods and kits of the invention may be used in selecting a plan for management of a systemic fungal infection.

Interferon activating disease

Interferon activating diseases (also referred to as “group 2” or “G2” in figures and tables) refer to a group of disorders characterized by the predominant activation of the interferon pathways. For the purposes of the present invention a suitable interferon activating disease may be selected from the group consisting of: active tuberculosis (TB); systemic lupus erythematosus (SLE); and a viral disease.

Thus, in a suitable embodiment, the interferon activating disease is a viral disease. Alternatively, in a suitable embodiment the interferon activating disease is active TB. In a further suitable embodiment, the interferon activating disease is SLE.

For the purposes of the present invention, a finding that a subject is “distinguished as having an interferon activating disease” may be taken as identifying that the subject’s fever is caused by an interferon activating disease. In particular, such a finding may be taken as identifying that the subject’s fever is caused by active TB, by SLE, or by viral disease.

The methods and kits of the invention may be used in selecting a regimen for treatment of an interferon activating disease. The methods and kits of the invention may be used in selecting a plan for management of an interferon activating disease.

Viral disease

For the purposes of the present disclosure, references to a viral disease may be taken as encompassing any viral disease which causes the activation of interferons during the immune response.

It will be appreciated that a viral disease which result in interferon activation may be caused by a wide variety of viruses. Merely by way of example, a virus capable of causing an interferon activating disease may be selected from the group consisting of: an influenza virus; a herpes simplex virus; a hepatitis C virus; and a vaccinia virus.

Thus, in a suitable embodiment the viral disease is caused by an influenza virus. Alternatively, in a suitable embodiment the viral disease is caused by a herpes simplex virus. In a further suitable embodiment, the viral disease is caused by a hepatitis C virus. In a still further suitable embodiment, the viral disease is caused by a vaccinia virus.

For the purposes of the present invention, a finding that a subject is “distinguished as having viral disease” may be taken as identifying that the subject’s fever is caused by a viral disease.

The methods and kits of the invention may be used in selecting a regimen for treatment of a viral disease. The methods and kits of the invention may be used in selecting a plan for management of a viral disease.

Active Tuberculosis (TB)

Active TB is an infectious disease of the lungs caused by bacteria such as *Mycobacterium tuberculosis*, and other species in the *Mycobacterium tuberculosis* complex.

Tuberculosis infection may exist in latent or active forms. Estimates suggest that up to a quarter of the world’s population may have latent TB. Approximately 10% of latent TB infections progress to active TB, and around half of active TB infections prove deadly if untreated. The active form of TB is known to activate host interferons, thereby being suitable for classification as an interferon activating disease for the purposes of the present invention.

The *Mycobacterium tuberculosis* complex comprises multiple species of *Mycobacterium*, all of which are capable of causing activation of interferons and hence all species are suitable for

classification as interferon activating. In a suitable embodiment, TB is caused by *Mycobacterium tuberculosis*. In a suitable embodiment, TB is caused by *Mycobacterium bovis*.

For the purposes of the present invention, a finding that a subject is “distinguished as having active TB” may be taken as identifying that the subject’s fever is caused by active TB.

The methods and kits of the invention may be used in selecting a regimen for treatment of active tuberculosis. The methods and kits of the invention may be used in selecting a plan for management of active tuberculosis.

Systemic lupus erythematosus (SLE)

SLE is a common worldwide chronic autoimmune disease which may affect every organ and tissue. The exact cause of SLE is still unknown but genetic predisposition, environmental triggers, and the hormonal milieu, interplay in disease development and activity.

For the purposes of the present invention, a finding that a subject is “distinguished as having SLE” may be taken as identifying that the subject’s fever is caused by SLE.

The methods and kits of the invention may be used in selecting a regimen for treatment of SLE. The methods and kits of the invention may be used in selecting a plan for management of SLE.

A subject with fever

The methods in accordance with the first to eleventh aspects of the invention are practiced in respect of a subject with fever. A subject with fever is one who is experiencing an elevated body temperature, as a result of an underlying medical condition or disease.

In a suitable embodiment, the subject is human. In a suitable embodiment, a subject is an adult. Suitably, in such an embodiment a subject is an individual who is 18 years old or older.

In a separate embodiment, a subject is a child. In a suitable example, a subject may be a child who is aged 17 or younger. In a suitable embodiment, a subject may be a child aged 5 years or younger.

In a suitable embodiment, the subject is a child aged 5 or under the and has clinical features, including fever, suggestive of Kawasaki disease. Embodiment of this sort, in which the subject is a child aged 5 or under, optionally with clinical features suggestive of Kawasaki disease, are particularly relevant in respect of methods in accordance with the second, third, fourth and eighth aspects of the invention.

In a suitable embodiment, the subject with fever is under the care of a clinician.

In a suitable embodiment, the subject has a fever with an unknown cause. In such an embodiment the subject may require a method by which the cause of their fever (which is to say the disease or condition responsible for the fever) may be distinguished from a number of alternative causes. In an embodiment of this sort, the subject may require identification of the cause of their fever, and a method of the invention may be used to provide or facilitate such an identification. Alternatively, or additionally, the subject may require selection of a regimen for treatment of their fever, and a method of the invention may be used to provide or facilitate such a selection. Alternatively, or additionally, the subject may require selection of a plan for management of their fever, and a method of the invention may be used to provide or facilitate such a selection.

In an alternative embodiment, the cause of a subject's fever may already have been identified. in which case a method of the invention may be useful in providing confirmation of the disease or condition causing the fever.

While the methods of the invention are well suited to use in human subjects, in a suitable embodiment, the subject is non-human. Suitably, the subject is an animal. Merely by way of example, the subject may be selected from the group consisting of: a cat; a dog; a bird; a reptile; and a fish.

Samples representative of gene expression

The methods in accordance with first to eleventh aspects of the invention are carried out using samples representative of gene expression in a subject. For the purposes of the present invention, a sample representative of gene expression in a subject may be any sample that suitable for providing information regarding gene expression within the subject in question.

In particular, the genes used in the RBBs employed in the methods and kits of the invention are host response genes. Accordingly, a suitable sample representative of gene expression

in a subject may be any sample that suitable for providing information regarding host response gene expression within the subject in question. The use of host response genes in this manner is advantageous, in that expression of host response genes is normally more readily detected than expression of genes by a pathogenic organism (for example a bacterium, fungus or virus associated with disease).

Suitably a sample representative of gene expression in a subject contains one or more target molecules indicative of expression of one or more corresponding gene(s).

A suitable target molecule indicative of gene expression may be a target molecule directly representative of gene expression. For example, a target molecule directly representative of gene expression may be an RNA target molecule. As used herein, an RNA target molecule may be any RNA sequence or structure, such as an RNA transcript, indicative of the expression of at least one of the genes which makes up an RBB disclosed in the present invention (the list of genes set out in Table 20).

Alternatively, a suitable target molecule indicative of gene expression may be a target molecule indirectly representative of gene expression. For example, a target molecule indirectly representative of gene expression may be a protein encoded by the gene in question. As used herein, a protein target molecule may be any protein, or fragment thereof, indicative of the expression of at least one of the genes which makes up an RBB disclosed in the present invention (the list of genes set out in Table 20).

Suitably, a sample representative of gene expression in a subject provides information about the gene expression levels within a specific context such as a subject suffering from a disease or condition. It will be recognised that gene expression may vary under different conditions, for example in different diseases. A sample may be obtained under the conditions relevant to the context to be investigated. In a suitable embodiment, a sample representative of gene expression is obtained from a subject with fever. In a suitable embodiment, a sample representative of gene expression is obtained from a healthy subject.

A suitable sample may be representative of gene expression in a subject with a first disease. A suitable sample may be representative of gene expression in a subject with a second disease. A suitable sample may be representative of gene expression in a subject with a third disease.

A suitable sample may be representative of gene expression in a subject without the first disease. A suitable sample may be representative of gene expression in a subject without the second disease. A suitable sample may be representative of gene expression in a subject without the third disease. A suitable sample may be representative of gene expression in a healthy control.

A suitable sample representative of gene expression in a subject may be selected with reference to the form of target molecule required for use in a method of the invention (for example, with the aim of using an RNA target molecule, or with the aim of using a protein target molecule).

In a suitable embodiment, a sample is a biological sample. Any biological sample that provides information regarding gene expression in a subject that is useful for the determination of the presence of a disease or condition in a subject with fever, or for distinguishing the presence of a disease or condition in a subject with fever, may be used.

Suitably a sample comprises biological cells. A sample representative of gene expression in a subject may comprise a plurality of different types of biological cells. Gene expression may be assessed with reference to some or all of the cell types present.

Alternatively, a suitable sample may comprise a single cell type. Such a sample may initially comprise a plurality of different cell types, before being processed to exclude unwanted cell types. In embodiments of this sort, gene expression may be assessed with reference to the single cell type present. Investigating a single cell type relevant to a particular disease or condition (for example a white blood cell of a type involved in the response to the disease or condition) can advantageously increase the specificity and accuracy of the methods of the invention.

In a suitable embodiment a sample representative of gene expression comprises one or more types of biological cells selected from the group consisting of: granulocytes (such as neutrophils, eosinophils, basophils, or mast cells) lymphocytes, monocytes, macrophages, T cells, B cells, dendritic cells, natural killer cells, and platelets.

In a suitable embodiment, a sample representative of gene expression in a subject comprises blood cells from which target molecules are obtained. In a suitable embodiment a sample representative of gene expression in a subject comprises neutrophils from which target

molecules are obtained. In a suitable embodiment, only target molecules obtained from neutrophils are investigated.

By way of a non-limiting example, such samples comprising biological cells may be selected from the group consisting of: a blood sample; a tissue biopsy; a saliva sample; or a sputum sample. Suitably, the sample representative of gene expression in the subject is a blood sample. In a suitable embodiment, the blood sample is a sample of whole blood.

A sample may be obtained by any appropriate means. Once a desired form of sample has been selected, a skilled person will readily identify appropriate means by which the sample may be obtained. By way of a non-limiting example, a sample may be obtained by blood draw, biopsy, skin swab, collection of urine or stool, or a lumbar puncture.

Analysing gene expression in a sample

Gene expression may be analysed by any appropriate method. Merely by way of example, gene expression may be analysed by quantifying the amount of target molecules representative of gene expression present within a sample. Suitable target molecules may be directly representative of gene expression, such as RNA transcripts, or indirectly representative of gene expression, such as proteins.

In a suitable embodiment, a reagent capable of specifically binding to a target molecule is added to a sample, to allow binding between the target molecule (if present) and reagent. The extent of binding occurring may be quantified, to provide an indication of the level of expression of a gene of interest (which for the present purposes may be a gene in an RBB) in the sample.

Suitably, gene expression is analysed by investigating an RNA target molecule. Analysis of gene expression using RNA target molecules is also known as transcriptomics.

Suitably, gene expression is analysed by investigating a protein target molecule. Analysis of gene expression using protein target molecules is also known as proteomics.

The skilled person will be aware of many methods that may be employed in transcriptomics or proteomics, and any of these may suitably be used to analyse gene expression in the context of the present invention. The method may be quantitative, and approaches by which

such techniques may be used quantitatively will be known to those seeking to practice the invention.

Merely by way of example, in the case of a technique making use of an RNA target molecule, gene expression may suitably be analysed by a technique selected from the group consisting of: qPCR, digital PCR, RNAseq, a DNA microarray assay, a DNA hybridization assay, a nanostring assay, and an enzyme-mediated quantification assay (such as Loop-mediated isothermal amplification – LAMP).

In the case of a technique making use of a protein target molecule, gene expression may suitably be analysed by a technique selected from the group consisting of: western blotting, flow cytometry and ELISA.

In a suitable embodiment of a method in accordance with the first to eleventh aspects of the present invention, gene expression is analysed by investigating a single cell type or subpopulation of blood cells in peripheral blood. In a suitable embodiment, gene expression is analysed by investigating peripheral blood neutrophils.

Ratio-based biomarkers

A ratio-based biomarker (RBB) as referred to herein is a biomarker that is a ratio between expression levels of two or more genes. The invention describes methods in which such RBBs may be used to distinguish between different diseases or conditions, and methods in which such RBBs may be used to determine the presence of a particular disease or condition in a subject, particularly in a subject with a fever. The invention also describes methods by which genes for use in such RBBs may be identified.

Although reference genes, commonly housekeeping genes, have previously been used in RBBs, their prior use has merely been to normalize the amount of RNA used in quantification experiment. Accordingly, they have not previously been used for any statistical property, as they are in the present invention.

The use of RBBs in the methods of the invention offer a number of notable advantages which are discussed elsewhere in this specification.

An RBB of use in the present invention comprise at least one numerator gene and at least one denominator gene. Suitably an RBB of use in the present invention comprises a single numerator gene and a single denominator gene.

As described further below, comparison of RBBs (particularly in respect of which RBB is higher, and which lower) enables different diseases or conditions to be distinguished, or their presence determined.

Numerator genes

The term “numerator gene,” as used herein, refers to one of the genes involved in the ratio calculation in an RBB. The numerator gene is selected based on the gene’s relevance to the disease or condition being studied and the level of expression of the numerator gene can discriminate between diseased and non-diseased states.

The inventors of the present invention have identified a plurality of numerator genes useful in methods of the invention as shown in Table 21.

A numerator gene may be a positive predictor of a disease. A “positive predictor” as used herein refers to a numerator gene whose expression is associated with an increased likelihood the presence a particular disease or condition. Details of numerator genes that may be used as positive predictors in specific embodiments of the methods or kits of the invention are set out elsewhere in this specification.

A numerator gene may be a negative predictor of a disease. A “negative predictor” as used herein refers to a numerator gene whose expression is associated with a decreased likelihood the presence a particular disease or condition. Details of numerator genes that may be used as negative predictors in specific embodiments of the methods or kits of the invention are set out elsewhere in this specification.

ANXA3 encodes for the protein Annexin A3, a calcium-dependent phospholipid-binding protein. ANXA3 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ANXA3 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, ANXA3 is a positive predictor gene for non-interferon activating disease. In a further example, ANXA3 may be used as the numerator gene in a method in accordance with the second aspect of the present

invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, ANXA3 may be used as a numerator gene in a method in accordance with the eighth aspect of the invention for determining the presence of Kawasaki disease. In a suitable embodiment, ANXA3 is a positive predictor gene for Kawasaki disease.

ALPL encodes a member of the alkaline phosphatase family of proteins. ALPL may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ALPL may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, ALPL is a positive predictor gene for non-interferon activating disease. In a further example, ALPL may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, ALPL may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, ALPL is a positive predictor gene for Kawasaki disease.

IL1R2 encodes a cytokine receptor that belongs to the interleukin 1 receptor family. IL1R2 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, IL1R2 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a further example, IL1R2 may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, IL1R2 may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, IL1R2 is a positive predictor gene for Kawasaki disease.

ARG1 encodes for arginase 1 which catalyses the hydrolysis of arginine to ornithine and urea. ARG1 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ARG1 may be used as a numerator gene in a method in accordance with methods of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, ARG1 is a positive predictor gene for non-interferon activating disease. Suitably, ARG1 may be used as a numerator gene in a

method in accordance with methods of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, ARG1 is a positive predictor gene for Kawasaki disease.

MMP9 encodes for a matrix metalloproteinase (MMP) family member involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in disease processes, such as arthritis and metastasis. MMP9 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, MMP9 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, MMP9 is a positive predictor gene for non-interferon activating disease. In a further example, MMP9 may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, MMP9 may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, MMP9 is a positive predictor gene for Kawasaki disease.

FCER1A encodes for an immunoglobulin epsilon receptor (IgE receptor) which is the initiator of the allergic response. FCER1A may be used as a numerator gene in methods or kits of the invention. By way of example, FCER1A may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, FCER1A may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, FCER1A is a negative predictor gene for Kawasaki disease. Furthermore, FCER1A may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable example, FCER1A may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE and viral disease. Alternatively, FCER1A may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. In a suitable embodiment, FCER1A is a negative predictor gene for SLE. In a suitable embodiment, FCER1A is a negative predictor gene for viral disease. In a suitable example, FCER1A expression in a subject with SLE is minimally expressed.

CCR3 encodes for a receptor for C-C type chemokines. CCR3 may be used as a numerator gene in methods or kits of the invention. By way of example, CCR3 may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, CCR3 may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, CCR3 is a negative predictor gene for Kawasaki disease. In a suitable embodiment, CCR3 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable example, CCR3 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE and viral disease. Alternatively, CCR3 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. In a suitable embodiment, CCR3 is a negative predictor gene for SLE. In a suitable embodiment, CCR3 is a negative predictor gene for viral disease. In a suitable example, CCR3 expression in a subject with SLE is minimally expressed.

HDC encodes a member of the group II decarboxylase family and forms a homodimer that converts L-histidine to histamine in a pyridoxal phosphate dependent manner. HDC may be used as a numerator gene in methods or kits of the invention. By way of example, HDC may be used as the numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. Suitably, HDC may be used as a numerator gene in a method in accordance with the eighth aspect of the present invention for determining the presence of Kawasaki disease. In a suitable embodiment, HDC is a negative predictor gene for Kawasaki disease. In a suitable embodiment, HDC may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable example, HDC may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE and viral disease. Alternatively, HDC may be used as a numerator gene in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. In a suitable embodiment, HDC is a negative predictor gene for SLE. In a suitable embodiment, HDC is a negative predictor gene for viral disease. In a suitable example, HDC expression in a subject with SLE is minimally expressed.

IFIT1 encodes for an interferon induced protein which may inhibit viral replication and translational initiation. IFIT1 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, IFIT1 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, IFIT1 is a positive predictor gene for interferon activating disease. In a further example, IFIT1 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, IFIT1 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, IFIT1 may be used as a numerator gene in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, IFIT1 may be used as a numerator gene in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, IFIT1 may be used as a numerator gene in accordance with the ninth aspect of the present invention for determining the presence of viral disease. In a suitable embodiment, IFIT1 is a positive predictor gene for a viral disease.

RSAD2 encodes an interferon-inducible antiviral protein that belongs to the S-adenosyl-L-methionine (SAM) superfamily of enzymes. RSAD2 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, RSAD2 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, RSAD2 is a positive predictor gene for interferon activating disease. In a further example, RSAD2 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, IFIT1 may be used as a numerator gene in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, RSAD2 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable embodiment, RSAD2 may be used as a numerator gene in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, RSAD2 may be used as a numerator gene in a method in accordance with the ninth aspect of the present invention for determining the presence of viral disease. In a suitable embodiment, RSAD2 is a positive predictor gene for a viral disease.

IFIT3 encodes an interferon induced protein which enables identical protein binding activity. IFIT3 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, IFIT3 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, IFIT3 is a positive predictor gene for interferon activating disease. In a further example, IFIT3 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, IFIT3 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, IFIT3 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable embodiment, IFIT3 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, IFIT3 may be used as a numerator gene in a method in accordance with the ninth aspect of the present invention for determining the presence of viral disease. In a suitable embodiment, IFIT3 is a positive predictor gene for a viral disease.

HERC5 encodes a member of the HERC family of ubiquitin ligases. HERC5 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, HERC5 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, HERC5 is a positive predictor gene for interferon activating disease. In a further example, HERC5 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, HERC5 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, HERC5 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, HERC5 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, HERC5 may be used as a numerator gene in a method in accordance with the ninth aspect of the present invention for determining the

presence of viral disease. In a suitable embodiment, HERC5 is a positive predictor gene for a viral disease.

PI3 encodes an elastase-specific inhibitor that functions as an antimicrobial peptide against Gram-positive and Gram-negative bacteria, and fungal pathogens. PI3 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, PI3 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, PI3 is a negative predictor gene for interferon activating disease. In a further example, PI3 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, PI3 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, PI3 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, PI3 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, PI3 may be used as a numerator gene in a method in accordance with the ninth aspect of the present invention for determining the presence of viral disease. In a suitable embodiment, PI3 is a negative predictor gene for a viral disease.

ADGRE3 encodes a member of the class B seven-span transmembrane (TM7) receptor family expressed predominantly by cells of the immune system. ADGRE3 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ADGRE3 may be used as a numerator gene in a method in accordance with the first aspect of the present invention for distinguishing between non-interferon activating disease and interferon activating disease. In a suitable embodiment, ADGRE3 is a negative predictor gene for interferon activating disease. In a further example, ADGRE3 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). In a suitable embodiment, ADGRE3 may be used as a numerator gene in a method in accordance with the fifth aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, ADGRE3 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, ADGRE3 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between

SLE, viral disease, and active TB. Suitably, ADGRE3 may be used as a numerator gene in a method in accordance with the ninth aspect of the present invention for determining the presence of viral disease. In a suitable embodiment, ADGRE3 is a negative predictor gene for a viral disease.

ANKRD22 encodes an Ankyrin Repeat Domain-Containing Protein. ANKRD22 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ANKRD22 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, ANKRD22 is a positive predictor gene for systemic fungal infection. In a further example, ANKRD22 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, ANKRD22 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, ANKRD22 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, ANKRD22 may be used as a numerator gene in a method in accordance with the tenth aspect of the invention for determining the presence of TB. In a suitable embodiment, ANKRD22 is a positive predictor gene for active TB.

BATF2 encodes a basic leucine zipper transcriptional factor which enables DNA-binding transcription factor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. BATF2 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, BATF2 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, BATF2 is a positive predictor gene for systemic fungal infection. In a suitable embodiment, BATF2 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, BATF2 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable embodiment, BATF2 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, BATF2 may be used

as a numerator gene in a method in accordance with the tenth aspect of the invention for determining the presence of TB. In a suitable embodiment, BATF2 is a positive predictor gene for active TB.

ETV7 encodes a member of the ETS family of transcription factors, which is a large group of evolutionarily conserved transcriptional regulators that play an important role in a variety of cellular processes throughout development and differentiation and are involved in oncogenesis as well. ETV7 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, ETV7 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, ETV7 is a positive predictor gene for systemic fungal infection. In a suitable embodiment, ETV7 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, ETV7 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable embodiment, ETV7 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, ETV7 may be used as a numerator gene in a method in accordance with the tenth aspect of the invention for determining the presence of TB. In a suitable embodiment, ETV7 is a positive predictor gene for active TB.

GBP5 encodes a member of the TRAFAC class dynamin-like GTPase superfamily. GBP5 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, GBP5 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, GBP5 is a positive predictor gene for systemic fungal infection. In a suitable embodiment, GBP5 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, GBP5 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable example, GBP5 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, GBP5 may be used as a numerator gene in a method in accordance with the tenth aspect of the invention for

determining the presence of TB. In a suitable embodiment, GBP5 is a positive predictor gene for active TB.

SERPING1 encodes a highly glycosylated plasma protein involved in the regulation of the complement cascade. SERPING1 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, SERPING1 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, SERPING1 is a positive predictor gene for systemic fungal infection. In a suitable embodiment, SERPING1 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, SERPING1 may be used as a numerator gene in a method in accordance with the sixth aspect of the invention for distinguishing between viral disease and active TB. In a suitable embodiment, SERPING1 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. Suitably, SERPING1 may be used as a numerator gene in a method in accordance with the tenth aspect of the invention for determining the presence of TB. In a suitable embodiment, SERPING1 is a positive predictor gene for active TB.

CEACAM8 encodes a carcinoembryonic antigen-related cell adhesion molecule which enables protein heterodimerization activity. CEACAM8 may be used as a numerator gene in methods or kits of the invention. Merely by way of example, CEACAM8 may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, CEACAM8 is a positive predictor gene for systemic fungal infection. In a suitable embodiment, CEACAM8 may be used as a numerator gene in a method in accordance with the third aspect of the present invention for distinguishing between bacterial infection and Kawasaki disease. In a suitable embodiment, CEACAM8 is a positive predictor gene for bacterial infection.

CAMP encodes a cathelicidin antimicrobial peptide. CAMP may be used as a numerator gene in methods or kits of the invention. Merely by way of example, CAMP may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of:

bacterial infection and Kawasaki disease. In a suitable embodiment, CAMP is a positive predictor gene for systemic fungal infection.

LTF encodes a member of the transferrin family, an important component of the non-specific immune system. LTF may be used as a numerator gene in methods or kits of the invention. In a suitable embodiment, LTF may be used as a numerator gene in a method in accordance with the second aspect of the present invention for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease. In a suitable embodiment, LTF is a positive predictor gene for systemic fungal infection.

CCN3 encodes a secreted cysteine-rich protein and a member of the CCN family of regulatory proteins. CCN3 may be used as a numerator gene in methods or kits of the invention. In a suitable embodiment, CCN3 may be used as a numerator gene in a method in accordance with the fourth aspect of the invention for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE). Suitably, CCN3 may be used as a numerator in a method in accordance with the fifth aspect of the present invention for distinguishing between SLE and viral disease. Alternatively, CCN3 may be used as a numerator gene in a method in accordance with the seventh aspect of the invention for distinguishing between SLE, viral disease, and active TB. In a suitable embodiment, CCN3 is a negative predictor gene for SLE. In a suitable embodiment, CCN3 is a negative predictor gene for viral disease. In a suitable example, CCN3 expression in a subject with SLE is minimally expressed.

IFITM3 encodes an interferon induced transmembrane protein. Interferon-induced transmembrane (IFITM) proteins are a family of interferon induced antiviral proteins. IFITM3 may be used as a numerator gene in methods or kits of the invention. In a suitable example, IFITM3 may be used as a numerator gene in a method in accordance with the third aspect of the present invention for distinguishing between a bacterial infection and Kawasaki disease. In a suitable embodiment, IFIT3 is a positive predictor gene for Kawasaki disease.

Denominator genes

The term “denominator gene” as used herein refers to the gene other than a numerator gene (as discussed) above involved in the ratio calculation in an RBB. A denominator gene may be referred to elsewhere in the specification as an origin-proxy reference gene (or “OP reference gene”). Origin-proxy reference genes suitable for use as denominator genes in RBB-based

methods may be identified using methods in accordance with the forty-ninth aspect of the invention.

The inventors of the present invention have identified denominator genes as set out in Table 3, that are useful in the methods or kits of the present invention. Notably, the denominator genes set out in Table 3 are distinct from the well-known housekeeping genes which have previously been described for use as reference genes in quantitative PCR.

The use of the denominator genes set out in Table 3 in the methods or kits of the invention offers a number of notable advantages.

A denominator gene used in methods of the invention can act as a common denominator gene. The same denominator gene can be used for each RBB provided in a method of the invention which reduces the number of biomarker genes that need to be quantified during routine application of RBBs. In a suitable embodiment, the same denominator gene is used for each RBB provided in a method of the invention.

Furthermore, in a method in accordance with the eleventh aspect of the invention, a denominator gene can act as the common denominator gene at each step of a triage pathway. The same denominator gene can be used for each RBB provided in the eleventh aspect of the invention. This reduces the number of biomarker genes that need to be quantified during the method, as well as providing the other benefits considered below.

In this regard, it is worth noting that utilizing a common denominator gene confers multiple notable advantages in practice.

When a common gene is used in multiple RBBs, it adds redundancy to the system. Redundancy can increase the robustness and reliability of the RBBs, reducing susceptibility to errors and variations.

Utilizing a common gene in multiple biomarkers can simplify the application of RBBs. Instead of analysing a large number of genes separately, it is possible to focus on a single gene shared by multiple RBBs, thereby reducing the complexity of analysis and comparison.

RBBs based on a common gene can be easier to interpret and communicate to relevant parties, such as clinicians and patients. Since there is a clear link between the RBBs, they can collectively provide a more straightforward picture.

It will be appreciated that different denominator genes may be identified for use in assays based on analysis of gene expression in different cell types. The inventors of the present invention have identified denominator reference genes useful in assays based on analysis of gene expression in granulocytes. The inventors of the present invention have identified denominator reference genes useful in assays based on analysis of gene expression in monocytes.

Details of denominator genes that may be used in the first to eleventh aspects of the invention are set out in Table 3. In a suitable embodiment, the denominator gene of an RBB for use in the present invention (whether in a method, kit, or other aspect) may be selected from Table 3. Suitably embodiment, the denominator gene of an RBB of use in the present invention may be any of those genes set out in Table 3. In a suitable embodiment, the denominator gene of such an RBB is SAT1. Alternatively, the denominator gene is SRGN. In a further example, the denominator gene is NCF1.

SAT1 encodes for Spermine N1-Acetyltransferase 1 which belongs to the acetyltransferase family, and is a rate-limiting enzyme in the catabolic pathway of polyamine metabolism. In a suitable embodiment, SAT1 may be used as the denominator gene for at least one RBB utilised in a method or kit of the invention. In a suitable embodiment, SAT1 may be used as the denominator gene in each RBB (common denominator gene) utilised in a method or kit of the invention. For example, SAT1 may be used as a common denominator gene in each RBB of a method according to the eleventh aspect of the invention.

SRGN encodes a protein best known as a hematopoietic cell granule proteoglycan. In a suitable embodiment, SRGN may be used as the denominator gene for at least one RBB utilised in a method or kit of the invention. In a suitable embodiment, SRGN may be used as the denominator gene in each RBB (common denominator gene) utilised in a method or kit of the invention.

NCF1 encodes a cytosolic subunit of neutrophil NADPH oxidase. In a suitable embodiment, NCF1 may be used as the denominator gene for at least one RBB utilised in a method or kit of the invention. In a suitable embodiment, NCF1 may be used as the denominator gene in each RBB (common denominator gene) utilised in a method or kit of the invention.

Comparison of RBBs

As described herein, “comparing” or “comparison” of a RBB refers to assessing the similarity between the determined ratio of a RBB to one or more of:

- a reference level of the same RBB,
- reference data, or
- the determined ratio of at least one other RBB of interest.

The “at least other RBB of interest” may, for example, refer to a first RBB, a second RBB, or a third RBB utilised in methods in accordance with the invention.

In a suitable embodiment of a method in accordance with the first aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a non-interferon activating disease. In an alternative suitable embodiment of a method in accordance with the first aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a non-interferon activating disease. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a non-interferon activating disease, this indicates that the subject with fever is distinguished as having a non-interferon activating disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a non-interferon activating disease, this indicates that the subject with fever is distinguished as having a non-interferon activating disease.

In a suitable embodiment of a method in accordance with the first aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with an interferon activating disease. In an alternative suitable embodiment of a method in accordance with the first aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without an interferon activating disease. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with an interferon activating disease, this indicates that the subject with fever is distinguished as having an interferon activating disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject

without an interferon activating disease, this indicates that the subject with fever is distinguished as having an interferon activating disease.

In a suitable embodiment of a method in accordance with the second aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a systemic fungal infection. In an alternative suitable embodiment of a method in accordance with the second aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a systemic fungal infection. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a systemic fungal infection, this indicates that the subject with fever is distinguished as having a systemic fungal infection. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a systemic fungal infection, this indicates that the subject with fever is distinguished as having a systemic fungal infection.

In a suitable embodiment of a method in accordance with the second aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a bacterial infection or Kawasaki disease. In an alternative suitable embodiment of a method in accordance with the second aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a bacterial infection or Kawasaki disease. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a bacterial infection or Kawasaki disease, this indicates that the subject with fever is distinguished as having a bacterial infection or Kawasaki disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a bacterial infection or Kawasaki disease, this indicates that the subject with fever is distinguished as having a bacterial infection or Kawasaki disease.

In a suitable embodiment of a method in accordance with the third aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject

with a bacterial infection. In an alternative suitable embodiment of a method in accordance with the third aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a bacterial infection. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a bacterial infection, this indicates that the subject with fever is distinguished as having a bacterial infection. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a bacterial infection, this indicates that the subject with fever is distinguished as having a bacterial infection.

In a suitable embodiment of a method in accordance with the third aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with Kawasaki disease. In an alternative suitable embodiment of a method in accordance with the third aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without Kawasaki disease. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with Kawasaki disease, this indicates that the subject with fever is distinguished as having Kawasaki disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject Kawasaki disease, this indicates that the subject with fever is distinguished as having Kawasaki disease.

In a suitable embodiment of a method in accordance with the fourth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with active TB. In an alternative suitable embodiment of a method in accordance with the fourth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without active TB. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with active TB, this indicates that the subject with fever is distinguished as having active TB. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a

subject without active TB, this indicates that the subject with fever is distinguished as having active TB.

In a suitable embodiment of a method in accordance with the fourth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease or SLE. In an alternative suitable embodiment of a method in accordance with the fourth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease or SLE. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease or SLE, this indicates that the subject with fever is distinguished as having a viral disease or SLE. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease or SLE, this indicates that the subject with fever is distinguished as having a viral disease or SLE.

In a suitable embodiment of a method in accordance with the fifth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with SLE. In an alternative suitable embodiment of a method in accordance with the fifth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without SLE. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with SLE, this indicates that the subject with fever is distinguished as having SLE. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without SLE, this indicates that the subject with fever is distinguished as having SLE.

In a suitable embodiment of a method in accordance with the fifth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease. In an alternative suitable embodiment of a method in accordance with the fifth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease. Suitably, in the case where the

determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease, this indicates that the subject with fever is distinguished as having a viral disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease, this indicates that the subject with fever is distinguished as having a viral disease.

In a suitable embodiment of a method in accordance with the sixth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease. In an alternative suitable embodiment of a method in accordance with the sixth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with a viral disease, this indicates that the subject with fever is distinguished as having a viral disease. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease, this indicates that the subject with fever is distinguished as having a viral disease.

In a suitable embodiment of a method in accordance with the sixth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with active TB. In an alternative suitable embodiment of a method in accordance with the sixth aspect of the invention comparison of the first RBB to the second RBB indicates that the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without active TB. Suitably, in the case where the determined ratio of the first and second RBBs is similar to a reference value representative of gene expression in a subject with active TB, this indicates that the subject with fever is distinguished as having active TB. Suitably, in the case where the determined ratio of the first and second RBBs is not similar to a reference value representative of gene expression in a subject without active TB, this indicates that the subject with fever is distinguished as having active TB.

In a suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and second RBBs or first and third RBBs is similar to a reference value representative

of gene expression in a subject with SLE. In an alternative suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and second RBBs or first and third RBBs is not similar to a reference value representative of gene expression in a subject without SLE. Suitably, in the case where the determined ratio of the first and second RBBs or first and third RBBs is similar to a reference value representative of gene expression in a subject with SLE, this indicates that the subject with fever is distinguished as having SLE. Suitably, in the case where the determined ratio of the first and second RBBs or first and third RBBs is not similar to a reference value representative of gene expression in a subject without SLE, this indicates that the subject with fever is distinguished as having SLE.

In a suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and second RBBs or second and third RBBs is similar to a reference value representative of gene expression in a subject with a viral disease. In an alternative suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and second RBBs or second and third RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease. Suitably, in the case where the determined ratio of the first and second RBBs or second and third RBBs is similar to a reference value representative of gene expression in a subject with a viral disease, this indicates that the subject with fever is distinguished as having a viral disease. Suitably, in the case where the determined ratio of the first and second RBBs or second and third RBBs is not similar to a reference value representative of gene expression in a subject without a viral disease, this indicates that the subject with fever is distinguished as having a viral disease.

In a suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and third RBBs or second and third RBBs is similar to a reference value representative of gene expression in a subject with active TB. In an alternative suitable embodiment of a method in accordance with the seventh aspect of the invention comparison of the first RBB, second RBB and third RBB indicates that the determined ratio of the first and third RBBs or second and third RBBs is not similar to a reference value representative of gene expression in a subject without active TB. Suitably, in the case where the determined ratio of the first and third RBBs or second and third RBBs is similar to a reference value representative of gene expression in a subject with active TB, this indicates that the subject with fever is distinguished as having active TB. Suitably, in the case where the determined ratio of the first and third

RBBs or second and third RBBs is not similar to a reference value representative of gene expression in a subject without active TB, this indicates that the subject with fever is distinguished as having active TB.

In a suitable embodiment of a method in accordance with the eighth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is similar to a reference value of the RBB representative of gene expression in a subject with Kawasaki disease. In a suitable embodiment of a method in accordance with the eighth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is not similar to a reference value of the RBB representative of gene expression in a subject with Kawasaki disease. Suitably, in the case where the standardised RBB is similar to a reference value representative of gene expression in a subject with Kawasaki disease, this indicates that the subject with fever is distinguished as having Kawasaki disease. Suitably, in the case where the standardised RBB is not similar to a reference value representative of gene expression in a subject without Kawasaki disease, this indicates that the subject with fever is distinguished as having Kawasaki disease.

In a suitable embodiment of a method in accordance with the ninth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is similar to a reference value of the RBB representative of gene expression in a subject with a viral disease. In a suitable embodiment of a method in accordance with the ninth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is not similar to a reference value of the RBB representative of gene expression in a subject with a viral disease. Suitably, in the case where the standardised RBB is similar to a reference value representative of gene expression in a subject with a viral disease, this indicates that the subject with fever is distinguished as having a viral disease. Suitably, in the case where the standardised RBB is not similar to a reference value representative of gene expression in a subject without a viral disease, this indicates that the subject with fever is distinguished as having a viral disease.

In a suitable embodiment of a method in accordance with the tenth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is similar to a reference value of the RBB representative of gene expression in a subject with active TB. In a suitable embodiment of a method in accordance with the tenth aspect of the invention comparison of the standardised RBB to a reference level of the RBB indicates that the standardised RBB is not similar to a reference value of the RBB representative of gene expression in a subject with active TB. Suitably, in the case where the

standardised RBB is similar to a reference value representative of gene expression in a subject with active TB, this indicates that the subject with fever is distinguished as having active TB. Suitably, in the case where the standardised RBB is not similar to a reference value representative of gene expression in a subject without active TB, this indicates that the subject with fever is distinguished as having active TB.

In a suitable embodiment the comparison of the first RBB and the second RBB of a method in accordance with the first to sixth aspects of the invention comprises determining the ratio of the first RBB to the second RBB and assessing the similarity of this determined ratio to reference data.

Suitably, the comparison of the first RBB, second RBB and third RBB of a method in accordance with the seventh aspect of the invention comprises determining at least two ratios selected from the group consisting of:

- the ratio of the first RBB to the second RBB;
- the ratio of the first RBB to the third RBB; and
- the ratio of the second RBB to the third RBB;

and assessing the similarity of the at least two determined ratios to reference data.

Details of particular comparisons of RBBs in the context of the present invention that enable distinction or determination of particular diseases or conditions are set out further in the following paragraphs.

Further embodiments of methods of the invention

In a suitable embodiment there is provided a method according to the first aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1 and MMP9; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a non-interferon activating disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a non-interferon activating disease;

- this indicates that the subject has a non-interferon activating disease.

In a suitable embodiment there is provided a method according to the first aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3, and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with an interferon activating disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without an interferon activating disease;
- this indicates that the subject has an interferon activating disease.

As noted above, the second aspect of the invention, there is provided a method for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease in a subject with fever.

In a suitable embodiment there is provided a method according to the second aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5, SERPING1, CEACAM8, CAMP and LTF; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a systemic fungal infection; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a systemic fungal infection;
- this indicates that the subject has a systemic fungal infection.

In a suitable embodiment there is provided a method according to the second aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1, MMP9, FCER1A, CCR3 and HDC; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a bacterial infection or Kawasaki disease; or

- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a bacterial infection or Kawasaki disease;
- this indicates that the subject has a bacterial infection or Kawasaki disease.

In a suitable embodiment, the systemic fungal infection is Candidemia.

In a suitable embodiment there is provided a method according to the third aspect of the invention, wherein:

- when the numerator gene of the first RBB is CEACAM8; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a bacterial infection; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a bacterial infection;
- this indicates that the subject has bacterial infection.

In a suitable embodiment there is provided a method according to the third aspect of the invention, wherein:

- when the numerator gene of the second RBB is IFITM3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with Kawasaki disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without Kawasaki disease;
- this indicates that the subject has Kawasaki disease.

In a suitable embodiment there is provided a method according to the fourth aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB;

- this indicates that the subject has active TB.

In a suitable embodiment there is provided a method according to the fourth aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3, ADGRE3, FCER1A, HDC, CCR3 and CCN3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease or SLE; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease or SLE;
- this indicates that the subject has a viral disease or SLE.

In a suitable embodiment there is provided a method according to the fifth aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: FCER1A, HDC, PI3, CCR3 and CCN3; and
- the determined ratio of the first and RBBs is assessed as similar to a reference value representative of gene expression in a subject with SLE; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without SLE;
- this indicates that the subject has SLE.

In a suitable embodiment there is provided a method according to the fifth aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

In a suitable embodiment, there is provided a method according to the fifth aspect of the invention wherein the subject has been distinguished as having SLE or viral disease by a method according to the fourth aspect of the invention.

In a suitable embodiment there is provided a method according to the sixth aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

In a suitable embodiment there is provided a method according to the sixth aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB;
- this indicates that the subject has active TB.

In a suitable embodiment, there is provided a method wherein the subject has been distinguished as having viral disease or active TB by a method according to the fourth aspect of the invention.

In a suitable embodiment, there is provided a method, wherein the subject has been identified as having interferon activating disease by a method according to the first aspect of the invention.

In a suitable embodiment there is provided a method according to the seventh aspect of the invention, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: FCER1A, HDC, CCR3, CCN and PI3; and
- the determined ratio of the first RBB and second RBBs or the first and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with SLE; or
- the determined ratio of the first and second RBB or the first and third RBBs is assessed as not similar to the reference value representative of gene expression in a subject without SLE;
- this indicates that the subject has SLE.

In a suitable embodiment there is provided a method according to the seventh aspect of the invention, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs or the first and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the second RBB to the first RBB and/or third RBB is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

In a suitable embodiment there is provided a method according to the seventh aspect of the invention, wherein:

- when the numerator gene of the third RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and third RBBs or the second and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and third RBBs or second and third RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB;
- this indicates that the subject has active TB.

In a suitable embodiment, there is provided a method according to the seventh aspect of the invention, wherein the subject has been identified as having viral disease, SLE or active TB by a method according to the fourth, fifth or sixth aspects of the invention.

In a suitable embodiment there is provided a method according to the eighth aspect of the invention, wherein:

- when the numerator gene is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1, MMP9, FCER1A, CCR3 and HDC; and
- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with Kawasaki disease; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject without Kawasaki disease;
- this indicates that the subject has Kawasaki disease.

In a suitable embodiment, there is provided a method according to the eighth aspect of the invention, wherein the subject is a child under the age of 5 and has clinical features suggestive of Kawasaki disease.

In a suitable embodiment, there is provided a method according to the eighth aspect of the invention, wherein the subject has been identified as having non-interferon activating disease by a method according to the first aspect of the invention.

In a suitable embodiment there is provided a method according to the ninth aspect of the invention, wherein:

- when the numerator gene is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

In a suitable embodiment, there is provided a method according to the ninth aspect of the invention, wherein the subject has been identified as having an interferon activating disease by a method according to the first aspect of the invention.

In a suitable embodiment there is provided a method according to the tenth aspect of the invention, wherein:

- when the numerator gene is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and

- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject with active TB;
- this indicates that the subject has active TB.

In a suitable embodiment, there is provided a method according to the tenth aspect of the invention, wherein the subject has been identified as having an interferon activating disease by a method according to the first aspect of the invention.

Combinations of methods of the invention

A plurality of methods of the first to tenth aspects of the invention may be combined, in order to provide a more comprehensive methodology for fever triage. By way of example, once a number of potential causes of a subject's fever have been distinguished by means of a first method of the invention, specific causes may further be distinguished by use of a second, and optionally a third or further, method of the invention. The combinations of methods described below may provide such advantages in practice, and other useful combinations of the methods of the invention will be apparent to those skilled in the art.

When multiple methods of the invention are to be practiced in combination, the same denominator gene may advantageously be used in the RBBs specified in the different aspects that are to be combined.

In a suitable embodiment, a subject with a fever distinguished as having a non-interferon activating disease by a method of the first aspect of the invention may then have potential causative non-interferon activating diseases further distinguished by means of a method of the second aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of systemic fungal infection and a group of diseases consisting of bacterial infection and Kawasaki disease in a method of the second aspect of the invention may have been identified as requiring such a distinction to be made by a method of the first aspect of the invention that distinguished them as having non-interferon activating disease.

In a suitable embodiment, a subject with a fever distinguished as having non-interferon activating disease by a method of the first aspect of the invention, or as having bacterial

infection or Kawasaki disease by a method of the second aspect of the invention, may then have these conditions further distinguished by means of a method of the third aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of bacterial infection and Kawasaki disease in a method of the third aspect of the invention may have been identified as requiring such a distinction to be made by a method of the first aspect of the invention (indicating that they have a non-interferon activating disease), or by a method of the second aspect of the invention.

In a suitable embodiment, a subject with a fever distinguished as having an interferon activating disease by a method of the first aspect of the invention may then have potential causative interferon activating diseases further distinguished by means of a method of the fourth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of active tuberculosis and a group of diseases in consisting of: viral disease and SLE a method of the fourth aspect of the invention may have been identified as requiring such a distinction to be made by a method of the first aspect of the invention that distinguished them as having an interferon activating disease.

In a suitable embodiment, a subject with a fever distinguished as having SLE or viral disease by a method of the fourth aspect of the invention may then have these conditions further distinguished by means of a method of the fifth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of SLE and viral disease in a method of the fifth aspect of the invention may have been identified as requiring such a distinction to be made by a method of the fourth aspect of the invention.

In a suitable embodiment, a subject with a fever distinguished as having viral disease or active TB by a method of the first aspect of the invention (indicating that the subject has fever caused by an interferon activating disease), or by a method of the fourth aspect of the invention, may then have these conditions further distinguished by means of a method of the sixth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of viral disease and active TB in a method of the sixth aspect of the invention may have been identified as requiring such a distinction to be made by a method of the first aspect of the invention (indicating that the subject has fever caused by an interferon activating disease), or by a method of the fourth aspect of the invention.

In a suitable embodiment, a subject with a fever distinguished as having SLE, or viral disease, or active TB by a method of the first aspect of the invention (indicating that the subject has fever caused by an interferon activating disease), by a method of the fourth aspect of the invention, by a method of the fifth aspect of the invention, or by a method of the sixth aspect of the invention, may then have these conditions further distinguished by means of a method of the seventh aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring a distinction to be made in respect of SLE, viral disease and active TB may have been identified as requiring such a distinction to be made by a method of the first aspect of the invention (indicating that the subject has fever caused by an interferon activating disease), by a method of the fourth aspect of the invention, by a method of the fifth aspect of the invention, or by a method of the sixth aspect of the invention.

In a suitable embodiment, a subject with a fever identified as potentially having Kawasaki disease by a method of the first aspect of the invention (indicating that they have non-interferon activating disease), or by a method of the second aspect of the invention, or of the third aspect of the invention, may then have the presence of Kawasaki disease determined by means of a method of the eighth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring the presence of Kawasaki disease to be determination in a method of the eighth aspect of the invention may have been identified as requiring such a determination to be made by a method of the first aspect of the invention (indicating that they have a non-interferon activating disease), or by a method of the second aspect of the invention, or of the third aspect of the invention.

In a suitable embodiment, a subject with a fever identified as potentially having a viral disease by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth aspect of the invention, or by a method of the fifth aspect of the invention, or by a method of the sixth aspect of the invention, or by a method of the

seventh aspect of the invention, may then have the presence of a viral disease determined by means of a method of the ninth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring the presence of a viral disease to be determination in a method of the ninth aspect of the invention may have been identified as requiring such a determination to be made by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth aspect of the invention, or by a method of the fifth aspect of the invention, or by a method of the sixth aspect of the invention, or by a method of the seventh aspect of the invention.

In a suitable embodiment, a subject with a fever identified as potentially having TB by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth aspect of the invention, by a method of the sixth aspect of the invention, or by a method of the seventh aspect of the invention, may then have the presence of TB determined by means of a method of the tenth aspect of the invention.

Similarly, in a suitable embodiment, a subject with fever requiring the presence of TB to be determination in a method of the tenth aspect of the invention may have been identified as requiring such a determination to be made by a method of the first aspect of the invention (indicating that they have interferon activating disease), or by a method of the fourth aspect of the invention, or by a method of the sixth aspect of the invention, or by a method of the seventh aspect of the invention.

Suitable embodiments of methods of the eleventh aspect of the invention

In a suitable embodiment of a method of the eleventh aspect of the invention, the denominator gene is the same in each RBB provided. Suitably, in such an embodiment, the denominator gene is selected from the group consisting of: SAT1, SRGN and NCF1. Suitably, the common denominator gene used in such an embodiment is SAT1.

Comparisons employed in the methods of the eleventh aspect of the invention may be the same as those used in embodiments of the first to fifth aspects of the invention. For example, a comparison employed in step (b) of a method of the eleventh aspect of the invention may be the same as a comparison described in connection with the first aspect of the invention. Similarly, a comparison employed in step (c) of a method of the eleventh aspect of the invention may be the same as a comparison described in connection with the second aspect of the invention, a comparison employed in step (d) of a method of the eleventh aspect of the

invention may be the same as a comparison described in connection with the third aspect of the invention, a comparison employed in step (e) of a method of the eleventh aspect of the invention may be the same as a comparison described in connection with the fourth aspect of the invention, and a comparison employed in step (f) of a method of the eleventh aspect of the invention may be the same as a comparison described in connection with the fifth aspect of the invention.

In a suitable embodiment of a method of the eleventh aspect of the invention, the manner of distinguishing employed in step (b) of a method of the eleventh aspect of the invention may be the same as a manner of distinguishing described in connection with the first aspect of the invention. Similarly, a manner of distinguishing employed in step (c) of a method of the eleventh aspect of the invention may be the same as a manner of distinguishing described in connection with the second aspect of the invention, a manner of distinguishing employed in step (d) of a method of the eleventh aspect of the invention may be the same as a manner of distinguishing described in connection with the third aspect of the invention, a manner of distinguishing employed in step (e) of a method of the eleventh aspect of the invention may be the same as a manner of distinguishing described in connection with the fourth aspect of the invention, and a manner of distinguishing employed in step (f) of a method of the eleventh aspect of the invention may be the same as a manner of distinguishing described in connection with the fifth aspect of the invention.

In a suitable embodiment of a method of the eleventh aspect of the invention, the numerator genes selected from Tables 1 and 2 may be as described in connection with embodiments of the first aspect of the invention. Similarly, numerator genes selected from Tables 8 and 9 may be as described in connection with embodiments of the second aspect of the invention, numerator genes selected from Tables 10 and 11 may be as described in connection with embodiments of the third aspect of the invention, numerator genes selected from Tables 12 and 13 may be as described in connection with embodiments of the fourth aspect of the invention, and numerator genes selected from Tables 14 and 15 may be as described in connection with embodiments of the 5 aspect of the invention.

Kits of the invention

Kits of the present invention are set forth in the twelfth to the twenty-first aspects of the invention. Kits of the present invention as set forth in the twelfth to the twenty-first aspects of the invention may be used as companion diagnostic test kits for the corresponding methods

selecting treatment regimens, methods of treatment, and medical uses defined in other aspects or embodiments of the invention.

Kits of the present invention contain reagents capable of specifically binding to specified target molecules (target molecules indicative of expression of a gene found in a recited RBB). Such reagents enable the kits of the invention to be used in analysing gene expression in a sample representative of gene expression in the subject, and thereby practicing a method of the invention.

The term “specifically binding” as used herein refers to the ability of the recited reagent to selectively bind to the target molecule in question. By this is meant that the binding of the reagent is able to allow a distinction to be made between the presence of the specified target molecule, and the presence of an alternative target molecule. Accordingly, a specific binding reagent exhibits a greater extent of binding to the specified target molecule than to a comparable control target molecule.

By way of example, specific binding may be demonstrated by a binding affinity between a reagent and corresponding target molecule that is at least 10-fold increased as compared to binding to a comparable control target molecule. Indeed, specific binding may be demonstrated by an affinity of binding that is at least 50-fold, at least 100-fold, at least 250-fold, at least 500-fold, or at least 1000-fold, or more increased as compared to binding to a comparable control target molecule.

Specific binding may be demonstrated by a degree of cross-reactivity between the reagent and a comparable control target molecule that is less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01%.

A “reagent capable of specifically binding to a target molecule”, for the purposes of the present invention, may be any substance which demonstrates the required degree of specificity in its interactions with a target molecule. It will be appreciated that suitable reagents for use in kits of the present invention enable the detection, measurement, or analysis of the presence or quantity of a specific target molecule in a sample representative of gene expression in a subject.

The term “binding” as used herein refers to the interaction between a target molecule and a reagent of a kit in accordance with twelfth to the twenty-first aspects of the invention. In a suitable embodiment, such binding may be by any suitable binding interaction. Merely by way

of example, specific binding may be by hydrogen bonding. Alternatively, specific binding may be by electrostatic interactions. In a suitable example, specific binding may be by covalent bonding. Alternatively, specific binding may be by hydrophobic interactions.

The term “target molecule indicative of gene expression” as used herein refers to any suitable molecule that is indicative of expression of a gene of interest. It will be appreciated that a wide range of types of target molecule may be employed in the methods of the invention, and hence a wide range of reagents capable of binding to such target molecules may be employed in the kits of the invention. Suitably, the target molecule may be an RNA target molecule. Suitably, the target molecule may be a peptide target molecule.

In a suitable embodiment, the reagent is capable of specifically binding to an RNA target molecule. It will be appreciated that there are many types of reagents which are capable of binding to an RNA target molecule. Such reagents may be utilised in the kits of the invention.

In a suitable embodiment a reagent capable of specifically binding to an RNA target molecule is selected from the group consisting of: a primer; a primer pair; a probe; or a primer set and a probe. Thus, a kit of the invention may comprise a reagent capable of specifically binding to an RNA target molecule that is a primer. A kit of the invention may comprise a reagent capable of specifically binding to an RNA target molecule that is a primer pair. A kit of the invention may comprise a reagent capable of specifically binding to an RNA target molecule that is a probe. A kit of the invention may comprise a reagent capable of specifically binding to an RNA target molecule comprising a primer set and a probe.

In a suitable embodiment, the primer is selected from a group consisting of: a qPCR primer, a digital PCR primer, a RNAseq primer, a DNA microarray primer, a DNA hybridization primer, and a RNA hybridization primer. In a suitable embodiment, a kit of the invention comprises a qPCR primer. In a suitable embodiment, a kit of the invention comprises a digital PCR primer. In a suitable embodiment, a kit of the invention comprises a RNAseq primer. In a suitable embodiment, a kit of the invention comprises a DNA microarray primer. In a suitable embodiment, a kit of the invention comprises a DNA hybridization primer. In a suitable embodiment, a kit of the invention comprises an RNA hybridization primer.

It will be appreciated, that the design and/or sequence of a suitable primer will depend on the target molecule. Merely by way of example, Table 34 sets out primers which can be used to determine the transcript abundance of relevant genes of interest within the RBBs.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 1 and 2 is used in a method of the invention in which expression of SAT1 is to be analysed, or is provided in a kit of the invention for the analysis of SAT1 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 3 and 4 is used in a method of the invention in which expression of SRGN is to be analysed, or is provided in a kit of the invention for the analysis of SRGN as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 5 and 6 is used in a method of the invention in which expression of ANXA3 is to be analysed, or is provided in a kit of the invention for the analysis of ANXA3 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 7 and 8 is used in a method of the invention in which expression of RSAD2 is to be analysed, or is provided in a kit of the invention for the analysis of RSAD2 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 9 and 10 is used in a method of the invention in which expression of IFIT1 is to be analysed, or is provided in a kit of the invention for the analysis of IFIT1 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 11 and 12 is used in a method of the invention in which expression of FCER1A is to be analysed, or is provided in a kit of the invention for the analysis of FCER1A as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 13 and 14 is used in a method of the invention in which expression of CCR3 is to be analysed, or is provided in a kit of the invention for the analysis of CCR3 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 15 and 16 is used in a method of the invention in which expression of PSAP is to be analysed, or is provided in a kit of the invention for the analysis of PSAP as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 17 and 18 is used in a method of the invention in which expression of VNN1 is to be analysed, or is provided in a kit of the invention for the analysis of VNN1 as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 19 and 20 is used in a method of the invention in which expression of IFI44L is to be analysed, or is provided in a kit of the invention for the analysis of IFI44L as part of an RBB.

In a suitable embodiment a primer pair comprising SEQ ID NOs: 21 and 22 is used in a method of the invention in which expression of IFI27 is to be analysed, or is provided in a kit of the invention for the analysis of IFI27 as part of an RBB.

In a suitable embodiment, the reagent is capable of specifically binding to a peptide target molecule. It will be appreciated that there are many types of reagents which are capable of binding to a peptide target molecule. Such reagents may be utilised in the kits of the invention.

In a suitable embodiment, a reagent capable of specifically binding to a peptide target molecule is selected from the group consisting of: an antibody or fragment thereof; a substrate; an aptamer; an avimer; a peptidomimetic; a receptor or fragment thereof; a ligand; and a cofactor. Thus, a kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is an antibody or fragment thereof. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is a substrate. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is an aptamer. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is an avimer. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is a peptidomimetic. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is a receptor or fragment thereof. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is a ligand. A kit according to the invention may comprise a reagent capable specifically binding to a peptide target molecule that is a cofactor.

In a suitable embodiment, an antibody capable of specifically binding to the target molecule may be a monoclonal antibody or a polyclonal antibody.

In a suitable embodiment, an antibody capable of specifically binding to the target molecule may be a fluorochrome-labelled antibody.

In a suitable embodiment, the reagent may be labelled with a detection moiety. Suitably the detection moiety may be a chemical substance. Merely by way of example, the detection moiety may be a fluorescent dye, or may be a chemiluminescent substrate.

In a suitable embodiment, the detection moiety may be a biological substance. Merely by way of example, the detection moiety may be an antibody or antigen, or may be an enzyme.

In a suitable embodiment, each reagent in a kit of the invention is of the same type. The types of reagents may be as considered above. For example, in an embodiment of a kit of the invention comprising reagents capable of specifically binding to RNA target molecules, suitably each reagent in the kit is a primer. Similarly, in an embodiment of a kit of the invention comprising reagents capable of specifically binding to protein target molecules, suitably each reagent in the kit is an antibody, or fragment thereof.

In a suitable embodiment, a kit in accordance with the present invention may comprise additional reagents that are capable of specifically binding to a comparable control target molecule but are not capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 20. In a suitable embodiment, no more than 50%, 40%, 30%, 20%, 10% of the reagents present in a kit of the invention may be such additional reagents. For example, such additional reagents may constitute no more than 5%, 4%, 3%, 2% or 1% of the total reagents present in a kit of the invention.

Suitably, in a kit of the invention, at least 50% of the reagents present are capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 20. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 20. It will be appreciated that such kits offer advantages in practicing the methods of the invention, not least because they avoid the need to produce and provide unnecessary reagents, or to obtain unnecessary quantities of a sample representative of gene expression in the subject with fever that would otherwise be required in order to allow sufficient sample for binding to such unnecessary reagents.

For example, in a kit in accordance with the twelfth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 1, Table 3 and Table 2. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%,

at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 1, Table 3 and Table 2.

For example, in a kit in accordance with the thirteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 8, Table 3 and Table 9. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 8, Table 3 and Table 9.

For example, in a kit in accordance with the fourteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 10, Table 3 and Table 11. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those set genes out in Table 10, Table 3 and Table 11.

For example, in a kit in accordance with the fifteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 12, Table 3 and Table 13. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 12, Table 3 and Table 13.

For example, in a kit in accordance with the sixteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule

indicative of expression of a gene from those set out in Table 14, Table 3 and Table 15. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 14, Table 3 and Table 15.

For example, in a kit in accordance with the seventeenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 15, Table 3 and Table 16. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 15, Table 3 and Table 16.

For example, in a kit in accordance with the eighteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 14, Table 3, Table 15 and Table 16. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 14, Table 3, Table 15 and Table 16.

For example, in a kit in accordance with the nineteenth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 17 and Table 3. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 17 and Table 3.

For example, in a kit in accordance with the twentieth aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 18 and Table 3. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 18 and Table 3.

For example, in a kit in accordance with the twenty-first aspect of the invention, at least 50% of the reagents present in the kit may be capable of binding specifically to a target molecule indicative of expression of a gene from those set out in Table 19 and Table 3. In a suitable embodiment of such a kit, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more, of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene from those genes set out in Table 19 and Table 3.

Kits of the invention may also comprise further constituents, such as a reference standard, or constituents for use in the assay in which the kit is to be used (for example, nucleoside triphosphates in the case of kits to be used in nucleic acid amplification-based methods, or buffered diluent solutions for kits to be used in protein blotting-based assays).

The term “reference standard” as used herein refers to a known and standardized level of the biomarker associated with the expression of the genes. Suitably, a kit in accordance with the invention comprises one or more reference standards indicating a relevant the reference level of the corresponding genes making up the RBB.

In a suitable embodiment, a kit of the invention may comprise a combination of one or more of the kits set out in the twelfth to the twenty-first aspects of the invention. Thus, such a kit may comprise a combination of a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3 with one, more, or all of the following:

- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2;

- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 16;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 17;
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 18; and
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 19.

A suitable kit in accordance with the invention may comprise:

- i. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1; and
- ii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2; and
- iii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8; and
- iv. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9; and
- v. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10; and

- vi. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11; and
- vii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12; and
- viii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13; and
- ix. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and
- x. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and
- xi. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3.

Such a kit may be of particular utility in practicing methods in accordance with the eleventh aspect of the invention.

As referred to elsewhere in this specification, methods in accordance with the first to eleventh aspects of the invention may be practiced using appropriate kits in accordance with twelfth to twenty-first aspects of the invention. Similarly, kits in accordance with the twelfth to twenty-first aspects of the invention may be used to practice appropriate methods in accordance with the first to eleventh aspects of the invention, or to identify patients who may benefit from the methods or medical uses of the twenty-second to forty-seventh aspects of the invention.

Marker identification methods of the invention

The forty-eighth and forty-ninth aspects of the invention respectively define methods of identifying genes for use in an RBB for determining presence of a disease, and methods of identifying an origin-proxy reference gene in respect of a state of interest.

Methods in accordance with either the forty-eighth or forty-ninth aspects of the invention may, for brevity, be referred to as a “marker identification method of the invention.”

The term “origin” as used herein is defined as the point at coordinate 0,0 within a LRA biplot. Thus, the term “closest to the origin” as used in the forty-eighth or forty-ninth aspects of the invention refers to genes which are found closest to the coordinate 0,0 in a LRA biplot.

It will be appreciated that there are various approaches to determine which genes are closest to the origin. Merely by way of example, up to the first 10 genes closest to the origin may be selected. For example, identifying a first group of genes closest to the origin in the LRA biplot may comprise identifying at least 10 genes, at least 9 genes, at least 8 genes, at least 7 genes, at least 6 genes, at least 5 genes, at least 4 genes, at least 3 genes, at least 2 genes or even at least 1 gene in closest proximity to the origin. For example, identifying a first group of genes closest to the origin in the LRA biplot may comprise identifying 10 genes, 9 genes, 8 genes, 7 genes, 6 genes, 5 genes, 4 genes, 3 genes, 2 genes or even 1 gene in closest proximity to the origin. In a suitable embodiment, identifying a group of genes closest to the origin in the LRA biplot further comprises identifying a number of genes from between 1 to 10 that are closest to the origin.

It is not typical in the field of gene panels to use genes close to the origin in a LRA biplot for dimension reduction. These genes have traditionally been deemed uninformative and often disregarded. This practice stemmed from variable selection methods primarily emphasizing a gene's contribution to overall variance, with components near the origin generally having low contributions and being routinely excluded from subsequent analyses.

Contrary to common practice, methods in accordance with the first to eleventh aspects of the present invention make use of these components genes as denominator genes in the formation of RBBs. The advantages of which have already been discussed.

Suitably, any of the genes identified in the first group of genes in a method according to the forty-eighth aspect of the invention or in the group of genes closest to the origin in a method of the forty-ninth aspect of the invention may be selected as the denominator gene in an RBB.

In a suitable embodiment, any of the genes identified in the second group of genes in a method according to the forty-eighth aspect of the invention may be selected as the numerator gene in an RBB.

In a suitable embodiment, a method in accordance with the forty-eighth aspect of the present invention, identifying a group of genes furthest from the first group of genes in the LRA biplot is determined with reference to the axis that furthest separates data representative of gene expression in patients from data representative of gene expression in controls.

In a suitable embodiment, a method in accordance with the forty-ninth aspect of the present invention, the group of genes closest to the origin in the LRA biplot is determined with reference to the axis that furthest separate patients from controls.

In a suitable embodiment, the first and/or the second set of data is obtained from a pre-defined gene list.

The term “pre-defined gene list” as used herein refers to a user-defined list of genes that are preferentially expressed in a relevant cell type. Merely by way of example, a pre-defined gene list may comprise genes that are preferentially expressed by granulocytes. Alternatively, a pre-defined gene list may comprise genes that are preferentially expressed by monocytes.

In a suitable embodiment, methods according to the forty-eighth and forty ninth aspects of the invention further comprise filtering the first group of genes, or origin-proxy reference genes, by implementing a gene expression threshold. In a suitable embodiment, the gene expression threshold is moderate to high gene expression. In a suitable embodiment, the gene expression threshold may be a value over 750 copies of transcript per million (TPM). For example, the gene expression threshold may be a value over about 750 TPM, over about 760 TPM, over about 770 TPM, over about 780 TPM, over about 790 TPM or even over 800 TPM.

In a suitable embodiment, methods according to the forty-eight and forty-ninth aspects of the invention further comprise dimension reduction analysis of the RBB. It will be appreciated that there are multiple techniques which would be suitable for dimension reduction analysis of the RBB, and any of these dimension reduction analysis techniques may be used in respect of the RBB. Merely by way of example, the dimension analysis may be by t-Distributed Stochastic Neighbor Embedding (t-SNE). Alternatively, the dimension analysis may be by Linear Discriminant Analysis (LDA). In a further example the dimension reduction analysis is by principal component analysis (PCA) to generate a PCA biplot. In a suitable example, the dimension reduction analysis is by Uniform Manifold Approximation and Projection (UMAP).

In a suitable embodiment, the dimension reduction analysis is by PCA to generate a PCA biplot, and further comprises a rotation of the PCA biplot axes.

In a suitable embodiment, the dimension reduction analysis is by PCA to generate a PCA biplot, and further comprises multiple rotation of the PCA biplot axes. For example, the dimension reduction analysis is by PCA to generate a PCA biplot and further comprises at

least one rotation, at least two rotations, at least three rotations or even at least four rotations of the PCA biplot axes.

The inventors of the present invention have developed marker identification methods according to the forty-eighth or forty-ninth aspects of the invention which use specific desirable properties of PCA of compositional data.

PCA of compositional data exhibits sub-compositional coherence and near-coherence. Sub-compositional coherence allows addition or removal of components (genes) in LRA and yet the analysis output remains coherent or similar.

Furthermore, sub-compositional coherence allows reduction of the dimensionality of the data while preserving the most important information.

Importantly, near-coherence offers the advantage of substantial dimension reduction while preserving a significant portion of critical information.

The inventors of the present invention have developed marker identification methods according to the forty-eighth or forty-ninth aspects of the invention which use specific desirable properties of PCA of compositional data.

PCA of compositional data are sub compositional coherent or near-coherent. Coherent results make it easier to interpret the principal components. When the loadings and scores align logically, it becomes more straightforward to attribute meaning to each component, aiding in the identification of patterns and relationships within the data.

Furthermore, PCA of compositional data have a link vector (a line joining vertex of 2 components) in the LRA biplots which represents log ratio of those two components. For example, the line BA joining the vertex of BATF2 to ANXA3 is the link vector representing the ratio of ANXA3 to BATF2 (Figure 4).

Additionally, methods of the invention solve the problem of ALR being asymmetric by using a new method to pick the denominator through a method according to the forty-ninth aspect of the invention (origin proxy reference gene) as the reference component for deriving another set of much less biased ALR for all other genes and to make it useful for RBB development.

The present invention uses, for the first time, a CoDa approach to identify the least biased reference genes as the common denominator for a panel of RBBs. Then, ALR is calculated with these reference genes for other genes in a gene list, which is followed by a PCA analysis of their log ratios against a denominator gene in an analysis of ALRs. ALR(s) that correlated with the PCA axis that maximally separate patients from controls can be used as RBB for that disease.

Selection of treatment regimens

The methods of the first to eleventh aspects of the invention, or combined methods of the invention as described herein, may also give rise to corresponding uses in the selection of treatment regimens.

Accordingly, the first aspect of the invention may provide a method for distinguishing between non-interferon activating disease and interferon activating disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for non-interferon activating disease in the event that the subject is distinguished as having non-interferon activating disease, or selecting a treatment regimen for interferon activating disease in the event that the subject is distinguished as having interferon activating disease. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having non-interferon activating disease, the treatment regimen may comprise provision of treatment with an anti-infective agent, while in the case of a subject distinguished as having interferon-activated disease, the treatment regimen may comprise provision of treatment with an immunomodulatory agent.

The second aspect of the invention may provide a method for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for systemic fungal infection in the event that the subject is distinguished as having systemic fungal infection, or selecting a treatment regimen for bacterial infection or Kawasaki disease in the event that the subject is distinguished as having one of these diseases. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having systemic fungal infection, the treatment regimen may comprise provision of treatment

with an anti-fungal agent, in the case of a subject distinguished as having bacterial infection, the treatment regimen may comprise provision of treatment with an antibiotic, while in the case of a subject distinguished as having Kawasaki disease, the treatment regimen may comprise provision of treatment with intravenous immunoglobulin.

The third aspect of the invention may provide a method for distinguishing between bacterial infection and Kawasaki disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for bacterial infection in the event that the subject is distinguished as having a bacterial infection, or selecting a treatment regimen for Kawasaki disease in the event that the subject is distinguished as having Kawasaki disease. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having bacterial infection, the treatment regimen may comprise provision of treatment with an antibiotic, while in the case of a subject distinguished as having Kawasaki disease, the treatment regimen may comprise provision of treatment with intravenous immunoglobulin.

The fourth aspect of the invention may provide a method for distinguishing between active TB and a group of diseases consisting of: viral disease and SLE in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB, or selecting a treatment regimen for viral disease or SLE in the event that the subject is distinguished as having one of these diseases. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having active TB, the treatment regimen may comprise provision of treatment with an anti-mycobacterial agent, in the case of a subject distinguished as having a viral disease the treatment regimen may comprise provision of treatment with an anti-viral agent, while in the case of a subject distinguished as having SLE, the treatment regimen may comprise provision of treatment with an anti-lupus therapeutic agent, optionally selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

The fifth aspect of the invention may provide a method for distinguishing between SLE and a viral disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for viral disease in the event that the subject is distinguished as

having a viral disease, or selecting a treatment regimen for SLE in the event that the subject is distinguished as having SLE. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having a viral disease, the treatment regimen may comprise provision of treatment with an anti-viral agent, while in the case of a subject distinguished as having SLE, the treatment regimen may comprise provision of treatment with an anti-lupus therapeutic agent, optionally selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

The sixth aspect of the invention may provide a method for distinguishing between viral disease and active TB in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for viral disease in the event that the subject is distinguished as having a viral disease, or selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having a viral disease, the treatment regimen may comprise provision of treatment with an anti-viral agent, while in the case of a subject distinguished as having active TB, the treatment regimen may comprise provision of treatment with an anti-mycobacterial agent (such as an antibiotic).

The seventh aspect of the invention may provide a method for distinguishing between SLE, viral disease, and active TB in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for SLE in the event that the subject is distinguished as having SLE, selecting a treatment regimen for viral disease in the event that the subject is distinguished as having a viral disease, or selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject distinguished as having SLE, the treatment regimen may comprise provision of treatment with a an anti-lupus therapeutic agent (optionally selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug), in the case of a subject distinguished as having a viral disease, the treatment regimen may comprise provision of treatment with an anti-viral agent, while in the case of a subject distinguished as having active TB, the treatment regimen may comprise provision of treatment with an anti-mycobacterial agent (such as an antibiotic).

The eighth aspect of the invention may provide a method for determining the presence of Kawasaki disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for Kawasaki disease in the event that the subject is determined as having Kawasaki disease present. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject determined as having Kawasaki disease present, the treatment regimen may comprise provision of intravenous immunoglobulin.

The ninth aspect of the invention may provide a method for determining the presence of a viral disease in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for a viral disease in the event that the subject is determined as having a viral disease present. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject determined as having a viral disease present, the treatment regimen may comprise provision of an anti-viral agent.

The tenth aspect of the invention may provide a method for determining the presence of a TB infection in a subject with fever, and selecting a suitable treatment regimen for the subject on the basis of this method. In such an embodiment, the method may further comprise selecting a treatment regimen for TB in the event that the subject is determined as having active TB present. In a suitable embodiment, such a method may further comprise the selected treatment regimen being provided to the subject. In the case of a subject determined as having active TB present, the treatment regimen may comprise provision of an anti-mycobacterial agent (such as an antibiotic).

Medical uses and methods of treatment of the invention

The methods of the invention are useful in identifying what therapeutic agents will be helpful in treating a subject's fever. The ability to stratify patients in this manner further gives rise to the twenty-second to forty-seventh aspects of the invention, which provide medical uses and methods of treatment.

In a twenty-second aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of a therapeutic agent for a non-interferon activating disease, wherein the subject has been

distinguished as having a non-interferon activating disease by means of a method in accordance with the first aspect of the invention. In a suitable embodiment, the therapeutic agent for a non-interferon activating disease is an anti-infective therapy. The anti-infective therapy may suitably be selected from the group consisting of: an antibiotic; an antifungal agent; and immunoglobulin for intravenous administration.

In a twenty-third aspect, the invention provides a therapeutic agent for a non-interferon activating disease for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a non-interferon activating disease by means of a method in accordance with the first aspect of the invention. In a suitable embodiment, the therapeutic agent for a non-interferon activating disease is an anti-infective agent. Suitably, the anti-infective agent is selected from the group consisting of: an antibiotic; an antifungal agent; and immunoglobulin for intravenous administration.

In a twenty-fourth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an therapeutic agent for an interferon activating disease, wherein the subject has been distinguished as having an interferon activating disease by means of a method in accordance with the first aspect of the invention. In a suitable embodiment, the therapeutic agent for an interferon activating disease may be an immunomodulatory therapeutic agent. In a suitable embodiment, the therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; an antiviral agent; and an antibiotic.

In a twenty-fifth aspect, the invention provides an immunomodulatory therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having an interferon activating disease by means of a method in accordance with the first aspect of the invention. In a suitable embodiment, the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; anti-inflammatory agent; an antiviral agent; and an antibiotic.

In a twenty-sixth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-fungal therapeutic agent, wherein the subject has been distinguished as having a systemic fungal infection by means of a method in accordance with the second aspect of the invention. In a suitable embodiment, the anti-fungal therapeutic agent is an anti-fungal drug.

In a twenty-seventh aspect, the invention provides an anti-fungal therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a systemic fungal infection by means of a method in accordance with the second aspect of the invention. In a suitable embodiment, the anti-fungal therapeutic agent is an antifungal drug.

In a twenty-eighth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-infective therapeutic agent, wherein the subject has been distinguished as having a disease selected from: bacterial infection; and Kawasaki disease by means of a method in accordance with the second aspect of the invention. In a suitable embodiment, the anti-infective therapeutic agent is selected from the group consisting of: an antibiotic; and immunoglobulin for intravenous administration.

In a twenty-ninth aspect, the invention provides an anti-infective therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having one of a group of diseases consisting of: bacterial infection; and Kawasaki disease by means of a method in accordance with the second aspect of the invention. In a suitable embodiment, the anti-infective therapeutic agent is selected from the group consisting of: an antibiotic; and immunoglobulin for intravenous administration.

In a thirtieth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-Kawasaki disease therapeutic agent, wherein the subject has been distinguished as having Kawasaki disease by means of a method in accordance with the third aspect of the invention. In a suitable embodiment, the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

In a thirty-first aspect, the invention provides an anti-Kawasaki disease therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having Kawasaki disease by means of a method in accordance with the third aspect of the invention. In a suitable embodiment, the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

In a thirty-second aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-bacterial therapeutic agent, wherein the subject has been distinguished as having a bacterial

infection by means of a method in accordance with the third aspect of the invention. In a suitable embodiment, the anti-bacterial therapeutic agent is an antibiotic.

In a thirty-third aspect, the invention provides an anti-bacterial therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a bacterial infection by means of a method in accordance with the third aspect of the invention. In a suitable embodiment, the anti-bacterial therapeutic agent is an antibiotic.

In a thirty-fourth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-tuberculosis therapeutic agent, wherein the subject has been distinguished as having active TB by means of a method in accordance with the fourth, sixth or seventh aspect of the invention. In a suitable embodiment, the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent (for example, an antibiotic).

In a thirty-fifth aspect, the invention provides an anti-tuberculosis therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having active TB by means of a method in accordance with the fourth, sixth or seventh aspect of the invention. In a suitable embodiment, the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent, such as an antibiotic.

In a thirty-sixth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an immunomodulatory therapeutic agent, wherein the subject has been distinguished as having a disease selected from: viral disease; and SLE by means of a method in accordance with the fourth aspect of the invention. In a suitable embodiment, the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; and an antiviral agent.

In a thirty-seventh aspect, the invention provides an immunomodulatory therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having one of a group of diseases consisting of: viral disease; and SLE by means of a method in accordance with the fourth aspect of the invention. In a suitable embodiment, the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; and an antiviral agent.

In a thirty-eighth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-lupus therapeutic agent, wherein the subject has been distinguished as having SLE by means of a method in accordance with the fifth or seventh aspect of the invention. In a suitable embodiment, the anti-lupus therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

In a thirty-ninth aspect, the invention provides an anti-lupus therapeutic agent for use in treatment of a subject with a fever, wherein the subject has been distinguished as having SLE by means of a method in accordance with the fifth or seventh aspect of the invention. In a suitable embodiment, the anti-lupus therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

In a fortieth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-viral therapeutic agent, wherein the subject has been distinguished as having a viral disease by means of a method in accordance with the fifth, sixth or seventh aspect of the invention.

In a forty-first aspect, the invention provides an anti-viral therapeutic agent for use in treatment of a subject with a fever, wherein the subject has been distinguished as having a viral disease by means of a method in accordance with the fifth, sixth or seventh aspect of the invention.

In a forty-second aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-Kawasaki disease therapeutic agent, wherein the subject has been determined as having Kawasaki disease by means of a method in accordance with the eighth aspect of the invention. In a suitable embodiment, the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

In a forty-third aspect, the invention provides an anti-Kawasaki disease therapeutic for use in the treatment of a subject with a fever, wherein the subject has been determined as having Kawasaki disease by means of a method in accordance with the eighth aspect of the invention. In a suitable embodiment, the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

In a forty-fourth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-viral agent, wherein the subject has been determined as having a viral disease by means of a method in accordance with the ninth aspect of the invention.

In a forty-fifth aspect, the invention provides an anti-viral agent for use in treatment of a subject with a fever, wherein the subject has been determined as having a viral disease by means of a method in accordance with the ninth aspect of the invention.

In a forty-sixth aspect, the invention provides a method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-tuberculosis therapeutic agent, wherein the subject has been determined as having active TB by means of a method in accordance with the tenth aspect of the invention. In a suitable embodiment, the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent (such as an antibiotic).

In a forty-seventh aspect, the invention provides an anti-tuberculosis therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been determined as having active TB by means of a method in accordance with the tenth aspect of the invention. In a suitable embodiment, the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent (such as an antibiotic).

It will be appreciated that medical uses and methods of treatment according to the twenty-second to forty-seventh aspects of the invention represent the selection of a suitable empirical treatment regimen.

The invention will now be further described with reference to the accompanying Examples.

EXAMPLES

Example 1 - Identification of origin-proxy reference genes in granulocytes with LRA using multiple datasets

In order to identify robust origin-proxy reference genes, various gene expression datasets from a wide spectrum of diseases, were analysed by modified CoDa. Almost all datasets investigated were gene expression datasets of whole blood (WB) samples unless indicated

otherwise. Gene expression quantification was performed by a variety of methodology ranging from microarray to RNA-sequencing as shown in Table 22.

To identify OP reference genes for granulocytes, LRA biplots of the 4 KD datasets in Table 22 were completed and analysed as shown in Figure 3. A list of 40 genes expressed predominantly by granulocytes were used in the LRA biplots to identify OP reference genes for granulocytes (as listed in the patent: *Determination of gene expression levels of a cell type*, US9589099B2). Loadings of PC1 and PC2 of each gene were used to calculate their distance from the origin. The list of 10 genes closest to the origin were identified for each dataset. These 10 genes were then filtered for those genes with moderate to high expression in blood samples (with Transcript per million (TPM) value above 750 in any blood cells in the NCBI GEO dataset GSE107011 (Monaco et al., 2019)). Only those genes that ranked among the top 10 in both datasets (GSE73461 and GSE68004) were further considered as potential origin-proxy reference genes.

A list of the 5 OP reference genes was decided upon by identifying which reference genes had a moderate to high expression in blood cells and which were also ranked top 10 by criteria for selection of origin-proxy reference gene in both datasets (GSE73461 and GSE68004). These top 5 gene are located in Table 23.

After looking at gene expression levels, only 3 genes, SAT1, SRGN and NCF1 showed a high expression level in peripheral blood (based on The Human Protein Atlas (HPA) website (*The Human Protein Atlas*, 2022)). Therefore, only these 3 genes were further used as OP reference gene specific for granulocytes in peripheral blood samples and denominators to derive RBB in subsequent examples and experiments.

Example 2 - Identification of origin-proxy reference genes in monocytes with LRA using multiple datasets

Using the same approach as discussed in Example 1, OP reference genes were determined from a list of 17 genes predominantly expressed by monocyte in peripheral blood. LRA biplots of these genes (Figure 3E) in GSE63881 dataset were analysed.

Both, CTSS and PSAP genes were located close to the origin (Figure 3E) and also have a sufficiently high expression level. For comparison, CD14 which is a well-known cell specific marker for monocyte, was located further away from the origin than these two monocyte OP reference genes.

The usefulness of monocyte OP reference genes is validated in two independent datasets which contain gene expression data of both peripheral whole blood and monocytes purified from the same samples. Typically, the CD14 gene would be used as the denominator gene as it is a well-known cell marker for monocyte. However, this RBB does not have good correlation with gene expression (e.g. LYZ) in purified monocytes (Figure 3F, $R^2=0.074$).

On the other hand, RBBs using OP reference genes as identified here are more revealing and showed better correlation with target gene (LYZ, numerator in RBB) expression in purified monocytes. Figure 3G shows a much higher correlation ($R^2=0.7$) between a RBB determined in whole blood samples using an monocyte OP reference gene as denominator (log of the ratio of LYZ to CTSS in whole blood, Y axis) and gene expression in purified monocytes (log of the ratio of LYZ to a housekeeping gene (B2M), X axis). The results are obtained from GSE60424 dataset.

Similarly, RBB of another target gene (VNN1) using another OP reference gene PSAP (Figure 3I) as the denominator shows a much higher ($R^2=0.77$ vs $R^2=0.29$) correlation with VNN1 expression purified monocytes than RBB using CD14 (Figure 3H) as the denominator in GSE163605 PBMC dataset.

As results were consistent across gene expression analysis platforms as shown in Example 1 and Example 2, the results are robust and can be translated into clinical application. As whole blood (WB) samples are composed of all types of blood cells, the same analysis methodology could also be applied to blood samples after enrichment of certain cell populations, such as peripheral blood mononuclear cells (PBMC) and other sample types after cell isolation.

Example 3 - A hierarchical decision tree classification into Group 1: Non-interferon activating group and Group 2: Interferon activation group (Figure 2)

The RBB analysis approach of the invention revealed that various diseases of interest fall into one of the two common gene expression CoDa profiles. The two principal profiles are named as (1) Group 1: non-interferon activating group and (2) Group 2: interferon activating group. The 2 groups are named as such because the former are consisting of various non-interferon activating diseases (bacterial infection, Kawasaki disease, and systemic fungal infection) and the latter are characterized by conditions associated with interferon activation (including viral disease, systemic lupus erythematosus (SLE), and active tuberculosis (TB)).

Gene expression data belonging to group 1 was obtained from patients with bacterial infection, fungal infection, or Kawasaki disease in the datasets of GSE176260, GSE176261, GSE63990, GSE73461 and GSE73463 (Table 22). Data belonging to group 2 was obtained from with viral disease, active tuberculosis or SLE in the datasets of GSE176260, GSE176261, GSE73461, GSE100150, GSE63990, GSE49454, GSE65391, GSE107994, GSE107991, and GSE83456 (Table 22).

A hierarchical classification approach is used to triage a patient into one of the 6 diseases or conditions. The first step is to classify the patient into these 2 major groups by the gene expression profile. It can be performed by using as few as two RBBs represented by 3 genes (the denominator gene is common to the two RBBs) to classify patients into 2 major groups as the first step (Figure 2). For example, patients belonging to group 1 have high group 1 RBB results and normal or low group 2 RBB results. On the other hand, patients in group 2 have high group 2 RBB results together with a normal or low group 1 RBB results.

In the following example, the method to define group 1 RBBs using Kawasaki disease (KD) as an example of group 1 is shown.

Example 4 - The method to define Ratio-Based Biomarkers (RBB) using the example KD patients (as an example of Group 1 Non-interferon activating diseases)

The potential denominators (OP reference genes) identified in the previous section were used here namely SAT1, SRGN and NCF1. Here, potential target genes (used as the numerator in RBB) were determined by performing another PCA of ALR (additive log ratios of genes in a given granulocyte genes list) using one of the OP reference gene as the chosen reference component (as the denominator of ALR). Kawasaki disease (KD) was used as a prototype model disease in the Group 1 (non-interferon activating diseases) to demonstrate the development of RBB by using this new modification of CoDa methods.

GSE73461 had blood gene expression data of KD patients and controls and was used in PCA analysis of ALR. ALR were derived for all candidate target genes in a given granulocyte gene list using one OP reference gene as the denominator. Then, PCA was used to generate a biplot showing both the vectors of each ALR and the locations of patients and controls according to their scores on the PC.

A list of genes predominantly expressed in granulocytes in peripheral blood was used. This list of potential target genes (numerators in the RBBs) can be formed from a list of genes

categorized by functional annotation genes, gene co-expression network or genes attributable to expression by certain cell types (Huang et al., 2021; L. S. N. Tang, 2017). Other lists of potential target (numerator) genes could also be used. For example, immune cell-type expression enrichment according to cell lineage or differentiation presented in The Human Protein Atlas (<https://www.proteinatlas.org/humanproteome/immune+cell>).

In a PCA analysis (Figure 4) of ALRs using SAT1 as the origin-proxy reference gene (denominator), ALR of the target gene ANXA3 = $\log(\text{ANXA3} / \text{SAT1}) = \log \text{ANXA3} - \log \text{SAT1}$. PCA biplot was used to show the loading of ALRs and location coordinates (also called scores) of each individual data point (for example patients and controls) as projected on the same PCA biplot. Figure 4 shows a PCA biplot of ALR for KD patients and controls in dataset GSE73461. Group centroid was determined for both KD and controls and Figure 4 shows the PCA plot after rotation of PC1 (principal component 1 or Dimension 1) and PC2 (principal component 2 or Dimension 2) to maximally separate KD and control along PC1. KD samples were distributed on the right side of the biplot. The projection of ALR of ANXA3 target gene is labelled in the PCA biplot by an arrow-line. Similarly, ALR of other target genes can be derived accordingly. Furthermore, the 3 OP reference genes (SAT1, NCF1, SRGN) were used in turn as the reference in ALR and similar PCA analysis can be performed which gave comparable projections (Figure 5B, 5C, 5D).

Two triangles (shown by dashed line in Figure 4) were superimposed into the biplot to demarcate those ALRs with higher loading in PC1 than PC2. ALRs inside the right triangle mark are potential RBBs (e.g. ANXA3 / SAT1) of positive predictor genes (e.g. ANXA3). On the other hand, ALRs inside the left triangles mark are potential RBBs (e.g. FCER1A / SAT1) of negative predictor genes (e.g. FCER1A) which have negative loadings on the PC1. ALRs and RBBs supportive of group 1 disease are located within either these left or right triangles. Vectors of RBBs specific for group 2 disease (e.g. IFIT1 / SAT1, see example 5) are pointing downward and are not included in both dashed line triangles in the PCA biplot due to a higher loading in PC2 than in PC1.

Each ALR (e.g. ANXA3/SAT1) corresponds to one RBB (e.g. the ratio of gene expression or transcript abundance between ANXA2 and SAT1 which can be measured in patient samples). Discriminating ALR (or RBB) are those showing maximal projection along PC1. They can be further validated by box plots and receiver-operator curve (ROC) analysis together with data from additional datasets (e.g. in Figure 6).

For clarity, only 31 selected granulocyte predictor genes are shown in the ALR PCA biplot in Figure 5A using the same dataset GSE73461 as in Figure 4. The location of the vector of ALRs was comparable to Figure 4 indicating the advantage property of sub compositional coherent of CoDa. Similar to Figure 4, AXNA3 / SAT1 again has the maximal positive loading on PC1 and FCER1A / SAT1 has the most negative projection on PC1, while group 2 specific RBB (such as IFIT1 / SAT1 and RSAD2 / SAT1) are predominantly projected on PC2 located outside the 2 triangles marked by dash line and on the top of the chart.

This ALR PCA biplot and its results were validated in other datasets (GSE68004) in Figure 5B. In Figures 5C and 5D, other OP reference genes (NCF1 and SRGN, respectively) were used as the reference component (denominator) for ALR PCA. The results are highly comparable to Figure 5A in which SAT1 is used as the reference component, indicating that the 3 OP reference genes can be interchangeable to develop the RBB.

ALRs with top-ranked positive loading (RBB of positive predictor genes) or top-ranked negative loading (RBB of negative predictor genes) in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups along PC1 of dataset GSE73461 are shown in Table 24 and Figure 5A.

ALRs with top-ranked positive or negative loading in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups along PC1 of dataset GSE68004 are shown in Table 25 and Figure 5B.

It is evident that the list of top-ranked ALRs (with high positive and negative loadings) were highly comparable from the 2 separate datasets which supports the high reproducibility or robustness of this new CoDa approach in identifying RBB.

Expressing RBB in term of multiple of control group median (MoM)

As different gene expression assays generate results on a different scale, a method to normalize results obtained from various experimental platforms or datasets using the different scale is required for the shortlisted RBBs. One approach is to express results relative to the median value among all healthy controls. RBB data of only healthy controls were used to define a median value for each RBB in each dataset which is called the median of normal samples (or median in short). The results of the RBBs of all individuals in the dataset (including individual patients and control subjects) were expressed as multiples of this normal (control) group median value. This method of data representation is also called multiple of control group

median (MoM). Use of MoM also allows different assays to be compared as it does not require that assays are based on the same calibrators, methodology, quantification scale or other performance characteristics. As RBBs are represented in log scale as ALR, MoM (in log scale) is obtained by subtraction of the RBB result of an individual subject from the control group median. Data used in this example have been taken natural log so one unit on the MoM axis (e.g. Figure 6) represents ~2.7 fold higher expression than the control group median.

These selected ALRs (Table 24) are readily used as RBB to detect KD or other Group 1 non-interferon activating diseases. Figure 6 shows the distribution of 8 RBBs of KD and controls by box plots together with classification performance receiver operating characteristic curve (ROC) of those RBBs using SAT1 as the denominator in dataset GSE73461. The best positive and negative predictor RBB are ANXA3/SAT1 (Figure 6D, Area under the ROC Curve, AUC=0.96) and FCER1A/SAT1 (Figure 6A, AUC=0.98), respectively.

The classification utility of RBB is validated across datasets. For example, in Figure 7, MoM distribution of another positive predictor gene RBB (IL1R2/SAT1) of control and KD samples are shown as box plots for 4 datasets. The difference of this RBB is statistically significant across all datasets (non-parametric Wilcoxon test for group difference, p values ranged from 7e-10 to 2.2e-16).

The OP reference gene of the RBB is interchangeable. Figure 6D shows the box plot distribution of RBB of ANXA3 predictor gene (using SAT1 as the OP reference gene, and the denominator in the calculation of RBB) and classification performance by ROC. When the other 2 OP reference genes (SRGC or NCF, Figure 8) are used as the denominator to calculate RBB for the positive predictor gene ANXA3, case and control MoM distribution and classification performance (AUC=0.97 and 0.96 for OP reference genes SRGN and NCF1, respectively) were comparable to that obtained with SAT1 as the OP reference gene (Figure 8 and Figure 6D).

The classification performance of RBB using various OP reference genes are highly comparable. AUC of ROC using various predictor genes and various origin-proxy reference genes in the 3 datasets of KD, GSE73461, GSE73463, and GSE68004 are shown in Table 26. Various predictor genes (numerators of RBB) are shown in the first column and OP reference genes (denominator of RBB) are shown in first header row of Table 26. For each RBB (cell in the table 26), 3 AUC values are shown representing the ROC of that RBB in the classification of KD from control for the 3 datasets GSE73461, GSE73463, and GSE68004,

respectively. For example, RBB FCER1A/SAT1 has AUC of 0.98, 0.95, 0.97 in the 3 datasets GSE73461, GSE73463, GSE68004, respectively.

Example 5 -Combinational use of 2 or more RBB using 2-Dimensional classification plot (2D plot)

Using KD as an example of Group 1 diseases (non-interferon activating diseases), there were more positive predictor RBBs than negative predictor RBBs. In most published biomarker sets reported so far, positive predictors represent the great majority of biomarkers and very few negative predictors have been proposed or used (Bodkin et al., 2022). They are also different from previously reported biomarkers found by DEG methodology in KD (Hoang et al., 2014; Kuiper et al., 2022; Wright et al., 2018).

In example 4, there were several positive biomarkers (RBB) for KD, such as a positive RBB represented by MoM of log ratio of ANXA3 to SAT1. In some cases, it is preferred to combine information of more than one positive predictor RBB to make the classification.

Alternatively, a new method is proposed here to use 2 or more RBB. When 2 RBBs are used for classification, they can be presented in a 2D plot or bivariate plot. These plots show results of 2 RBBs for every patient in which individual's data appears as a data point using 2 RBBs as the coordinates on the plot. For example, MoM of one RBB is shown on the X-axis and that of another RBB is shown on the Y-axis. Classification regions for patient groups can be defined by classification decision boundaries, quadrants, or specific regional areas on the graph. Here, 2 RBB results are presented in 2D plots to enhance classification performance (Figure 9).

Using KD as an example of Group 1 diseases (non-interferon activating diseases), the results of a negative predictor RBB (e.g., MoM of log ratio of FCER1A to SAT1) and that of a positive predictor RBB (e.g., MoM of log ratio of ANXA3 to SAT1) were plotted in 2D to show the distribution of KD and controls. In Figure 9, the MoM of log (ANXA3 / SAT1) was plotted against MoM of log (FCER1A / SAT1). KD and controls were distributed in 2 different regions in the chart and formed 2 different clusters, such that KD samples were distributed in the FCER1A low and ANXA3 high area. The corresponding area in the 2D plot can then be used to discriminate KD from controls when data is obtained from a new patient for identification of the cause of their fever. It is evident that the classification region for KD is highly reproducible in both KD datasets, GSE73461 and GSE73463 (Figure 9).

The new findings of a negative predictor (FCER1A) and its associated RBB (e.g. MoM of log ratio of FCER1A to SAT1) provided another new possibility for the differentiation of KD from controls.

Example 6 - Group 2 interferon-activating diseases are differentiated from Group 1 non-interferon activating diseases

Viral diseases were used as a prototype to represent Group 2 interferon-activating diseases. ALRs based on either positive predictive genes or negative predictive genes are derived from viral disease (labelled as Definite Viral or adenovirus infection, HAdV) samples in 2 datasets (GSE73461, GSE68004) using PCA.

Top-ranked positive or negative loading in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups (VD vs control) along PC1 in dataset GSE73461 as shown in Table 27 and Figure 10.

Classification performance of RBBs in 2 viral disease (VD) datasets are shown in Table 28. Various predictor genes (the numerator of RBB) are shown in the first column and OP reference genes (the denominator of RBB) are shown in first row of Table 28. For each RBB (cell in the table 28), 2 AUC values are shown representing the ROC of that RBB in the classification of VD from control for the 2 datasets GSE73461, GSE68004. For example, RBB ADGRE3/SRGN has AUC of 0.90 and 0.81 (underlined in table 28) in the 2 datasets GSE73461, GSE68004, respectively. AUC values in Bold font correspond to those shown in Figures 11 and 12.

Group-wise comparison (VD vs control) in dataset GSE73461 of these RBBs expressed in term of MoM are shown in Figure 11 using NCF1 as the OP reference gene and their classification performance are shown by receiver-operator curve (ROC) analysis in Figure 11. Figure 12 shows the performance of RBB using another origin-proxy reference genes (SAT1) as denominators (OP reference gene). The AUC in ROC analysis were comparable when these 2 origin-proxy reference genes were used as the denominator of the RBB.

Example 7 - Using 2D plot or Bayesian probability calculation to differentiate KD and Viral disease by 2 RBBs

The classification performance can be enhanced by using more than one RBB. Here are examples on how to summate information from 2 RBBs. Two approaches are shown, by (a)

using 2D plot and setup classification boundary and/or (b) Bayesian probability method using post-test probability.

(a) using 2D plot and setup classification boundary

After identifying the useful granulocytes RBBs that can differentiate group 1 non-interferon activating group (KD as an example) and group 2 interferon activation group (VD as an example) from healthy controls, they are applied to differentiate these 2 groups (group 1 vs group 2) from each other in this example.

As the clinical features of KD is similar to viral disease in a paediatric setting, it is helpful to make their differentiation by blood biomarkers. As the RBB of blood gene expression was very different between KD and viral disease, they could be used to make a probability-based assessment if the patient has KD or viral disease.

Figure 13 show that a 2D plot using two RBB can very well differentiate KD from viral diseases (VD) and their respective classification boundaries. Therefore, a biomarker to differentiate KD from viral disease will be very useful in clinical applications.

When using 2 RBBs, the subjects of 2 classes can be displayed on 2-dimensional (2D) plots. Decision boundaries can be derived by various discriminant functions to divide the 2 classes. These decision boundaries could be linear, non-linear, or regional depends on the methods used for their definition. Commonly used methods include logistic regression, random forest, Naïve Bayes, and support vector machine (SVM). Naïve Bayes Classifier (Zhang, 2016) is used here as a default method (e.g. Figure 14 and Table 29) to calculate classification performance (including AUC in ROC analysis and balance accuracy).

(b) Bayesian probability method is used to assess the post-test probability of having a disease

The risk (probability) of disease in a patient can be modelled by Bayesian approach and its extension to use likelihood ratios (Bolin & Lam, 2013; Ranganathan & Aggarwal, 2018). Here, the probability of KD before this transcriptome blood test can be expressed as pre-test probability or pre-test odds. With a set of given results of the transcriptome biomarker tests, likelihood ratios (LR) of KD are determined by the ratio of true positive rate to false positive rate which is also expressed as sensitivity/(1-specificity).

Formulae:

Post-test odds = Pre-test odds x likelihood ratio(s)

Odds = Probability / (1-Probability)

Likelihood ratio for a positive test result (LR+) = true positive rate / false positive rate
(can also express as) = sensitivity / (1-specificity).

Probability (e.g. post-test probability) = odds (e.g. post-test odds) / (1 + odds)

Illustrative example of how it works:

Pre-test probability of having a disease can be determined from patient's characteristics and signs. For example, the probability of a paediatric patient with fever and rash of the typical age of KD would be estimated to be around 1 in 100 as KD is a rare disease. So the pre-test odds for KD is $0.01 / (1-0.01) \sim 0.01$.

Testing Scenario 1: When only 1 RBB is done (ANXA3/SAT1),

Test results: log (ANXA3/SAT1) is above the cutoff of 1.5 MoM

The sensitivity and specificity was both at 0.9 when a cutoff value at 1.5 MoM was used. Thus, the likelihood ratio for a positive test is equal to $0.9 / (1-0.9) = 9$.

Post-test odds = $0.01 \times 9 = 0.09$.

And conversion to probability:

Probability of KD of this patient after getting a positive RBB test (i.e. ANXA3/SAT1 is above 1.5 MoM) = $0.09 / (1+0.09) = 0.083$ (8.3 in 100).

Test Scenario 2: When 2 RBB are done (ANXA3/SAT1) and (LY6E/SAT1),

Test result-1: log (ANXA3/SAT1) is above the cutoff of 1.5 MoM. The LR for KD = 9 (as given in Scenario 1).

Test result-2: log (LY6E/SAT1) is below the cutoff of -0.7 MoM. The sensitivity and specificity for KD below this cutoff are both ~ 0.8 . Therefore, the LR for KD is $0.8 / (1-0.8) = 4$.

Post-test odds (after 2 RBB was done, and both in keeping with KD) = $0.01 \times 9 \times 4 = 0.36$.

And conversion to probability:

Probability of KD of this patient after doing 2 RBB = $0.36 / (1+0.36) = 0.26$ (26 in 100 or ~1 in 4).

This high chance of KD will support the clinician to prescribe intravenous immunoglobulin, the specific form of treatment for KD as the risk of adverse effect of untreated KD will outweigh treatment complications.

The values of pre-test odds can be stored and assigned according to local prevalence of the disease. Therefore, a central computer server can be used to handle these data. In one embodiment, the test works together with such computer system and further details are provided in a subsequent section (Figure 21).

(c) other multivariate classifiers

In addition to using likelihood ratio classification to combine information of multiple RBBs, other multivariate classifier methods can also be used. For example, linear models for classification are a battery of discriminant functions using multiple features to classify objects (Bishop, 2016).

Example 8 -Differentiation between Group 1 (non-interferon activating) and Group 2 (interferon activating) diseases

(a) by using granulocyte RBBs alone (2 RBBs)

In the previous example, only one disease is used as a prototype representing these 2 groups of diseases for classification. Here, all patients with Group 1 diseases (bacterial, fungal infections and KD) or Group 2 diseases (viral disease, active tuberculosis and SLE) in various datasets listed in Table 22 were pooled together for classification.

Firstly, the classification can be based on only 2 granulocyte RBBs and is shown in 2D plots (Figure 14). One of the two RBBs is chosen among RBBs of Group 1 disease. Group 1 non-interferon activating group RBBs can be found among ALRs (representing RBBs) shown in Table 24. The numerator of these RBBs are predictor genes and the denominator can be chosen among the OP reference genes, SAT1, NCF1 and SRGN. Similarly, Group 2 interferon activation group RBBs is found among ALRs shown in Table 27 and one of the three OP reference genes (SAT1, NCF1 and SRGN) could be used as the denominator. As FCER1A is

a negative predictor gene in both group 1 and group 2 disease, it was not used here to differentiate group 1 from group 2 patients.

Figure 14A shows a 2D scatter plot of a Group 2 RBB vs Group 1 RBB; namely MoM of log (RSAD2/SAT1) vs MoM of log (ANXA3 / SAT1). Group 1 patients are located in the lower right part of the plot while group 2 patients are located in the upper left portion of the chart. Various classification boundaries can be applied to the 2D plot (Figure 14A). The classification performance shown in Figure 14B was obtained by a Naïve Bayes Classifier which had a balanced accuracy of over 0.85. This figure represents a low-end estimate as other more robust multivariate classification methods can also be used (such as SVM, logistic regression, Bayesian likelihood ratio, neural network, etc) which are recognized to perform better than simple Naïve Bayes Classifier.

(b) by using both granulocyte RBBs and monocyte RBBs (4 RBBs)

Further RBBs can be added to improve the classification. For example, adding 2 monocyte RBB (first shown in Figure 3E of example 1), MoM of log (VNN1/ PSAP) and MoM of log (IFI27/PSAP) into the classification increases the balanced accuracy from 0.85 (as mentioned above in Figure 14B) to 0.879 (Figure 15A and 15B). And the Area Under Curve (AUC) is up to 0.95 (Figure 15A). Similarly, MoM of log (IFI44L/PSAP) can be used in place of MoM of log (IFI27/PSAP) to archive similar classification performance (AUC=0.95, balanced accuracy = 0.877, Figure 15C and 15D).

Example 9 - Differentiation among diseases within the Group 2 Interferon activation diseases – SLE

A robust method that can differentiate important diseases causing interferon activation (Group 2) is still lacking. Diseases in this group include viral illness, active tuberculosis, and SLE.

Activation of genes in the interferon pathway is well-recognized in SLE (Baechler et al., 2003; Banchereau et al., 2016; Feng et al., 2015; Mackay et al., 2016). A panel of genes is commonly used to determine an interferon score in SLE patients as a biomarker for disease activity (e.g., LY6E, OAS1, OASL, MX1 and ISG15) (Feng et al., 2015). On the other hand, there was no consensus on how many genes should be used to form the panel, as there have been SLE gene expression signatures based on a few genes to 100 genes (Ding et al., 2018). A more

difficult task is to differentiate between SLE and viral disease, both conditions are known to induce a similar profile of interferon-stimulated genes (Xu et al., 2021).

RBBs which use negative predictor genes (one or more from the list of these genes: FCER1A, HDC, CCN3, PI3 or CCR3) of VD can robustly differentiate SLE from VD (Figure 16). Here, for the first time, RBBs of these negative predictor genes were found to be suppressed in VD and TB but only minimally suppressed in SLE. Therefore, their RBB (for example CCN3 / SAT1) are good classifier to differentiate SLE from viral illness.

Classification performance of differentiating SLE from VD in 2D plots using the results of using a RBB of negative predictor genes with log (RSAD2/SAT1) in the differentiation of SLE patients from VD is shown in Table 29. The analysis was performed on SLE and VD samples in GSE100150.

Example 10 - Differentiation among diseases within the Group 2 Interferon activation diseases - Active Tuberculosis (TB)

TB is an important cause of febrile illness and needs to be picked up early for proper management and potential requirement of household screening and isolation. However, the culture of the causative mycobacteria takes a very long time, commonly requiring about 1 month to complete. Active TB induces interferon response in leukocytes and blood transcriptome gene panels have been developed for the diagnosis of TB (Berry et al., 2010; Blankley et al., 2016; Gupta et al., 2020; Sweeney, Braviak, et al., 2016; Turner et al., 2020). However, the latest evaluation of such panels showed that their performance was inadequate for clinical applications.

Here, RBBs are found to be useful in the identification of active TB patients. Figure 17, based on dataset GSE100150, shows 3 RBB of predictor genes (ANKRD222, SERPING1, BATF2) that can differentiate TB from VD in 2D plots together with either MoM log (RSAD2/SAT1) or MoM log (IFIT1/SAT1). In Figure 17A, the sample distribution of ANKRD22/SAT1 was plotted against RSAD2/SAT1. Active TB patients (filled squares symbol) had a higher ANKRD22/SAT1 and viral disease patients (open square symbols) had a higher RSAD2/SAT1. A classification region was draw as a rectangle and viral disease patients were mostly within this rectangle. The balanced accuracy of this classifier was above 0.9. In Figure 17B, another pair of RBBs are used. In this plot of SERPING1/SAT1 vs IFIT1/SAT1, TB patients are distributed in the left upper corner and viral disease patients were found mostly in the lower right. A linear discriminant boundary was shown, and the balanced accuracy was

again well above 0.8 in differentiating TB patients from viral disease. Similarly, in Figure 17C of BATF2/SAT1 vs RSAD2/SAT1 also archived a balanced accuracy of over 0.8 in differentiating TB patients from viral disease.

Results of using 2 RBB in differentiation of TB from VD using TB and VD samples in GSE100150 are shown in Table 30.

These RBBs are useful to differentiate TB and VD in samples pooled from multiple datasets, including GSE107991, GSE107994, GSE84356 and GSE100150 when used in a multivariate approach (see below, Example 13, Figure 20). The results confirmed the results in Figure 17 and Table 30 that these RBBs could be used to differentiate TB from VD.

Example 11 - Using multiple RBB in high dimensional classification of group 2 interferon-activating patients into viral disease, SLE and active TB

The three common diseases of the group 2 interferon-activating diseases (TB, viral illness and SLE) can be differentiated by a multivariate approach with using 3 or more RBB at the same time. Figure 18A shows 3 RBBs in a multivariate 3D plot with each axis showing one RBB, namely MoM of logs (RSAD2 / SAT1), (FCER1A / SAT1) and (ANKRD22 / SAT1). Using dataset GSE100150, patients with the 3 different diseases are clearly demarcated in the 3D plot, ANKRD22/NCF1 (as x axis), FCER1A/NCF1 (as y-axis) and RSAD2/NCF1 (as z-axis), illustrating the triage utility of the 3 RBB.

In this case, Naïve Bayesian classifier was applied using the data of these 3 RBBs. Other multivariate algorithms could also be used, such as t-SNE, UMAP, machine learning etc. When the 3 RBBs were evaluated at the same time, the AUC of ROC in the identification of TB was as high as 0.996 and 22 out of 23 active TB patients were correctly identified (Figure 18B and 18C). It was less accurate in identifying viral illness.

Example 12 - Other causes of the Group 1 non-interferon activating group could also be differentiated from bacterial infection by using additional RBB

Some RBBs mentioned in Example 11 above are also useful to delineate diseases inside the Group 1 non-interferon activating group. Although bacterial infection is the most common cause in Group 1, other pathogens can cause febrile illness occasionally. These less common pathogens causing Group 1 non-interferon activating disease are called atypical infections here. Candidemia (Systemic fungal infection) is used as an example of atypical non-interferon

activating disease here and it induces expression of some additional genes and thus their RBBs could be used as biomarker for systemic fungal infection (as an example of atypical non-interferon activating diseases in Group 1).

In group 1 diseases, to differentiate Candidemia from bacterial infection, one or more of these RBBs can be used. Activation of these RBBs also suggests atypical causes of non-interferon activating illness, and therefore pathogens in addition to bacterial pathogens should also be investigated.

Classification performance to differentiate candidemia from bacterial disease using Candidemia and Bacterial infection samples in GSE176260 is shown in Table 31.

KD is another disease inside the group 1. In order to differentiate KD from bacterial infection, one or more of these RBBs can be used: (IFITM3 / SAT1) and (CEACAM8/SAT1). Figure 19E shows a 2D plot of MoM log (IFITM3 / SAT1) vs MoM (CEACAM8 / SAT1). Patients with bacterial infection tend to have a higher value of the (CEACAM8 / SAT1) and a lower (IFITM3 / SAT1).

Example 13 - Using Classification methods or multivariate analysis approaches other than hierarchical classification can also use RBBs to triage febrile patients.

Multivariate approach such as using 3D plots is an example other than hierarchical classification that can utilize RBBs to triage patients (as shown in Figure 18). In addition, other methods can be equally well applied. High dimensional mapping such as t-SNE or U-Map mapping, can handle multivariate data and illustrate distance among data points (patients). They could be used as a method of clustering which allows the demarcation of patients to make classification.

Using SAT1 as the OP reference genes, these RBBs are used to build a t-SNE, U-Map or other multivariate approaches (Figure 20A and 20B). Figure 20 used all samples from all datasets listed in Table 22 except GSE63990 as it does not have data for some genes listed in Table 32.

19 RBBs are used to build a multivariate analysis for differential identification of the cause of fever are shown in Table 32. Together with the OP reference gene, their data separate patients into clusters of various etiologies (Figure 20)

In another example, only one RBB was taken from each classification node. Therefore, expression data of only 7 genes were used to build a multivariate map. Therefore, a minimal panel for quantification of transcript abundance in blood is 7 genes. A reduced set of 6 RBBs, as shown in Table 33, can also be used in multivariate classification or clustering.

Example 14 – Modelling the risk of disease

Using a computer system to incorporate clinical parameters, positive gene RBB and negative gene RBB to report a disease risk for new patients pending identification of the cause of their fever in question is shown in Figure 21

The risk (probability) of disease in a patient can be modelled by Bayesian approach and its extension to use likelihood ratios (Bolin & Lam, 2013; Ranganathan & Aggarwal, 2018).

In this embodiment, the probability of Kawasaki Disease before this transcriptome blood test can be expressed as pre-test probability or pre-test odds. With a set of given results of the transcriptome biomarker tests, likelihood ratios (LR) of KD is determined by the ratio of true positive rate to false positive rate which is also expressed as sensitivity/(1-specificity).

Post-test odds = Pre-test odds x likelihood ratio(s)

Odds = Probability / (1-Probability)

Likelihood ratio for a positive test result (LR+) = true positive rate / false positive rate
(can also express as) = sensitivity / (1-specificity).

Probability (e.g. post-test probability) = odds (e.g. post-test odd) / (1 + odds)

Pre-test probability of having a disease can be determined from patient's characteristics and signs. They are entered by doctor in-charge of the patient (1100) into a client computer in the hospital (1600). This pre-test probability is converted as pre-test odds (1500). This pre-test odds can be modified by hospital or other factors like if it is currently peak season of the disease and such information are entered by hospital or department administrator (1200). Then a blood test is performed for the patient (1300) and the results are converted to MoM (1400). Whenever possible, an overall median score or similar indication is determined to summarize MoM of all positive predictor genes (1400). In addition, another MoM of negative predictor genes (2400) is also determined. Patient's test results MoMs and other blood results

are then transmitted to test kit manufacturer's servers (or cloud server, 1820) to obtain the likelihood ratios corresponding to the results of RBBs. Post-test odds (1520, and probability) can then be determined by the above equation. All these data entry and calculation can be performed in a client computer (1600) in doctor's office except those procedures marked by broken line (1800) which represents procedures performed in the manufacturer's servers (or cloud server, 1820). This server keeps the datasets of all patients and controls. So, it will convert patients' test results into MoM and provides the likelihood ratio corresponding specific RBB levels.

As noted elsewhere, the methods of the invention may be non-diagnostic in their character. However, with agreement of the user, clinicians and hospital, the user can also later enter the final diagnosis when it is apparent after further laboratory tests and disease phenotype. So, the manufacturer can update likelihood ratios correspond to RBB levels of this patient in the server (or cloud server, 1820).

Example 15 – General laboratory procedure for quantification of transcript abundance of RBBs genes and reagent kit composition

Primers will need to be designed to determine the transcript abundance of these B lymphocyte informative genes. Some example primers that could be used in quantitative PCR (qPCR) are provided here for reference in Table 34. They can be used in the presence a SYBR green dye in qPCR reaction to get the threshold cycle data which could be used to determine the delta-CT, delta-delta CT or Efficiency corrected delta-CT as the biomarker parameters using relative quantification assays for transcript abundance of RNA in blood samples (Dorak, 2007). Plasma RNA (also known as circulating RNA) is also a possible sample type of cellular RNA that could be used to analyse host cell response by this method.

Firstly, to quantify transcript abundance, RNA was extracted from various blood samples using Trizol. Then, RNA was reverse transcribed into cDNA by a reverse transcriptase. Specific gene targets are quantified by a method of the choice of the user, which include qPCR, digital PCR, RNA-sequencing, microarray, and hybridization assays. Although PCR based method is mentioned in this embodiment, other hybridization-based assays, like barcoded probes commonly known as Nanostring or enzyme-mediated quantification assays, e.g. Loop-mediated isothermal amplification (LAMP), could also be used to quantify the transcript abundance of the RBB. Besides, some quantification methods that do not need to carry out reverse transcription could also be used.

The methods of using that cDNA samples can be used for quantification of transcript abundance of both genes forming the RBB. Primers useful for quantification of some of these genes are listed in Table 34. The biomarker parameter will be the delta-CT, delta-delta CT or Efficiency corrected delta-CT obtained from qPCR of such pair of genes constituting the RBB. In fact, the value of delta-delta CT using a calibration of pooled blood samples from multiple controls will be comparable to that of the MoM as the pooled blood samples could represent the median value of RBB of the control subjects. However, minor adjustments on the cut-off values are required as there is variation of the reagent lots and qPCR machines.

Furthermore, absolute quantification approaches with qPCR, digital PCR, hybridization, or sequencing assays could also be used and can perform the same classification utility.

Example 16 - Application to other animals.

As the gene sequences of relevant numerator and denominator gene are highly similar between humans and various other animals. This technology can therefore be applied to analyse blood samples of animals to differentiate the cause of infectious diseases and illness.

The high level of identity of gene sequences of the relevant numerator and denominator genes among human and other animal species is shown in Table 35. The % value shown is the level of identity of gene sequence (cDNA) between human gene and the orthologue gene of another animal species.

It is clear that there is a high level of identity between cDNA sequence between human and other vertebrates. For example, the sequence of ANXA3 is 89% identical between human and dog. Furthermore, SAT1 was 92% identical between both Human vs Dog and Human vs Cat.

TABLES

Table 1: List of G1 non-interferon activating diseases numerator genes for distinction from G2 Interferon activating diseases.

Non-interferon activating disease numerator gene(s)	Nature of the numerator gene in RBB	Examples that the numerators were originally from	Overlap with Group 2 interferon activating disease

ANXA3	Positive predictor gene	G1 (Kawasaki) vs G2 (viral), Kawasaki disease vs Control	No
ALPL	Positive predictor gene	G1 (Kawasaki) vs G2 (viral), Kawasaki disease vs Control	No
IL1R2	Positive predictor gene	G1 (Kawasaki) vs G2 (viral), Kawasaki disease vs Control	No
ARG1	Positive predictor gene	G1 (Kawasaki) vs G2 (viral), Kawasaki disease vs Control	No
MMP9	Positive predictor gene	G1 (Kawasaki) vs G2 (viral), Kawasaki disease vs Control	No

Table 2: List of G2 Interferon activating diseases numerator genes for distinction from G1 Non-interferon activating diseases.

Interferon activating disease numerator gene(s)	Nature of the numerator gene in RBB	Examples that the numerators were originally from	Overlap with Group 1 Non-interferon activating disease
IFIT1	Positive predictor gene	Viral disease vs Control, G1 (Kawasaki) vs G2 (viral)	No
RSAD2	Positive predictor gene	Viral disease vs Control, G1 (Kawasaki) vs G2 (viral)	No
IFIT3	Positive predictor gene	Viral disease vs Control, G1 (Kawasaki) vs G2 (viral)	No
HERC5	Positive predictor gene	Viral disease vs Control	No
PI3	Negative predictor gene	Viral disease vs Control	No
ADGRE3	Negative predictor gene	Viral disease vs Control	No

Table 3: List of denominator genes (OP reference genes for granulocytes in WB sample).

Denominator gene(s)
SAT1
SRGN
NCF1

Table 4: List of non-interferon activating numerator genes using Kawasaki disease as a prototype to find numerator genes for distinction from controls / healthy controls.

Non-interferon activating disease numerator gene(s)	Nature of the numerator gene in RBB
ANXA3	Positive predictor gene
ALPL	Positive predictor gene
IL1R2	Positive predictor gene
ARG1	Positive predictor gene
MMP9	Positive predictor gene
FCER1A	Negative predictor gene
CCR3	Negative predictor gene
HDC	Negative predictor gene

Table 5: List of viral disease numerator genes (as a prototype of G2 diseases) for distinction from G1 non-interferon activating diseases.

Viral disease numerator gene(s)	Nature of the numerator gene in RBB
IFIT1	Positive predictor gene
RSAD2	Positive predictor gene
IFIT3	Positive predictor gene
HERC5	Positive predictor gene
PI3	Negative predictor gene
ADGRE3	Negative predictor gene

Table 6: List of SLE numerator genes for distinction among G2 interferon activating diseases.

SLE numerator gene(s)	Nature of the numerator gene in RBB
FCER1A	Negative predictor gene (Viral will suppress expression but SLE fail to suppress)
HDC	Negative predictor gene (Viral will suppress expression but SLE fail to suppress)
CCR3	Negative predictor gene (Viral will suppress expression but SLE fail to suppress)
CCN3	Negative predictor gene (Viral will suppress expression but SLE fail to suppress)

Table 7: List of active TB numerator genes for distinction among G2 interferon activating diseases.

Active TB numerator gene(s)	Nature of the numerator gene in RBB
ANKRD22	Positive predictor gene
BATF2	Positive predictor gene
ETV7	Positive predictor gene
GBP5	Positive predictor gene
SERPING1	Positive predictor gene

Table 8: List of systemic fungal infection numerator genes for distinction among G1 non-interferon activating diseases.

Systemic fungal infection numerator gene(s)	Nature of the numerator gene in RBB
ANKRD22	Positive predictor gene
BATF2	Positive predictor gene
ETV7	Positive predictor gene
GBP5	Positive predictor gene
SERPING1	Positive predictor gene
CEACAM8	Positive predictor gene
CAMP	Positive predictor gene
LTF	Positive predictor gene

Table 9: List of common bacterial infection and Kawasaki disease numerator genes for detection of the presence of bacterial infection or Kawasaki disease.

Bacterial and Kawasaki disease numerator gene(s)	Nature of the numerator gene in RBB
ANXA3	Positive predictor gene
ALPL	Positive predictor gene
IL1R2	Positive predictor gene
ARG1	Positive predictor gene
MMP9	Positive predictor gene
FCER1A	Negative predictor gene
CCR3	Negative predictor gene
HDC	Negative predictor gene

Table 10: List of bacterial infection numerator genes for distinction from Kawasaki disease.

Bacterial infection numerator gene(s)	Nature of the numerator gene in RBB
CEACAM8	Positive predictor gene

Table 11: List of Kawasaki disease numerator genes for distinction from bacterial infection.

Kawasaki disease numerator gene(s)	Nature of the numerator gene in RBB
IFITM3	Positive predictor gene

Table 12: List of active TB numerator genes for detection of the presence of active TB

Active TB numerator gene(s)	Nature of the numerator gene in RBB
ANKRD22	Positive predictor gene
BATF2	Positive predictor gene
ETV7	Positive predictor gene
GBP5	Positive predictor gene
SERPING1	Positive predictor gene

Table 13: List of viral disease and SLE numerator genes for detection of the presence of viral disease or SLE

Viral disease and SLE numerator gene(s)	Nature of the numerator gene in RBB
IFIT1	Positive predictor gene
RSAD2	Positive predictor gene
IFIT3	Positive predictor gene
LY6E	Positive predictor gene
HERC5	Positive predictor gene
PI3	Negative predictor gene
ADGRE3	Negative predictor gene
FCER1A	Negative predictor gene
HDC	Negative predictor gene
CCR3	Negative predictor gene
CCN3	Negative predictor gene

Table 14: List of SLE numerator genes for distinction from viral disease and/or active TB.

SLE numerator gene(s)	Nature of the numerator gene in RBB
FCER1A	Negative predictor gene
HDC	Negative predictor gene
CCR3	Negative predictor gene
CCN3	Negative predictor gene
PI3	Negative predictor gene

Table 15: List of viral disease numerator genes for distinction from SLE and/or active TB.

Viral disease numerator gene(s)	Nature of the numerator gene in RBB
IFIT1	Positive predictor gene
RSAD2	Positive predictor gene
IFIT3	Positive predictor gene
HERC5	Positive predictor gene
PI3	Negative predictor gene
ADGRE3	Negative predictor gene

Table 16: List of active TB numerator genes for distinction from viral disease and/or SLE.

Active TB numerator gene(s)	Nature of the numerator gene in RBB
ANKRD22	Positive predictor gene
BATF2	Positive predictor gene
ETV7	Positive predictor gene
GBP5	Positive predictor gene
SERPING1	Positive predictor gene

Table 17: List of Kawasaki disease numerator genes for detection of Kawasaki disease.

Kawasaki disease numerator gene(s)	Nature of the numerator gene in RBB
ANXA3	Positive predictor gene
ALPL	Positive predictor gene
IL1R2	Positive predictor gene
ARG1	Positive predictor gene
MMP9	Positive predictor gene
FCER1A	Negative predictor gene
CCR3	Negative predictor gene
HDC	Negative predictor gene

Table 18: List of viral disease numerator genes for detection of viral disease.

Viral disease numerator gene(s)	Nature of the numerator gene in RBB
IFIT1	Positive predictor gene
RSAD2	Positive predictor gene
IFIT3	Positive predictor gene
HERC5	Positive predictor gene
PI3	Negative predictor gene
ADGRE3	Negative predictor gene

Table 19: List of active TB numerator genes for detection of active TB.

Active TB numerator gene(s)	Nature of the numerator gene in RBB
ANKRD22	Positive predictor gene
BATF2	Positive predictor gene
ETV7	Positive predictor gene
GBP5	Positive predictor gene
SERPING1	Positive predictor gene

Table 20: List of all potential numerator and denominator genes

Numerator and denominator gene(s)
ANXA3
ALPL
IL1R2
ARG1
MMP9
FCER1A
CCR3
HDC
IFIT1
RSAD2
IFIT3
HERC5
PI3
ADGRE3
ANKRD22
BATF2
ETV7
GBP5
SERPING1
CEACAM8
CAMP
LTF
CCN3
IFITM3
SAT1
SRGN
NCF1

Table 21: List of all potential numerator genes

Numerator gene(s)
ANXA3
ALPL
IL1R2
ARG1
MMP9
FCER1A
CCR3
HDC
IFIT1
RSAD2
IFIT3
HERC5
PI3
ADGRE3
ANKRD22
BATF2
ETV7
GBP5
SERPING1
CEACAM8
CAMP
LTF
CCN3
IFITM3

Table 22: Examples of gene expression datasets used to determine the OP reference gene and other RBBs.

Dataset identifier	Disease groups used in the analysis and sample size used in analysis (some samples may not be used if they failed QC as described in Huang et al., 2021)	Used to identify OP reference genes	References
GSE73461 (Illumina microarray, HumanHT-12)	• Definite Bacterial infection (n=52), • Kawasaki Disease (n=78), • Definite Viral disease (n=94), • Inflammatory diseases (n=84), • uncertain of bacterial or viral disease (n=96).	Yes	(Wright et al., 2018)
GSE68004 (Illumina microarray, HumanHT-12)	• Kawasaki Disease (n=87, including ◦ complete KD, and incomplete KD), • Bacterial infection (17 patients with Group A streptococcal disease (GAS, GAS/SF) disease) • Viral, adenovirus infection (n=19, HAdV)	Yes	(Jaggi et al., 2018)
GSE73463 (Illumina HumanHT-12 V4.0 beadchip)	• Kawasaki Disease (Acute, n=146 and Convalescent, n=87)	Yes	(Wright et al., 2018)
GSE63881 (Illumina HumanHT-12 V4.0 beadchip)	• Kawasaki Disease (Acute and Convalescent, total n=341)	Yes	(Hoang et al., 2014)
GSE100150 (Illumina microarray, HumanWG-6)	• Flu, (viral disease, n=24) • SLE (n=54) • TB (n=23)	No	(Altman et al., 2021)

GSE176260 (RNA-seq)	• Bacterial (n=34) • Candidemia (fungal infection, n=22) • Viral (n=48)	No	(Steinbrink et al., 2021)
GSE176261 (RNA-seq)	• Bacterial (n=8) • Candidemia (fungal infection, n=18) • Viral (n=10)	No	(Steinbrink et al., 2021)
GSE63990 (Affymetrix Human Genome U133A 2.0 Array)	• Bacterial (n=71) • Viral (n=115)	No	(Tsalik et al., 2016)
GSE49454 (Illumina HumanHT-12 V4.0 beadchip)	• SLE (n=156)	No	(Chiche et al., 2014)
GSE65391 (Illumina HumanHT-12 V4.0 beadchip)	• SLE (n=920)	No	(Banchereau et al., 2016)
GSE107994 and GSE107991 (RNA-seq Illumina HiSeq 4000)	• TB (n=50 and n=21)	No	(Singhania et al., 2018)
GSE83456 (Illumina HumanHT-12 V4.0 beadchip)	• TB (n=91, PTB and EPTB)	No	(Blankley et al., 2016b)

Table 23: Top 5 genes identified during selection of OP reference genes.

	Highest Expression level in GSE107011, TPM (Monaco et al., 2019)	Expression level (nTPM) in immune cell of HPA website (<i>The Human Protein Atlas</i> , 2022)
SAT1	>1000	34233
SRGN	>3000	16945
NCF1	>3000	3262
NCF1B	>750	n.a.
NCF1C	>750	n.a.

Table 24: ALRs with top-ranked positive loading (RBB of positive predictor genes) or top-ranked negative loading (RBB of negative predictor genes) in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups along PC1 of dataset GSE73461 (Figure 5A).

ALR	Loadings on PC1 after rotation	Nature of the numerator gene in RBB
FCER1A / SAT1	-1.0	Negative predictor gene
CCR3 / SAT1	-0.69	Negative predictor gene
HDC / SAT1	-0.69	Negative predictor gene
ANXA3 / SAT1	0.83	Positive predictor gene
IL1R2 / SAT1	0.83	Positive predictor gene
MMP9 / SAT1	0.70	Positive predictor gene
ALPL / SAT1	0.62	Positive predictor gene
ARG1 / SAT1	0.60	Positive predictor gene

Table 25: ALRs with top-ranked positive or negative loading in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups along PC1 of dataset GSE68004 (Figure 5B).

ALR	Loading on PC1 after rotation	Nature of the numerator gene
FCER1A / SAT1	-0.97	Negative predictor gene
CCR3 / SAT1	-0.37	Negative predictor gene
HDC / SAT1	-0.39	Negative predictor gene
ANXA3 / SAT1	0.85	Positive predictor gene
IL1R2 / SAT1	0.73	Positive predictor gene
MMP9 / SAT1	0.91	Positive predictor gene
ALPL / SAT1	0.78	Positive predictor gene
ARG1 / SAT1	0.84	Positive predictor gene

Table 26: Classification performance of RBBs in 3 KD datasets. 3 AUC values are shown representing the ROC analysis using RBBs of each numerator gene paired with an OP reference gene specified in the column header. The 3 KD datasets analysed are GSE73461, GSE73463 and GSE68004, respectively.

	SAT1	SRGN	NCF
FCER1A	0.98, 0.95, 0.97	0.98, 0.96, 0.97	0.96, 0.92, 0.97
CCR3	0.90, 0.84, 0.83	0.87, 0.81, 0.74	0.81, 0.80, 0.77
HDC	0.92, 0.88, 0.92	0.89, 0.86, 0.88	0.87, 0.85, 0.88
ANXA3	0.96, 0.94, 0.99	0.97, 0.95, 0.99	0.96, 0.92, 0.99
IL1R2	0.85, 0.85, 0.83	0.87, 0.84, 0.89	0.91, 0.86, 0.89
MMP9	0.88, 0.92, 0.95	0.90, 0.92, 0.96	0.94, 0.94, 0.98
ALPL	0.91, 0.92, 0.98	0.92, 0.92, 0.99	0.96, 0.92, 0.98
ARG1	0.84, 0.90, 0.91	0.87, 0.91, 0.94	0.89, 0.88, 0.93

Table 27: Top-ranked positive or negative loading in Dimension 1 (PC1) after rotation to maximize the separation of centroids of the 2 sample groups (VD vs control) along PC1 in dataset GSE73461 (Figure 10).

ALR	Loading on PC1 after rotation in GSE73461	Nature of the numerator gene
FCER1A / SAT1	-0.77	Negative predictor gene
PI3 / SAT1	-0.86	Negative predictor gene
ADGRE3 / SAT1	-0.70	Negative predictor gene
IFIT1 / SAT1	0.86	Positive predictor gene
RSAD2 / SAT1	0.87	Positive predictor gene
IFIT3 / SAT1	0.69	Positive predictor gene
HERC5 / SAT1	0.66	Positive predictor gene

Table 28: Classification performance of RBB in 2 viral disease (VD) datasets. 2 AUC values are shown representing the ROC analysis using RBBs of each numerator gene paired with an OP reference gene specified in the column header. The 2 VD datasets analysed are GSE73461 and GSE68004, respectively.

	SAT1 (also shown in Figure 12)	SRGN	NCF1 (also shown in Figure 11)
PI3	0.80 , 0.75	0.77, 0.67	0.74 , 0.70
ADGRE3	0.91 , 0.91	<u>0.90, 0.81</u>	0.86 , 0.81
IFIT1	0.77 , 0.74	0.78, 0.78	0.82 , 0.77
RSAD2	0.79 , 0.76	0.79, 0.82	0.83 , 0.80
IFIT3	0.74 , 0.77	0.76, 0.81	0.80 , 0.79
LY6E	0.74 , 0.82	0.76, 0.82	0.83 , 0.83
HERC5	0.73 , 0.72	0.76, 0.77	0.80 , 0.75

Table 29: Classification performance of differentiating SLE from VD in 2D plots using a RBB of negative predictor genes with log (RSAD2/SAT1). The analysis was performed on SLE and VD samples in GSE100150.

Ratio-based biomarkers	Balanced Accuracy in differentiation of SLE from viral illness by using 2 RBBs. The RBB shown in the left column was used together with RSAD2/SAT1 by a Naïve Bayes Classifier	AUC of ROC when the RBB shown in the left column was used together with RSAD2/SAT1 by a Naïve Bayes Classifier
CCN3/SAT1	0.84	0.94
CCR3/SAT1	0.74	0.87
HDC/SAT1	0.66	0.87
FCER1A/SAT1	0.64	0.74
PI3/SAT1	0.56	0.74

Table 30: Results of using 2 RBB in differentiation of TB from VD. The analysis was performed on TB and VD samples in GSE100150.

Ratio-based biomarkers	Balanced Accuracy by a Naïve Bayes Classifier using the RBB shown in the left column together with RSAD2/SAT1 in differentiation TB from viral illness	AUC in ROC differentiation by the same Naïve Bayes Classifier using the RBB shown in the left column together with RSAD2/SAT1 in differentiation TB from viral illness
GBP5/SAT1	0.92	0.98
ANKRD22/SAT1	0.91	0.95
SERPING1/SAT1	0.90	0.96
BATF2/SAT1	0.85	0.90
ETV7/SAT1	0.81	0.83

Table 31: Classification performance to differentiate candidemia from bacterial disease. The analysis was performed on Candidemia and Bacterial infection samples in GSE176260.

Ratio-based biomarkers	Balanced Accuracy by a Naïve Bayes Classifier using the RBB shown in the left column together with ANXA3/SAT1 in the differentiation of Candidemia from Bacterial infection	AUC in ROC differentiation by the same Naïve Bayes Classifier using the RBB shown in the left column together with ANXA3/SAT1 in the differentiation of Candidemia from Bacterial infection
CAMP/SAT1	0.902	0.888
ANKRD22/SAT1	0.894	0.918
CEACAM8/SAT1	0.888	0.951
LTF/SAT1	0.888	0.949
FCER1A/SAT1	0.886	0.884
IL5RA/SAT1	0.886	0.901
CCR3/SAT1	0.872	0.928
CCN3/SAT1	0.864	0.921
CLC/SAT1	0.864	0.928
HDC/SAT1	0.864	0.874
ALOX15/SAT1	0.864	0.917
BATF2/SAT1	0.857	0.889
GBP5/SAT1	0.857	0.876
SERPING1/SAT1	0.842	0.892
ETV7/SAT1	0.834	0.902

Table 32: 19 RBBs which can used to build a multivariate analysis for differential identification of the cause of a subject's fever.

RBB used to build a multivariate map of patients with difference diseases in the dataset. All RBB variables are expressed in term of MoM or equivalent.	Remarks
ANXA3 / SAT1	Group 1 / 2 differentiation
ALPL / SAT1	Group 1 / 2 differentiation
RSAD2 / SAT1	Group 1 / 2 differentiation
HERC5 / SAT1	Group 1 / 2 differentiation
ANKRD22 / SAT1	TB vs viral
BATF2 / SAT1	TB vs viral
ETV7 / SAT1	TB vs viral
GBP5 / SAT1	TB vs viral
SERPING1 / SAT1	TB vs viral
FCER1A / SAT1	SLE vs viral
CCR3 / SAT1	SLE vs viral
HDC / SAT1	SLE vs viral
CCN3 / SAT1	SLE vs viral
CEACAM8 / SAT1	Systemic fungal infection
LTF / SAT1	Systemic fungal infection
CAMP/SAT	Systemic fungal infection
LY6E /SAT1	KD vs Bacterial
IFITM3/SAT	KD vs Bacterial
IFIT1/SAT	KD vs Bacterial

Table 33: A reduced set of 6 RBBs that can also be used in multivariate classification or clustering.

RBB used to build a multivariate map of patients with different diseases in the dataset. All values are expressed as MoM	Remarks
ANXA3 / SAT1	Group 1 / 2 differentiation
RSAD2 / SAT1	Group 1 / 2 differentiation
BATF2 / SAT1	TB
FCER1A / SAT1	SLE
CEACAM8 / SAT1	Systemic fungal infection
LY6E / SAT1	KD

Table 34: List of primers useful in quantification of gene expression in whole blood samples to determine the RBB.

Gene	Primer 1	Primer 2
SAT1	CTAAATTCGTGATCCGCCCAG SEQ ID NO: 1	ATTCATATTTAGCCAGCTCCTTGAT SEQ ID NO: 2
SRGN	TCGAAC TACTTCCAGGTGAATCC SEQ ID NO: 3	TCCTGGATTCTCGTCTTTGGAAAA SEQ ID NO: 4
ANXA3	ACTTACTGTTGGCCATAGTTAATTG SEQ ID NO: 5	AGGCTCGATGCAGTCTTTTCG SEQ ID NO: 6
RSAD2	GCCTGAATCTAACCAGAAGATGAAA SEQ ID NO: 7	CAGGATGGACTTGGAAGGGTC SEQ ID NO: 8
IFIT1	ATTTACAGCAACCATGAGTACAAA SEQ ID NO: 9	TCGTCATCAATGGATAACTCCCA SEQ ID NO: 10
FCER1A	CCTTACTGTTCTTCGCTCCAGAT SEQ ID NO: 11	CATGGAGGGTTCAAGGAGAC SEQ ID NO: 12
CCR3	GCCTTACCCTTGCAGCTTACAG SEQ ID NO: 13	GTCCACTCTGTGGTCTGTTCA SEQ ID NO: 14
PSAP	ATGGCCGACATATGCAAGAA SEQ ID NO: 15	TCTCCTTGGGTTGCATGTGCA SEQ ID NO: 16
VNN1	GCAGATCAGGGTGCGCATATT SEQ ID NO: 17	GGTCTGGCCAAATCTGTTACG SEQ ID NO: 18
IFI44L	TGGGCCGTCAGTATTTGGAATGTGAAG SEQ ID NO: 19	TGAAACCAAGTCTGCATAGGG SEQ ID NO: 20
IFI27	TCTCCTTCTTTGGGTCTGGC SEQ ID NO: 21	TGGTCACTGCTGATGAGGTG SEQ ID NO: 22

Table 35: The high level of identity of gene sequences of the relevant numerator and denominator genes among human and other animal species. The % value shown is the level of identity of gene sequence (cDNA) between human gene and the orthologue gene of another animal species.

	Cat (<i>Felis catus</i>)	Dog (<i>Canis lupus dingo</i>)	Bird (<i>Gallus gallus</i>)	Reptile (<i>Chelonoidis abingdonii</i>)	Fish (<i>Carassius auratus</i>)
SAT1	92%	92%	77%	78%	73%
SRGN	76%	79%	50%	54%	36%
ANXA3	60%	89%	Not found	78%	63%
RSAD2	81%	81%	75%	73%	67%
IFIT1	69%	75%	53%	56%	39%
FCER1A	70%	62%	35%	32%	23%
CCR3	73%	75%	62%	62%	51%
PSAP	64%	85%	64%	62%	57%
VNN1	84%	84%	66%	51%	69%
IFI44L	68%	62%	Not found	50%	54%

Specific aspects and embodiments of the invention

The following paragraphs do not constitute claims, but do set out details of specific aspects, embodiments, and combinations of subject matter in respect of which the Applicants may wish to seek protection.

1. A method of indicating the cause of a subject's fever, the method comprising:
 - (a) analysing gene expression in a sample representative of gene expression in the subject with fever, and, based on this analysis:
 - (b) providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3; and
providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;
comparing the first RBB and second RBB; and
distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison; wherein
if non-interferon activating disease is distinguished further step (c) and optionally a still further step (d) is performed based on the analysis in (a), and
if interferon activating disease is distinguished further step (e) and optionally a still further step (f) is performed based on the analysis in (a);
 - (c) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3, and
providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;
comparing the third RBB and fourth RBB; and
distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease on the basis of this comparison; wherein
if systemic fungal disease is distinguished, the method indicates systemic fungal disease is the cause of the subject's fever; and
if the group of diseases consisting of: bacterial disease and Kawasaki disease is distinguished, a further step (d) is performed;

(d) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3; and

providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;

comparing the fifth RBB and sixth RBB; and

distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison; wherein

if bacterial disease is distinguished, the method indicates bacterial disease is the cause of the subject's fever; and

if Kawasaki disease is distinguished, the method indicates Kawasaki disease is the cause of the subject's fever;

(e) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3; and

providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;

comparing the third RBB and fourth RBB; and

distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison; wherein

if active TB is distinguished, the method indicates active TB is the cause of the subject's fever; and

if the group of diseases consisting of: viral disease and SLE is distinguished, a further step (f) is performed;

(f) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3; and

providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;

comparing the fifth RBB and sixth RBB; and

distinguishing between SLE and viral disease on the basis of this comparison; wherein

if SLE is distinguished, the method indicates SLE is the cause of the subject's fever;

and

if viral disease is distinguished, the method indicates viral disease is the cause of the subject's fever.

2. A method for distinguishing between non-interferon activating disease and interferon activating disease in a subject with fever, the method comprising:

- c) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
 - providing a first ratio-based biomarker (RBB) which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;
- d) comparing the first RBB and second RBB; and

distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison.

3. A method for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease in a subject with fever, the method comprising:

- c) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
 - providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;
- d) comparing the first RBB and second RBB; and

distinguishing between systemic fungal infection and the group of diseases consisting of: bacterial infection and Kawasaki disease on the basis of this comparison.

4. A method according to paragraph 3, wherein the subject has been distinguished as having non-interferon activating disease by a method according to paragraph 2.

5. A method for distinguishing between bacterial infection and Kawasaki disease in a subject with fever, the method comprising:

c) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3;
- providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;

d) comparing the first RBB and second RBB; and

distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison.

6. A method according to paragraph 5, wherein the subject has been distinguished as having bacterial infection or Kawasaki disease by a method according to paragraph 3 or 4.

7. A method for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE) in a subject with fever, the method comprising:

c) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3;
- providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;

d) comparing the first RBB and second RBB; and

distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison.

8. A method according to paragraph 7, wherein the subject has been distinguished as having an interferon-activated disease by a method according to paragraph 2.

9. A method for distinguishing between SLE and viral disease in a subject with fever, the method comprising:

c) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
- d) comparing the first RBB and second RBB; and
distinguishing between SLE and viral disease on the basis of this comparison.
10. A method according to paragraph 9, wherein, the subject has been distinguished as having SLE or a viral disease by a method according to paragraph 7 or paragraph 8.
11. A method according to any of paragraphs 1 to 10, wherein analysing gene expression in a sample is practiced *in vitro*.
12. A method according to any of paragraphs 1 to 11, wherein the denominator gene in each provided RBB is SAT1.
13. A method according to any of paragraphs 1 to 11, wherein the numerator gene from Table 1 in a provided RBB is ANXA3, and the numerator gene from Table 2 provided in an RBB is RSAD2.
14. A method according to any of paragraphs 1 to 13, wherein at least one RBB provided comprises a numerator gene selected from the group consisting of: BATF2; FCER1A; and CEACAM8.
15. A method according any of paragraphs 1 to 14, wherein the sample representative of gene expression in a subject comprises neutrophils.
16. A method according to paragraph 15, wherein the sample representative of gene expression in a subject comprises whole blood.
17. A method according to any of paragraphs 1 to 16, wherein gene expression is analysed by a technique selected from the group consisting of: qPCR, digital PCR, RNAseq, a DNA microarray assay, a DNA hybridization assay, a nanostring assay, and an enzyme-mediated quantification assay (such as Loop-mediated isothermal amplification – LAMP).

18. A method according to any of paragraphs 1 to 17, wherein the subject is a child.
19. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2.
20. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9.
21. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11.
22. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13.
23. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and

ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and

iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15.

24. A kit comprising:

xii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1; and

xiii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2; and

xiv. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8; and

xv. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9; and

xvi. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10; and

xvii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11; and

xviii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12; and

xix. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13; and

xx. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and

xxi. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and

xxii. at least one reagent capable of binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3.

25. A kit according to any of paragraphs 19 to 24, comprising a reagent capable of specifically binding to an RNA target molecule selected from the group consisting of: a primer; a primer pair; a probe; or a primer set and a probe.

CLAIMS

1. A method of indicating the cause of a subject's fever, the method comprising:
 - (a) analysing gene expression in a sample representative of gene expression in the subject with fever, and, based on this analysis:
 - (b) providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3; and
providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;
comparing the first RBB and second RBB; and
distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison; wherein
if non-interferon activating disease is distinguished further step (c) and optionally a still further step (d) is performed based on the analysis in (a), and
if interferon activating disease is distinguished further step (e) and optionally a still further step (f) is performed based on the analysis in (a);
 - (c) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3, and
providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;
comparing the third RBB and fourth RBB; and
distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease on the basis of this comparison; wherein
if systemic fungal disease is distinguished, the method indicates systemic fungal disease is the cause of the subject's fever; and
if the group of diseases consisting of: bacterial disease and Kawasaki disease is distinguished, a further step (d) is performed;
 - (d) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3; and
providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;

comparing the fifth RBB and sixth RBB; and
distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison; wherein
if bacterial disease is distinguished, the method indicates bacterial disease is the cause of the subject's fever; and
if Kawasaki disease is distinguished, the method indicates Kawasaki disease is the cause of the subject's fever;
(e) providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3; and
providing a fourth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;
comparing the third RBB and fourth RBB; and
distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison; wherein
if active TB is distinguished, the method indicates active TB is the cause of the subject's fever; and
if the group of diseases consisting of: viral disease and SLE is distinguished, a further step (f) is performed;
(f) providing a fifth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3; and
providing a sixth RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
comparing the fifth RBB and sixth RBB; and
distinguishing between SLE and viral disease on the basis of this comparison; wherein
if SLE is distinguished, the method indicates SLE is the cause of the subject's fever;
and
if viral disease is distinguished, the method indicates viral disease is the cause of the subject's fever.

2. A method for distinguishing between non-interferon activating disease and interferon activating disease in a subject with fever, the method comprising:
 - a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first ratio-based biomarker (RBB) which is the ratio between expression of a numerator gene selected from the group set out in Table 1 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 2 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between non-interferon activating disease and interferon activating disease on the basis of this comparison.
3. A method for distinguishing between systemic fungal infection and a group of diseases consisting of: bacterial infection and Kawasaki disease in a subject with fever, the method comprising:
- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 8 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 9 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
- distinguishing between systemic fungal infection and the group of diseases consisting of: bacterial infection or Kawasaki disease on the basis of this comparison.
4. A method for distinguishing between bacterial infection and Kawasaki disease in a subject with fever, the method comprising:
- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 10 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 11 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and

distinguishing between bacterial infection and Kawasaki disease on the basis of this comparison.

5. A method for distinguishing between active tuberculosis (TB) and a group of diseases consisting of: viral disease and systemic lupus erythematosus (SLE) in a subject with fever, the method comprising:

a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 12 and expression of a denominator gene selected from the group set out in Table 3;
- providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 13 and expression of a denominator gene selected from the group set out in Table 3;

b) comparing the first RBB and second RBB; and

distinguishing between active TB and the group of diseases consisting of: viral disease and SLE on the basis of this comparison.

6. A method for distinguishing between SLE and viral disease in a subject with fever, the method comprising:

a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3;
- providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;

b) comparing the first RBB and second RBB; and

distinguishing between SLE and viral disease on the basis of this comparison.

7. A method for distinguishing between viral disease and active TB in a subject with fever, the method comprising:

a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,

- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 16 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB and second RBB; and
distinguishing between viral disease and active TB on the basis of this comparison.
8. A method for distinguishing between SLE, viral disease, and active TB in a subject with fever, the method comprising:
- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a first RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 14 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a second RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 15 and expression of a denominator gene selected from the group set out in Table 3;
 - providing a third RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 16 and expression of a denominator gene selected from the group set out in Table 3;
- b) comparing the first RBB, second RBB and third RBB; and
distinguishing between SLE, viral disease, and active TB on the basis of this comparison.
9. A method of determining the presence of Kawasaki disease in a subject with fever, the method comprising:
- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
- providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 17 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and
determining the presence of Kawasaki disease on the basis of this comparison.

10. A method of determining the presence of viral disease in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
 - providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 18 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and

determining the presence of viral disease on the basis of this comparison.

11. A method of determining the presence of TB in a subject with fever, the method comprising:

- a) analysing gene expression in a sample representative of gene expression in the subject; and, based on this analysis,
 - providing a RBB which is the ratio between expression of a numerator gene selected from the group set out in Table 19 and expression of a denominator gene selected from the group set out in Table 3;
 - standardising the RBB;
- b) comparing the standardised RBB to a reference level of the RBB; and

determining the presence of active TB on the basis of this comparison.

12. The method according to any one of claims 1 to 7, wherein the comparison of the first RBB and the second RBB comprises determining the ratio of the first RBB to the second RBB and assessing the similarity of this determined ratio to reference data.

13. The method according to claim 8, wherein the comparison of the first RBB, second RBB and third RBB comprises determining at least two ratios selected from the group consisting of:

- the ratio of the first RBB to the second RBB;
- the ratio of the first RBB to the third RBB; and
- the ratio of the second RBB to the third RBB;

and assessing the similarity of the at least two determined ratios to reference data.

14. The method according to claims 12 or 13, wherein the reference data comprises reference values in respect of the determined ratio or ratios between RBBs in one or more of:

a sample representative of gene expression in a subject with a first disease; a sample representative of gene expression in a subject with a second disease; a sample representative of gene expression in a subject with a third disease; a sample representative of gene expression in a subject without the first disease; a sample representative of gene expression in a subject without the second disease; a sample representative of gene expression in a subject without the third disease; a sample representative of a healthy control.

15. The method according to claim 14, wherein the subject's disease is distinguished by assessing the similarity of the subject's determined ratio or ratios to the ratio of the same RBBs in the reference values.

16. The method according to claim 15, wherein the similarity is assessed in respect of a determined ratio by comparison of the determined ratio to a 2-dimensional plot of the reference data.

17. The method according to claim 15, wherein the similarity is assessed in respect of at least two determined ratios by comparison of the determined ratios to a 3-dimensional plot of the reference data.

18. The method according to claim 16, wherein the 2-dimensional plot of the reference data comprises one or more of: a geometric boundary and a linear boundary.

19. The method according to claim 18, wherein the 3-dimensional plot comprises one or more of: a 3D geometric boundary and a planar boundary.

20. The method according to claims 18 or 19, wherein the boundary is defined by a technique selected from the group consisting of: logistic regression; random forest; Naïve Bayes, support vector machine, Decision Tree, Nearest Neighbours and Neural network.

21. The method according to claim 20, wherein the boundary is defined by a Naïve Bayes classifier model.

22. The method according to any one of claims 18 to 21, wherein the assessment of similarity is with reference to a geometric boundary or 3D geometric boundary and comprises analysing whether a determined ratio or ratios in the subject is within the boundary.

23. The method according to claim 22, wherein presence of a determined ratio or ratios in the subject within the geometric boundary or 3D geometric boundary is indicative of a disease.

24. The method according to any of claims 18 to 21, wherein the linear boundary or planar boundary distinguishes at least two indicative areas independently selected from the group consisting of: a first disease; a second disease; a third disease; a subject without the first disease; a subject without the second disease; a subject without the third disease; and a healthy control.

25. The method according to claim 24, wherein the assessment of similarity comprises analysing whether a determined ratio or ratios in the subject is within an indicative area defined by the linear boundary or planar boundary.

26. A method according to any one of claim 1, 2, or claims 12, 14 to 16, 18, or 20 to 25 as dependent on claim 1 or 2, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1 and MMP9; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a non-interferon activating disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a non-interferon activating disease;
- this indicates that the subject has a non-interferon activating disease.

27. A method according to any one of claim 1, 2 or claims 12, 14 to 16, 18, or 20 to 25 as dependent on claim 1 or 2, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3, and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with an interferon activating disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without an interferon activating disease;
- this indicates that the subject has an interferon activating disease.

28. A method according to any one of claim 3, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 3, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5, SERPING1, CEACAM8, CAMP and LTF; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a systemic fungal infection; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a systemic fungal infection;
- this indicates that the subject has a systemic fungal infection.

29. A method according to any one of claim 3, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 3, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1, MMP9, FCER1A, CCR3 and HDC; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a bacterial infection or Kawasaki disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a bacterial infection or Kawasaki disease;
- this indicates that the subject has a bacterial infection or Kawasaki disease.

30. A method according to any one of claim 3, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 3, or claims 28 or 29, wherein the systemic fungal infection is Candidemia.

31. A method according to any one of claim 4, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 4, wherein:

- when the numerator gene of the first RBB is CEACAM8; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a bacterial infection; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a bacterial infection;
- this indicates that the subject has bacterial infection.

32. A method according to any one of claim 4, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 4, wherein:

- when the numerator gene of the second RBB is IFITM3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with Kawasaki disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without Kawasaki disease;
- this indicates that the subject has Kawasaki disease.

33. A method according to any one of claim 5, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 5, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB
- this indicates that the subject has active TB.

34. A method according to any one of claim 5, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 5, or claim 33, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3, ADGRE3, FCER1A, HDC, CCR3 and CCN3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease or SLE; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease or SLE;
- this indicates that the subject has a viral disease or SLE.

35. A method according to any one of claim 6, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 6, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: FCER1A, HDC, PI3, CCR3 and CCR3; and
- the determined ratio of the first and RBBs is assessed as similar to a reference value representative of gene expression in a subject with SLE; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without SLE;
- this indicates that the subject has SLE.

36. A method according to any one of claim 6, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 6, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

37. A method according to any one of claims 6, 35 to 36 wherein the subject has been distinguished as having SLE or viral disease by a method of any of claims 5 or 34.

38. A method according to any one of claim 7, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 7, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

39. A method according to any one of claim 7, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 6, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and second RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and second RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB;
- this indicates that the subject has active TB.

40. A method according to claims 38 or 39 wherein the subject has been distinguished as having viral disease or active TB by a method of any of claims 5 or 34.

41. A method according to any of claims 33 to 40, wherein the subject has been identified as having interferon activating disease by a method of any of claims 2 or 27.

42. A method according to any one of claim 8, or claims 13 to 15, 17, or 19 to 25 as dependent on claim 8, wherein:

- when the numerator gene of the first RBB is selected from the group consisting of: FCER1A, HDC, CCR3, CCN and PI3; and
- the determined ratio of the first RBB and second RBBs or the first and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with SLE; or
- the determined ratio of the first and second RBB or the first and third RBBs is assessed as not similar to the reference value representative of gene expression in a subject without SLE;
- this indicates that the subject has SLE.

43. A method according to any one of claim 8, or claims 13 to 15, 17 or 19 to 25 as dependent on claim 8, wherein:

- when the numerator gene of the second RBB is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the determined ratio of the first and second RBBs or the first and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the determined ratio of the second RBB to the first RBB and/or third RBB is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

44. A method according to any one of claim 8, or claims 13 to 15, 17 or 19 to 25 as dependent on claim 8, wherein:

- when the numerator gene of the third RBB is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the determined ratio of the first and third RBBs or the second and third RBBs is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the determined ratio of the first and third RBBs or second and third RBBs is assessed as not similar to a reference value representative of gene expression in a subject without active TB;
- this indicates that the subject has active TB.

45. A method according to any of claims 8 or 42 to 44, wherein the subject has been identified as having viral disease, SLE or active TB by a method of any of claims 5-7, 12, 14 to 16, 18, 20 to 25, or 33 to 41.

46. A method according any one of claim 9, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 9, wherein:

- when the numerator gene is selected from the group consisting of: ANXA3, ALPL, IL1R2, ARG1, MMP9, FCER1A, CCR3 and HDC; and
- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with Kawasaki disease; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject without Kawasaki disease;
- this indicates that the subject has Kawasaki disease.

47. A method according to anyone of claims 9 or 46, wherein the subject is a child under the age of 5 and has clinical features suggestive of Kawasaki disease.

48. A method according to any one of claims 9, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 9, or claims 46 or 47, wherein the subject has been identified as having non-interferon activating disease by a method of any of claims 1, 2 or 26.

49. A method according to any one of claim 10, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 10, wherein:

- when the numerator gene is selected from the group consisting of: IFIT1, RSAD2, IFIT3, HERC5, PI3 and ADGRE3; and
- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with a viral disease; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject without a viral disease;
- this indicates that the subject has a viral disease.

50. A method according to any one of claim 10, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 10, or 49, wherein the subject has been identified as having an interferon activating disease by a method of any of claims 1, 2, or 27.

51. A method according to any one of claim 11, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 11, wherein:

- when the numerator gene is selected from the group consisting of: ANKRD22, BATF2, ETV7, GBP5 and SERPING1; and
- the standardised RBB is assessed as similar to a reference value representative of gene expression in a subject with active TB; or
- the standardised RBB is assessed as not similar to a reference value representative of gene expression in a subject with active TB;
- this indicates that the subject has active TB.

52. A method according to any one of claim 11, or claims 12, 14 to 16, 18 or 20 to 25 as dependent on claim 11, or claim 51, wherein the subject has been identified as having an interferon activating disease by a method of any of claims 1, 2 or 27.

53. A method according to any preceding claim, wherein the sample representative of gene expression in the subject comprises granulocytes.

54. A method according to any preceding claim, wherein the sample representative of gene expression in the subject is a blood sample.

55. A method according to claim 54, wherein the blood sample is a sample of whole blood (WB)

56. A method according to any preceding claim, wherein gene expression is analysed by investigating a single cell type or subpopulation of blood cells in peripheral blood.

57. A method according to any preceding claim, wherein gene expression is analysed by investigating an RNA target molecule.
58. A method according to claim 57, wherein gene expression is analysed by a technique which is selected from a group consisting of: qPCR, digital PCR, RNAseq, DNA microarray assay, and a DNA hybridization assay.
59. A method according to any one of claims 1 to 56, wherein gene expression is analysed by investigating its peptide product as target molecule.
60. A method according to claim 59, wherein the peptide product of gene expression is analysed by a technique which is selected from a group consisting of: western blotting, flow cytometry and ELISA.
61. A method according to any preceding claim wherein the denominator gene is SAT1.
62. A method according to any preceding claim wherein the denominator gene is SRGN.
63. A method according to any preceding claim wherein the denominator gene is NCF1.
64. A method according to any preceding claim wherein the denominator gene is the same for each RBB provided.
65. A method according to any preceding claim, wherein the RBBs are standardised prior to comparison.
66. A method according to claim 63, wherein the standardisation of RBBs is by determination of multiple of control group medium (MoM) values for each RBB.
67. A method according to any one of claims 1 to 66, wherein the method further comprises selecting a suitable treatment regimen for the subject on the basis of this distinction or determination.
68. A method according to claim 67 as dependent on claim 1 or claim 2, wherein the method further comprises selecting a treatment regimen for non-interferon activating disease in the event that the subject is distinguished as having non-interferon activating disease, or

selecting a treatment regimen for interferon activating disease in the event that the subject is distinguished as having interferon activating disease.

69. A method according to claim 67 as dependent on claim 3, wherein the method further comprises selecting a treatment regimen for systemic fungal infection in the event that the subject is distinguished as having systemic fungal infection, or selecting a treatment regimen for bacterial infection or Kawasaki disease in the event that the subject is distinguished as having one of these diseases.

70. A method according to claim 67 as dependent on claim 4, wherein the method further comprises selecting a treatment regimen for bacterial infection in the event that the subject is distinguished as having a bacterial infection, or selecting a treatment regimen for Kawasaki disease in the event that the subject is distinguished as having Kawasaki disease.

71. A method according to claim 67 as dependent on claim 5, wherein the method further comprises selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB, or selecting a treatment regimen for viral disease or SLE in the event that the subject is distinguished as having one of these diseases.

72. A method according to claim 67 as dependent on claim 6, wherein the method further comprises selecting a treatment regimen for viral disease in the event that the subject is distinguished as having a viral disease, or selecting a treatment regimen for SLE in the event that the subject is distinguished as having SLE.

73. A method according to claim 67 as dependent on claim 7, wherein the method further comprises selecting a treatment regimen for viral disease in the event that the subject is distinguished as having a viral disease, or selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB.

74. A method according to claim 67 as dependent on claim 8, wherein the method further comprises selecting a treatment regimen for SLE in the event that the subject is distinguished as having SLE, selecting a treatment regimen for viral disease in the event that the subject is distinguished as having a viral disease, or selecting a treatment regimen for TB in the event that the subject is distinguished as having active TB.

75. A method according to claim 67 as dependent on claim 9, wherein the method further comprises selecting a treatment regimen for Kawasaki disease in the event that the subject is determined as having Kawasaki disease present.

76. A method according to claim 67 as dependent on claim 10, wherein the method further comprises selecting a treatment regimen for a viral disease in the event that the subject is determined as having a viral disease present.

77. A method according to claim 67 as dependent on claim 11, wherein the method further comprises selecting a treatment regimen for TB in the event that the subject is determined as having active TB present.

78. A method according to any one of claims 67 to 77, wherein the method further comprises the selected treatment regimen being provided to the subject.

79. A kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 1; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 2.

80. A kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 8; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 9.

81. A kit comprising:

- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 10; and
- ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and

- iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 11.
82. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 12; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 13.
83. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15.
84. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 16.
85. A kit comprising:
- i. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 14; and
 - ii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - iii. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 15; and
 - iv. a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 16.

86. A kit comprising:
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 17; and
 - a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - a reference standard indicating the reference level of the corresponding biomarker.
87. A kit comprising:
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 18; and
 - a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - a reference standard indicating the reference level of the corresponding biomarker.
88. A kit comprising:
- a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 19; and
 - a reagent capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 3; and
 - a reference standard indicating the reference level of the corresponding biomarker.
89. The kit of any one of claims 79 to 88, wherein the reagent is capable of specifically binding to an RNA target molecule.
90. The kit of claim 89, wherein the reagent capable of specifically binding to the target molecule is selected from the group consisting of: a primer; a primer pair; a probe; or a primer set and a probe.
91. The kit of claim 90, wherein the primer is selected from a group consisting of: a qPCR primer, a digital PCR primer, a RNAseq primer, a DNA microarray primer, a DNA hybridization primer, and a RNA hybridization primer.
92. The kit of any one of claims 79 to 88, wherein the reagent is capable of specifically binding to a peptide target molecule.
93. The kit of claim 92, wherein the reagent capable of specifically binding to the target molecule is selected from the group consisting of: an antibody or fragment thereof; a substrate;

an aptamer; an avimer; a peptidomimetic; a receptor or fragment thereof; a ligand; and a cofactor.

94. The kit of claim 93, wherein the antibody capable of specifically binding to the target molecule is a monoclonal antibody or a polyclonal antibody.

95. The kit according to any one of claims 92 to 94, wherein the antibody capable of specifically binding to the target molecule is a fluorochrome-labelled antibody.

96. The kit according to any of claims 79 to 95, wherein at least 50% of the reagents present in the kit are capable of specifically binding to a target molecule indicative of expression of a gene selected from the group set out in Table 20.

97. A method of identifying genes for use in an RBB for determining presence of a disease, the method comprising:

- obtaining a first set of data representative of gene expression in a sample of patients with the disease;
- obtaining a second set of data representative of gene expression in a sample of controls without the disease;
- performing an unweighted LRA analysis in respect of the first and second sets of data to generate an LRA biplot;
- identifying a first group of genes closest to the origin in the LRA biplot; and
- identifying a second group of genes furthest from the first group of genes in the LRA biplot, wherein
- the first group of genes provides the denominator of the RBB, and the second group of genes provides the numerator of the RBB.

98. A method according to claim 97, wherein identifying a second group of genes furthest from the first group of genes in the LRA biplot is along the axis that furthest separate patients from controls.

99. A method of identifying an origin-proxy reference gene in respect of a state of interest, the method comprising:

- obtaining a first set of data representative of gene expression in a sample of patients with the state of interest;
- obtaining a second set of data representative of gene expression in a sample of controls without the state of interest;

- performing an unweighted LRA analysis in respect of the first and second sets of data to generate an LRA biplot; and
- identifying the group of genes closest to the origin in the LRA biplot as origin-proxy reference genes.

100. A method according to claim 99, wherein the group of genes closest to the origin in the LRA biplot is along the axis that furthest separate patients from controls.

101. A method according to any one of claims 95 to 98, wherein obtaining the first and/or the second set of data is from a pre-defined gene list.

102. A method according to any of claims 97 to 101, wherein identifying a group of genes closest to the origin in the LRA biplot further comprises identifying a number of genes closest to the origin between 1 and 10.

103. A method according to any of claims 97 to 102, further comprising filtering the first group of genes by implementing a gene expression threshold.

104. A method according to claim 103, wherein the gene expression threshold is moderate to high gene expression.

105. A method according to claim 104, wherein the gene expression threshold is a value over 750 copies of transcript per million (TPM).

106. A method according to any of claims 97 to 105, further comprising dimension reduction analysis of the RBB.

107. A method according to claim 106, wherein the dimension reduction analysis is by principal component analysis (PCA) to generate a PCA biplot.

108. A method according to claim 107, further comprising a rotation of the PCA biplot axes.

109. A method according to claim 108, further comprising multiple rotations of the PCA biplot axes.

110. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of a therapeutic agent for a non-interferon

activating disease, wherein the subject has been distinguished as having a non-interferon activating disease by a method according to claim 1 or claim 2.

111. A method according to claim 110, wherein the therapeutic agent for a non-interferon activating disease is an anti-infective agent.

112. A method according to claim 111, wherein the anti-infective therapy is selected from the group consisting of: an antibiotic; an antifungal agent; and immunoglobulin for intravenous administration.

113. A therapeutic agent for a non-interferon activating disease for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a non-interferon activating disease by a method according to claim 1 or claim 2.

114. A therapeutic agent for a non-interferon activating disease for use according to claim 113, wherein the therapeutic agent is an anti-infective agent.

115. An anti-infective agent for use according to claim 114, wherein the anti-infective agent is selected from the group consisting of: an antibiotic; an antifungal agent; and immunoglobulin for intravenous administration.

116. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of a therapeutic agent for an interferon-activating disease, wherein the subject has been distinguished as having an interferon activating disease by a method according to claim 1 or claim 2.

117. A method according to claim 116, wherein the therapeutic agent for an interferon activating disease is an immunomodulatory therapeutic agent.

118. A method according to claim 116 or 117, wherein the therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; an antiviral agent; and an antibiotic.

119. An immunomodulatory therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having an interferon activating disease by a method according to claim 1 or claim 2.

120. An immunomodulatory therapeutic agent for use according to claim 119, wherein the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; an antiviral agent; and an antibiotic.

121. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-fungal therapeutic agent, wherein the subject has been distinguished as having a systemic fungal infection by a method according to claim 3.

122. A method according to claim 121, wherein the anti-fungal therapeutic agent is an anti-fungal drug.

123. An anti-fungal therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a systemic fungal infection by a method according to claim 3.

124. An anti-fungal therapeutic agent for use according to claim 123, wherein the anti-fungal therapeutic agent is an antifungal drug.

125. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-infective therapeutic agent, wherein the subject has been distinguished as having a disease selected from: bacterial infection; and Kawasaki disease by a method according to claim 3.

126. A method according to claim 125, wherein the anti-infective therapeutic agent is selected from the group consisting of: an antibiotic; and immunoglobulin for intravenous administration.

127. An anti-infective therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having one of a group of diseases consisting of: bacterial infection; and Kawasaki disease by a method according to claim 3.

128. An anti-infective therapeutic agent for use according to claim 127, wherein the anti-infective therapeutic agent is selected from the group consisting of: an antibiotic; and immunoglobulin for intravenous administration.

129. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-Kawasaki disease therapeutic agent, wherein the subject has been distinguished as having Kawasaki disease by a method according to claim 4 or claim 9.

130. A method according to claim 129, wherein the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

131. An anti-Kawasaki disease therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having Kawasaki disease by a method according to claim 4 or claim 9.

132. An anti-Kawasaki disease therapeutic agent for use according to claim 131, wherein the anti-Kawasaki disease therapeutic agent is immunoglobulin for intravenous administration.

133. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-bacterial therapeutic agent, wherein the subject has been distinguished as having a bacterial infection by a method according to claim 4.

134. A method according to claim 133, wherein the anti-bacterial therapeutic agent is an antibiotic.

135. An anti-bacterial therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having a bacterial infection by a method according to claim 4.

136. An anti-bacterial therapeutic agent for use according to claim 135, wherein the anti-bacterial therapeutic agent is an antibiotic.

137. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-tuberculosis therapeutic agent, wherein the subject has been distinguished as having active TB by a method according to any one of claims 5, 7, 8 or 11.

138. A method according to claim 137, wherein the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent.

139. A method according to claim 138, wherein the anti-mycobacterial agent is an antibiotic.

140. An anti-tuberculosis therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having active TB by a method according to any one of claims 5, 7, 8 or 11.

141. An anti-tuberculosis therapeutic agent for use according to claim 140, wherein the anti-tuberculosis therapeutic agent is an anti-mycobacterial agent.

142. An anti-tuberculosis therapeutic agent for use according to claim 141, wherein the anti-mycobacterial agent is an antibiotic.

143. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an immunomodulatory therapeutic agent, wherein the subject has been distinguished as having a disease selected from: viral disease; and SLE by a method according to claim 5.

144. A method according to claim 143, wherein the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; and an antiviral agent.

145. An immunomodulatory therapeutic agent for use in the treatment of a subject with a fever, wherein the subject has been distinguished as having one of a group of diseases consisting of: viral disease; and SLE by a method according to claim 5.

146. An immunomodulatory therapeutic agent for use according to claim 145, wherein the immunomodulatory therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-inflammatory agent; and an antiviral agent.

147. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-lupus therapeutic agent, wherein the subject has been distinguished as having SLE by a method according to any one of claims 6 or 8.

148. A method according to claim 147, wherein the anti-lupus therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

149. An anti-lupus therapeutic agent for use in treatment of a subject with a fever, wherein the subject has been distinguished as having SLE by a method according to any one of claims 6 or 8.

150. An anti-lupus therapeutic agent for use according to claim 149, wherein the anti-lupus therapeutic agent is selected from the group consisting of: an immunosuppressive agent; an anti-malarial agent; a steroid agent; and an anti-inflammatory drug.

151. A method of treating a subject with a fever, the method comprising providing the subject with a therapeutically effective amount of an anti-viral therapeutic agent, wherein the subject has been distinguished as having a viral disease by a method according to any one of claims 6, 7, 8 or 10.

152. An anti-viral therapeutic agent for use in treatment of a subject with a fever, wherein the subject has been distinguished as having a viral disease by a method according to any one of claims 6, 7, 8 or 10.