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(54) Title: METHODS FOR ASSESSING IMMUNE FITNESS

(57) Abstract: The present disclosure provides compositions and methods related to assessing the immune fitness of a subject. In particular, the present disclosure provides novel methods for assessing immune fitness of a subject through the measurement of immune cell differentiation, maturation, exhaustion, and senescence. Based on this assessment, the present disclosure provides prognostic information and risk/resilience stratification for surgical procedures and critical illness.

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METHODS FOR ASSESSING IMMUNE FITNESS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/451,998 filed March 14, 2023, which is incorporated herein by reference in its entirety and for all purposes.

FIELD

[0002] The present disclosure provides compositions and methods related to assessing the immune fitness of a subject. In particular, the present disclosure provides novel methods for assessing immune fitness of a subject through the measurement of immune cell differentiation, maturation, exhaustion, and senescence. Based on this assessment, the present disclosure provides prognostic information and risk/resilience stratification for surgical procedures and critical illness.

BACKGROUND

[0003] Humans endure numerous stresses, environmental insults, and immunological challenges throughout their lifespan. Their physiologic ability to adapt to these insults defines their resiliency, and evidence of prior injury and adaptation manifests in several ways. For example, clinicians intuitively estimate their patients' prior experience with injury and assess their suitability for subsequent injury by noting evidence of prior insults, such as physical scars, and ordered return to original structure and function. Practically, this is relevant when considering a patient for elective surgery; for example, surgeons noting that an unviolated abdomen is more permissive for surgical intervention than a scarred one, and preservation of form and function in previously insulted areas (minimal residual scar from prior surgeries) suggests less extensive prior injury and a general capability for ordered recovery following a subsequent operation.

[0004] These same principles can be applied to less obvious but perhaps more systemic signs of prior insult. Specifically, most insults are accompanied by an immune response, initiated with a generalized innate cell (neutrophil, natural killer cell or macrophage) response, recruiting the maturation of an adaptive (T and B) lymphocyte response, and culminating in specific protective immunity. Repeated adaptive responses are manifest through an accumulation of lymphocytes with phenotypic changes indicative of progressively advanced maturation from naïve cells to those that have encountered and responded to antigen. These

phenotypic changes include alterations in surface adhesion molecules and immune regulatory ligands that have been classified in terms of immune memory, prior antigen exposure, cellular exhaustion and ultimately senescence and death. Prior studies validating functional associations with particular surface phenotypes have established themselves in the viral response and cancer literature for memory, exhaustion, and senescence. More recently, markers of immune senescence and exhaustion have been associated with specific outcomes in transplantation and trauma. These changes accumulate over time, but are not time-dependent per se, in that people with severe childhood illness can exhibit advanced immune exhaustion early in life, and though aging populations drift toward immune maturation, there is considerable heterogeneity in the individual degree of immune maturation observed. As described further herein, a patient's history of immunological perturbation manifests as changes to the T-cell repertoire such that increases in prior immune activation portends progressively limited resilience to subsequent challenges.

SUMMARY

[0005] Embodiments of the present disclosure include a method of assessing a subject's immune fitness for a physiological stressor. In accordance with these embodiments, the method includes determining a frequency of CD4+ lymphocytes and a frequency CD8+ lymphocytes in population of cells isolated from a sample from a subject, and determining a frequency of at least one lymphocyte maturation marker in the population of cells isolated from the sample from the subject.

[0006] In some embodiments, determining the frequency of CD4+ lymphocytes, the frequency CD8+ lymphocytes, and/or the frequency of the at least one lymphocyte maturation marker comprises using flow cytometry to determine a relative frequency based on absolute count data.

[0007] In some embodiments, the at least one lymphocyte maturation marker comprises CD45RA, CCR7, CD27, CD28 CD57, CD244, and/or PD1.

[0008] In some embodiments, the method further comprises determining a ratio of CD4+ lymphocytes to CD8+ lymphocytes.

[0009] In some embodiments, the at least one lymphocyte comprises a T lymphocyte. In some embodiments, the at least one lymphocyte comprises a B lymphocyte.

[0010] In some embodiments, the method further comprises determining a frequency of CD3+ lymphocytes in the population of cells isolated from the sample from the subject, and

wherein the at least one lymphocyte maturation marker is determined from the population of CD3+ lymphocytes.

[0011] In some embodiments, the frequency of the at least one lymphocyte maturation marker is determined from the population of the CD4+ and/or CD8+ lymphocytes in the cells isolated from the sample from the subject.

[0012] In some embodiments, the sample is a blood sample, a serum sample, a tissue sample, a bone marrow sample, a lavage fluid sample, a cerebral spinal fluid sample, a urine sample, or a fecal sample.

[0013] In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a human.

[0014] In some embodiments, the physiological stressor comprises a surgical procedure performed on the subject. In some embodiments, the surgical procedure comprises head and neck surgery, hernia repair surgery, a mastectomy, or a thyroidectomy. In some embodiments, the surgical procedure comprises cardiovascular surgery, bariatric surgery, a colectomy, a cystectomy, a nephrectomy, a pancreatectomy, a prostatectomy, or a thoracotomy.

[0015] In some embodiments, the physiological stressor comprises a non-surgical medical procedure. In some embodiments, the physiological stressor comprises prolonged physical exertion.

[0016] In some embodiments, the method comprises assessing the subject's immune fitness within 60 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 30 days prior to exposure to the physiological stressor. In some embodiments, the method further comprises reassessing the subject's immune fitness after exposure to the physiological stressor. In some embodiments, the method further comprises assessing the subject's immune fitness before and after exposure to the physiological stressor.

[0017] In some embodiments, an increased frequency of CD57+ lymphocytes is indicative of immune system senescence and decreased immune fitness in the subject.

[0018] In some embodiments, an increased frequency of PD1+ lymphocytes is indicative of immune system exhaustion and decreased immune fitness in the subject.

[0019] In some embodiments, an increased frequency of CD244+ lymphocytes is indicative of immune system activation and decreased immune fitness in the subject.

[0020] In some embodiments, a decreased frequency of CD28+ lymphocytes is indicative of reduced naïve T cell activation and decreased immune fitness in the subject.

[0021] In some embodiments, a decreased frequency of CD45RA+ lymphocytes and/or CCR7+ lymphocytes is indicative of increased T cell maturation and decreased immune fitness in the subject.

[0022] In some embodiments, decreased immune fitness comprises an increased likelihood of at least one of hospital readmission, infection, system dysfunction, and/or post-surgical complications. In some embodiments, the method further comprises administering a treatment to the subject to increase immune fitness.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIGS 1A-1C: Age-related patterns of T-cell expression, boxplots showing relative frequency of CD8+ maturational T cell subsets at pre-surgery by decades of life (A). Boxplots showing relative frequency of CD4+ T-cell subsets expressing activation and exhaustion markers (B). Association of relative frequency of cell subsets pre-surgery with various post-surgery outcomes (C). Each row corresponds to a flow cytometry-gated cell subset. Cells in table are shaded by magnitude and direction of the test statistic (e.g., an increase in CD3+ CD4+ naïve cells was associated with a decreased odds of infection). The number in each cell corresponds to un-adjusted p-values with ** indicating that FDR was significant when adjusting for other tests within the same column. Clustering was performed using hierarchical clustering on a matrix of the Euclidean distance between rows (based on their test-statistics).

[0024] FIGS. 2A-2C: tSNE plots showing qualitative differences between groups (A). The first column displays information about cell density; all other columns display information about expression of a specific marker (from left to right: CD3, CD4, CD8, CD127, CD45RA, CCR7, CD57, CD244, PD-1, and CD28). Row 1 (Pooled) is pseudo-colored by marker expression levels using samples pooled across all outcomes and is provided to guide interpretation of the distribution of different cell types. All other rows show the relative difference, after density estimation and smoothing, in marker expression between two different pooled sample groups (from top to bottom: Pooled, Pre/Post Surgery, Age, Infection, Any Complication, System Dysfunction, and Readmission). Rows 4-7 groups (Infection, Any Complication, System Dysfunction, and Readmission) samples by various outcomes after surgery. The specific comparisons are post-surgery vs pre-surgery (row 2), age > median vs age <= median (row 3), infection vs no infection (row 4), any complication vs no complication (row 5), system dysfunction vs no system dysfunction (row 6), and readmission vs no readmission (row 7). For rows 2 onwards, Red = relative over-expression and Blue = relative underrepresent in the group name displayed in the left-hand row.

[0025] Association of the change in relative frequency from pre-surgery to post ("recovery") surgery observation and various clinical complications (B). Cells in table are shaded by size and direction of the test statistic (e.g., an increase in CD3+ CD4+ naïve cells was associated with a decreased odds of infection). The number in each cell corresponds to un-adjusted p-values with ** indicating that FDR was significant when adjusting for other tests within the same column. Clustering is done using hierarchical clustering on a matrix of the Euclidean distance between rows (based on their test-statistics).

[0026] Visualization of changes in three cell subsets from Pre-surgery to Recovery (C). Each color corresponds to a different cell subset. Plot is facet wrapped by procedure. Circles indicate pre-surgery observations, triangles indicate recovery.

[0027] FIGS. 3A-3B: Boxplot showing logit change of relative frequency of CD19+ lymphocytes at immediate post-surgery by visceral surgery (A). Boxplot showing logit change of relative frequency of cell subsets at immediate post-surgery by laparoscopic surgery (B).

[0028] FIG. 4: Boxplot showing logit change of relative frequency of cell subsets at immediate post-surgery by major surgery.

[0029] FIG. 5: TruCount and Phenoflow Gating strategies.

[0030] FIG. 6: Pie chart displaying number of patients by procedure. The legend labels the procedures in each slice of the pie chart going counterclockwise from Head and Neck (n=8) to Mastectomy (n=31).

[0031] FIG. 7: Flow tree for CD4+ and CD8+ subsets.

DETAILED DESCRIPTION

[0032] It has been reported that immune (inflammatory) aging creates a state of low chronic inflammation that can lead to higher susceptibility to infections and higher rates of morbidity and mortality. This has been mainly attributed to what is described as immune senescence, which is felt to be a gradual deterioration of both the innate and adaptive immune responses as a function of chronological age. However, it is becoming increasingly clear that heterogeneity in immune function occurs across all age groups and is clearly impacted by intrinsic and extrinsic variations (e.g., variable rates of thymic involution and differential environmental exposures, respectively). As such, loss of certain T-cell phenotypes arising from altered production or variations in differentiation are determinable, creating many age-independent paths to immune senescence or hyporesponsiveness. Furthermore, differing immunological backgrounds impact homeostatic challenges, including surgery and other physiological stressors.

[0033] The results described herein indicate that $CD4^+$ and $CD8^+$ naïve cells decrease as a patient ages. Additionally, after adjusting for age and other demographics, lower percentages of naïve T cells at baseline were associated with increased odds of postoperative complications. As evidence of prior immune activation increases, resilience to injury decreases. Another phenotypic characteristic of immune senescence is the expression of late-differentiated markers on memory cells reflecting the magnitude and diversity of antigens challenging the immune system throughout life. These memory cells display significant differences compared to naïve cells such as CD28 and CD27 expression, and increased expression of exhaustion and senescence markers such as CD57, CTLA-4, and PD-1. Results of the present disclosure indicate that higher levels of T cells with exhaustive/senescent phenotypes (e.g., $CD57^+$ or EM or EMRA subsets) at baseline were related to a higher likelihood of postoperative complications after elective surgery.

[0034] The clinical importance of cumulative immune experience, especially in the human response to injury and healing, has not been fully elucidated. Elective surgery in presumably immunologically balanced individuals is an opportunity to understand both the systemic and focal responses accompanying controlled and well-characterized injury to the human body. As the present disclosure illustrates, even in younger and seemingly healthy individuals, the immune response to controlled stress varies with immunological history, as reflected in immune cell phenotype. The effect of chronic stimulation of the immune system on survival has been studied in patients with CMV or HIV infection where chronic infection was clearly associated with all-cause mortality, independent of age, gender, race, ethnicity, and BMI. This phenotype shows features common to immune senescence, such as the filling of the immunological space with exhausted T cells expressing markers such as CD57 and PD-1, as well as by the decline in naive T-cell levels. The absence of findings related to a cancer diagnosis is worth clarifying. Patients approaching surgery after chemotherapy, or individuals with known immune deficiency were excluded from the experiments of the present disclosure. Thus, the present study was not designed or powered to assess any specific cancer type, or to explore the role of immune phenotype in cancer pathogenesis or prognosis, but rather to understand the relationship between prior, aggregate immune experience as a relevant parameter in acute surgical outcome.

[0035] Overall, the results and analysis described herein have discovered an age-independent relationship between baseline experience-driven immune phenotypes and acute outcomes following a physiological stressor (e.g., elective surgical procedures). Embodiments

of the present disclosure provide a more biologically precise means of pre-operative risk stratification and pre-emptive risk mitigation.

[0036] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0037] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0038] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0039] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0040] "Correlated to" as used herein refers to compared to.

[0041] The terms "administration of" and "administering" a composition as used herein refers to providing a composition of the present disclosure to a subject in need of treatment (e.g., antiviral treatment). The compositions of the present disclosure may be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, nebulization, or implant), by inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic

pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration.

[0042] As used herein, the term "subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (e.g., a monkey, such as a cynomolgus or rhesus monkey, chimpanzee, macaque, etc.) and a human). In some embodiments, the subject may be a human or a non-human. In one embodiment, the subject is a human. The subject or patient may be undergoing various forms of treatment.

[0043] As used herein, the term "sample" generally refers to a biological sample obtained from a subject. In some embodiments, the sample comprises a tissue and/or biological fluid obtained from a human patient. In some embodiments, the sample comprises whole blood, blood plasma, or serum. In some embodiments, the sample comprises cellular fluid, ascites, urine, feces, pancreatic fluid, fluid obtained during endoscopy, blood, mucus, or saliva. In some embodiments, the sample is a stool sample. Such samples can be obtained by any number of means known in the art, such as will be apparent to the skilled person. For instance, urine and fecal samples are easily attainable, while blood, ascites, serum, or pancreatic fluid samples can be obtained parenterally by using a needle and syringe, for instance. Cell free or substantially cell free samples can be obtained by subjecting the sample to various techniques known to those of skill in the art which include, but are not limited to, centrifugation and filtration.

[0044] As used herein, the term "treat," "treating" or "treatment" are each used interchangeably herein to describe reversing, alleviating, or inhibiting the progress of a disease and/or injury, or one or more symptoms of such disease, to which such term applies. Depending on the condition of the subject, the term also refers to preventing a disease, and includes preventing the onset of a disease, or preventing the symptoms associated with a disease (e.g., viral infection). A treatment may be either performed in an acute or chronic way. The term also refers to reducing the severity of a disease or symptoms associated with such disease prior to affliction with the disease. Such prevention or reduction of the severity of a disease prior to affliction refers to administration of a treatment to a subject that is not at the time of administration afflicted with the disease. "Preventing" also refers to preventing the recurrence of a disease or of one or more symptoms associated with such disease.

[0045] As used herein, "expression level" refers to the amount of a molecule expressed in a cell that corresponds to the physiological state of the cell. The expression level of a molecule can be represented by the amount of messenger RNA (mRNA) encoded by a gene, the amount

of polypeptide corresponding to a given amino acid sequence encoded by a gene, or the amount of biochemical forms of molecules expressed in a cell, including the amount of particular post-synthetic modifications of a molecule such as a polypeptide, nucleic acid or small molecule. As such, an expression level is intended to include a "gene expression level," a "cellular expression level," or both. The expression level can refer to an absolute amount of the molecule in a specimen or to a relative amount of the molecule. The expression level of a molecule can be determined relative to a control molecule in the specimen.

[0046] As used herein, an "expression profile" refers to a characteristic representation of the expression level of at least two molecules in a specimen such as a cell or tissue. The determination of an expression profile in a specimen from an individual is representative of the expression state of the individual. An expression profile reflects the gene expression level and/or cellular expression level of at least two molecules in a specimen such as a cell or tissue.

[0047] As used herein, a "reference expression level" refers to the expression level of a molecule that is correlated with an associated reference expression level. One skilled in the art can readily determine a reference expression level by determining the expression level of a molecule in a reference or control sample using appropriate standards. A reference expression level can be any level suitable for measuring and comparing expression levels of molecules between different samples.

[0048] The term "associated with" or "association with" refers to a change in one or more biomarkers (e.g., differences in frequency, level, or amount, e.g., greater or less than a reference) that occurs under a given set of conditions or parameters (e.g., as a correlation between the biomarker and the condition). The association can be made at any time point (e.g., prior to, during, or after development or onset of the condition(s)). The biomarker need not be causative of the condition, simply present at any point during the time course of the condition.

[0049]

[0050] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. Methods and Compositions for Assessing Immune Fitness

[0051] Embodiments of the present disclosure include compositions and methods related to assessing the immune fitness of a subject. In particular, the present disclosure provides novel methods for assessing immune fitness of a subject through the measurement of immune cell differentiation, maturation, exhaustion, and senescence. Based on this assessment, the present disclosure provides prognostic information and risk/resilience stratification for surgical procedures and critical illness.

[0052] As described further herein, the ability to characterize and quantify accumulating immunological alterations has been greatly improved by advances in multiparameter flow cytometry, and numerous changes in cell surface phenotype are now accepted as indicative of cellular lineage, antigen experience, effector function and cell fate. Embodiments of the present disclosure include the assessment of these changes (immune "scarring") as a systemic measure of accumulated insult to a patient's ability to sustain and recover from impending injury. Results of the present disclosure indicate that an assessment of a patient's T-cell maturation (e.g., quantifying relative accumulation of T cells with more advanced maturation states of memory, exhaustion and senescence) provides information relevant to the likelihood of the development of complications following a future physiological stressor (e.g., surgical intervention). As described further herein, embodiments of the present disclosure provide a prospective study of the relationship between peripheral immune phenotype and outcomes after elective surgery. Results indicate a significant association between pre-operative advanced immune experience and post-operative risk of complications.

[0053] In accordance with these embodiments, the present disclosure includes a method of assessing a subject's immune fitness for a physiological stressor. In some embodiments, the method includes determining a frequency of CD4+ lymphocytes and a frequency CD8+ lymphocytes in population of cells isolated from a sample from a subject. In some embodiments, the method includes determining a frequency of at least one lymphocyte maturation marker in the population of cells isolated from the sample from the subject.

[0054] In some embodiments, determining the frequency of CD4+ lymphocytes, the frequency CD8+ lymphocytes, and/or the frequency of the at least one lymphocyte maturation marker comprises using flow cytometry to determine a relative frequency based on absolute count data. In some embodiments, the at least one lymphocyte maturation marker comprises CD45RA, CCR7, CD27, CD28 CD57, CD244, and/or PD1.

[0055] In general, flow cytometry involves the passage of individual cells through the path of one or more laser beams. A scattering of a beam and excitation of any fluorescent molecule attached to, or found within, a cell is detected by photomultiplier tubes to create a readable output. Often optical filters and beam splitters direct various scattered light to detectors, which generate electronic signals proportional to intensity of light signals received. Data can be collected, stored in computer memory, and cell characteristics analyzed based on fluorescent and light scattering properties. In some instances, flow cytometry involves analysis of a single sample or involves high-throughput screening (e.g. 96-well or greater microtiter plates).

[0056] As would be recognized by one of ordinary skill in the art based on the present disclosure, cells may be labeled with one or more fluorophores and then excited by one or more lasers to emit light at the fluorophore emission frequency or frequencies. In some instances, fluorescence is measured as cells pass through multiple laser beams simultaneously. Several detection elements, e.g. fluorophore-conjugated antibodies or fluorescence markers, can be used simultaneously, so measurements made as one cell passes through a laser beam may consist of scattered light intensities as well as light intensities from each fluorophore. For example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more fluorescence markers are used. In some cases, a combination of fluorescence markers is used. Characterization of a single cell can comprise a set of measured light intensities that may be represented as a coordinate position in a multidimensional space (e.g., a feature coordinate space). A number of coordinate axes (the dimensions of the space) is often the number of fluorophores used plus one or more forward scatter or side scatter measurements.

[0057] Several types of fluorophores can be used as consistent with this application. Non-limiting examples are Alexa-Fluor dyes (e.g., Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 500, Alexa Fluor® 514, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 610, Alexa Fluor® 633, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, and Alexa Fluor® 750), APC, Cascade Blue, Cascade Yellow and R-phycoerythrin (PE), DyLight 405, DyLight 488, DyLight 550, DyLight 650, DyLight 680, DyLight 755, DyLight 800, FITC, Pacific Blue, PerCP, Rhodamine, Texas Red, Cy5, Cy5.5, and Cy7.

[0058] Alternately or in combination to fluorescence measurements, flow cytometry may measure at least one of cell size, cell volume, cell morphology, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of

intracellular proteins, or signaling or binding events in cells. In some instances, cell analysis by flow cytometry on the basis of fluorescent level is combined with a determination of other flow cytometry readable outputs, such as granularity or cell size to provide a correlation between the activation level of a multiplicity of elements and other cell qualities measurable by flow cytometry for single cells.

[0059] In some instances, flow cytometry data is presented as a single parameter histogram. Alternatively, or additionally, flow cytometry data is presented as 2-dimensional (2D) plots of parameters called cytograms. Often in cytograms, two measurement parameters are depicted such as one on an x-axis and one on a y-axis. In some instances, parameters depicted comprise at least one of side scatter signals (SSCs), forward scatter signals (FSCs), and fluorescence. In some instances, data in a cytogram is displayed as at least one of a dot plot, a pseudo-color dot plot, a contour plot, or a density plot. For example, data regarding cells of interest is determined by a position of the cells of interest in a contour or density plot. The contour or density plot can represent a number of cells that share a characteristic such as expression of particular biomarkers.

[0060] Flow cytometry data is conventionally analyzed by gating. Often sub-populations of cells are gated or demarcated within a plot. Gating can be performed manually or automatically. Manual gates, by way of non-limiting example, can take the form of polygons, squares, or dividing a cytogram into quadrants or other sectional measurements. In some instances, an operator can create or manually adjust the demarcations to generate new sub-populations of cells. Alternately or in combination, gating is performed automatically. Gating can be performed, in some part, manually or in some part automatically.

[0061] In various embodiments, the methods described herein comprise using a flow cytometry instrument (also referred to as a flow cytometer) to collect flow cytometry data. Flow cytometry is a technology for analyzing the physical and chemical characteristics of particles in a fluid that are passed in a stream through the beam of at least one laser. One way to analyze cell characteristics using flow cytometry is to label cells with a fluorophore and then excite the fluorophore with at least one laser to emit light at the fluorophore emission frequency. The fluorescence is measured as cells pass through one or more laser beams simultaneously. Up to thousands of cells per second can be analyzed as they pass through the laser beams in the liquid stream. Characteristics of the cells, such as their granularity, size, fluorescent response, and internal complexity, can be measured.

[0062] Flow cytometer instruments generally comprise three main systems: fluidics, optics, and electronics. The fluidic system may transport the cells in a stream of fluid through the laser

beams where they are illuminated. The optics system may be made up of lasers which illuminate the cells in the stream as they pass through the laser light and scatter the light from the laser. When a fluorophore is present on the cell, it will fluoresce at its characteristic frequency, which fluorescence is then detected via a lensing system. The intensity of the light in the forward scatter direction and side scatter direction may be used to determine size and granularity (i.e., internal complexity) of the cell. Optical filters and beam splitters may direct the various scattered light signals to the appropriate detectors, which generate electronic signals proportional to the intensity of the light signals they receive. Data may be thereby collected on each cell, may be stored in computer memory, and then the characteristics of those cells can be analyzed based on their fluorescent and light scattering properties. The electronic system may convert the light signals detected into electronic pulses that can be processed by a computer. Information on the quantity and signal intensity of different subsets within the overall cell sample can be identified and measured.

[0063] Flow cytometry data may be presented in the form of single parameter histograms or as 2-dimensional plots of parameters, generally referred to as cytograms, which display two measurement parameters, one on the x-axis and one on the y-axis, and the cell count as a density (dot) plot or contour map. FIGS. 4A and 4B show examples of 2-dimensional plots and some gates. In some embodiments, parameters are side scattering (SSC) intensity, forward scattering (FSC) intensity, or fluorescence. SSC and FSC intensity signals can be categorized as Area, Height, or Width signals (SSC-A, SSC-H, SSC-W and FSC-A, FSC-H, FSC-W) and represent the area, height, and width of the photo intensity pulse measured by the flow cytometer electronics. The area, height, and width of the forward and side scatter signals can provide information about the size and granularity, or internal structure, of a cell as it passes through the measurement lasers. In further embodiments, parameters, which consist of various characteristics of forward and side scattering intensity, and fluorescence intensity in particular channels, are used as axes for the histograms or cytograms. In some applications, biomarkers represent dimensions as well. Cytograms display the data in various forms, such as a dot plot, a pseudo-color dot plot, a contour plot, or a density plot. The data can be used to count cells in particular populations by detection of biomarkers and light intensity scattering parameters. A biomarker is detected when the intensity of the fluorescent emitted light for that biomarker reaches a particular threshold level.

[0064] In accordance with the above, embodiments of the methods of the present disclosure further comprises determining a ratio of CD4+ lymphocytes to CD8+ lymphocytes. In some

embodiments, determining a ratio of CD4+ lymphocytes to CD8+ lymphocytes is used to assess the immune fitness of a subject for a given physiological stressor.

[0065] In some embodiments, the at least one lymphocyte comprises a T lymphocyte. In some embodiments, the at least one lymphocyte comprises a B lymphocyte. In some embodiments, a population of T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a gradient or by counterflow centrifugal elutriation. The methods described herein can include selection of a specific subpopulation of lymphocytes, e.g., T cells, B cells, etc., and assessing these isolated lymphocytes for expression of at least one lymphocyte maturation marker comprises CD45RA, CCR7, CD27, CD28 CD57, CD244, and/or PD1.

[0066] In some embodiments, the methods of the present disclosure further comprise determining a frequency of CD3+ lymphocytes in a population of cells isolated from a sample from a subject. In accordance with these embodiments, at least one lymphocyte maturation marker is determined from the population of CD3+ lymphocytes. In some embodiments, the frequency of the at least one lymphocyte maturation marker is determined from the population of the CD4+ and/or CD8+ lymphocytes in the cells isolated from the sample from the subject. In some embodiments, determining a frequence comprises determining a relative frequency that is calculated using the respective absolute count data, with relative frequency equal to count+1 divided by parent count+1.

[0067] As described further herein, embodiments of the present disclosure include methods of assessing a subject's immune fitness for a physiological stressor by determining a frequency of at least one lymphocyte maturation marker in a population of cells isolated from a sample from a subject using flow cytometry. In accordance with these embodiments, the method includes assessing the isolated lymphocytes for expression of at least one lymphocyte maturation marker comprises CD45RA, CCR7, CD27, CD28 CD57, CD244, and/or PD1. In some embodiments, an increased frequency of CD57+ lymphocytes is indicative of immune system senescence and decreased immune fitness in the subject. In some embodiments, an increased frequency of PD1+ lymphocytes is indicative of immune system exhaustion and decreased immune fitness in the subject. In some embodiments, an increased frequency of CD244+ lymphocytes is indicative of immune system activation and decreased immune fitness in the subject. In some embodiments, a decreased frequency of CD28+ lymphocytes is indicative of reduced naïve T cell activation and decreased immune fitness in the subject. In some embodiments, a decreased frequency of CD45RA+ lymphocytes and/or CCR7+ lymphocytes is indicative of increased T cell maturation and decreased immune fitness in the

subject. In some embodiments, decreased immune fitness comprises an increased likelihood of at least one of hospital readmission, infection, system dysfunction, and/or post-surgical complications. In some embodiments, the method further comprises administering a treatment to the subject to increase immune fitness.

[0068] In some embodiments, a sample is obtained from a subject and used to isolate a population of cells for assessment, as described further herein. In some embodiments, the sample is a blood sample, a serum sample, a tissue sample, a bone marrow sample, a lavage fluid sample, a cerebral spinal fluid sample, a urine sample, or a fecal sample. In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a human.

[0069] As described further herein, embodiments of the present disclosure include methods of assessing a subject's immune fitness for a physiological stressor. As would be understood by one of ordinary skill in the art based on the present disclosure, the methods of the present disclosure can be used to assess the impact (e.g., prognostication) of any physiological stressor on a subject's immune fitness. In some embodiments, the physiological stressor comprises a surgical procedure performed on the subject. In some embodiments, the surgical procedure comprises head and neck surgery, hernia repair surgery, a mastectomy, or a thyroidectomy. In some embodiments, the surgical procedure comprises cardiovascular surgery, bariatric surgery, a colectomy, a cystectomy, a nephrectomy, a pancreatectomy, a prostatectomy, or a thoracotomy. In some embodiments, the physiological stressor comprises a non-surgical medical procedure. In some embodiments, the physiological stressor comprises prolonged physical exertion.

[0070] In some embodiments, the method comprises assessing the subject's immune fitness within 60 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 50 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 40 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 30 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 20 days prior to exposure to the physiological stressor. In some embodiments, the method comprises assessing the subject's immune fitness within 10 days prior to exposure to the physiological stressor. In some embodiments, the method further comprises reassessing the subject's immune fitness after exposure to the

physiological stressor. In some embodiments, the method further comprises assessing the subject's immune fitness before and after exposure to the physiological stressor.

[0071] Although the methods of the present disclosure generally involve the use of flow cytometry to assess a subject's immune fitness based on determining the expression of various immune system cell surface markers on a population of cells isolated from the subject, other means known in the art can be used for biomarker (e.g., protein or polypeptide) detection. For example, methods to measure biomarkers described herein, include, but are not limited to: Western blot, immunoblot, enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, liquid chromatography mass spectrometry (LC-MS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting (FACS), magnetic activated cell sorting (MACS), flow cytometry, laser scanning cytometry, hematology analyzer and assays based on a property of the protein including but not limited to DNA binding, ligand binding, or interaction with other protein partners.

[0072] The activity or level of a marker protein can also be detected and/or quantified by detecting or quantifying the expressed polypeptide. The polypeptide can be detected and quantified by any of a number of means well known to those of skill in the art. These can include analytic biochemical methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, or various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Western blotting, immunohistochemistry and the like. A skilled artisan can readily adapt known protein/antibody detection methods for use in determining the expression level of one or more biomarkers in a serum sample.

[0073] Another agent for detecting a polypeptide is an antibody capable of binding to a polypeptide corresponding to a marker described herein, e.g., an antibody with a detectable label. Antibodies can be polyclonal or monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary

antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

[0074] In another embodiment, the antibody is labeled, e.g., a radio-labeled, chromophore-labeled, fluorophore-labeled, or enzyme-labeled antibody. In another embodiment, an antibody derivative (e.g., an antibody conjugated with a substrate or with the protein or ligand of a protein-ligand pair (e.g., biotin-streptavidin)), or an antibody fragment (e.g., a single-chain antibody, an isolated antibody hypervariable domain, etc.) which binds specifically with a protein corresponding to the marker, such as the protein encoded by the open reading frame corresponding to the marker or such a protein which has undergone all or a portion of its normal post-translational modification, is used.

[0075] Proteins from cells can be isolated using techniques that are well known to those of skill in the art. The protein isolation methods employed can, for example, be such as those described in Harlow and Lane (Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

[0076] In accordance with the above embodiments, methods of the present disclosure can be applied as part of a clinical decision support algorithm. In some embodiments, the present disclosure provides algorithms and methods that can be used for diagnosis and monitoring of a subject's immune fitness before, during, and/or after exposure to one or more physiological stressors. For example, data obtained using the methods of the present disclosure can be input into a clinical decision support algorithm designed to normalize and or improve the reliability of the data, as well as integrate the data with other data pertaining to various aspects of the patient. In some embodiments, data analysis requires a computer or other device, machine or apparatus for application of the various algorithms described herein due to the large number of individual data points that are processed. A "machine learning algorithm" refers to a computational-based prediction methodology, also known to persons skilled in the art as a "classifier," employed for characterizing, for example, a biomarker profile or an immune fitness profile. Signals can correspond to certain biomarker levels and be input into the algorithm for processing. Supervised learning generally involves "training" a classifier to recognize the distinctions among classes and then "testing" the accuracy of the classifier on an independent test set. In some embodiments, meta-analysis can be used to generate the clinical support decision algorithm, such as that described by Fishel and Kaufman et al. 2007 *Bioinformatics* 23(13): 1599-606. In some cases, the algorithm may be supplemented with a meta-analysis approach such as a repeatability analysis. In some cases, the repeatability analysis selects biomarkers that appear in at least one predictive expression product marker set.

[0077] In some embodiments, the clinical support decision algorithm can include Bayesian analysis. The data can be subjected to a classification step, including any of the classification methods known in the art, and subsequently ranked according to a probability function. For example, a probability function may be derived from examining various patient parameters, and error rates may be calculated. These probabilities may then be combined with other patient profiling datasets, including the assessments of a patient's immune fitness according to the present disclosure. A statistical evaluation of the results of the patient profiling obtained using the clinical decision support algorithm may provide a quantitative value or values that can be presented directly to a physician in its most useful form to guide patient care. The results of the molecular profiling can be statistically evaluated using a number of methods known to the art including, but not limited to: the students T test, the two sided T test, pearson rank sum analysis, hidden markov model analysis, analysis of q-q plots, principal component analysis, one way ANOVA, two way ANOVA, LIMMA and the like. In some embodiments, accuracy may be determined by tracking the patient over time to determine the accuracy of an original diagnosis. In other cases, accuracy may be established in a deterministic manner or using statistical methods. For example, receiver operator characteristic (ROC) analysis may be used to determine the optimal assay parameters to achieve a specific level of accuracy, specificity, positive predictive value, negative predictive value, and/or false discovery rate. Methods for using ROC analysis in cancer diagnosis are known in the art and have been described for example in US Patent Application No. 2006/019615 herein incorporated by reference in its entirety.

3. Materials and Methods

[0078] *Patients and Clinical Assessments.* Two-hundred forth-eight (248) patients at Duke University Medical Center in a prospective, Institutional Review Board-approved trial facilitating comprehensive biospecimen and clinical data collection in patients scheduled to undergo elective surgery (Pro00110005); informed consent was obtained from all participants. The aim of the project was to evaluate baseline health at the time of elective surgical procedures, leveraging these controlled and anticipatable episodes of physiological stress to define the role of individual patient characteristics in their ability to maintain or return to homeostasis.

[0079] Clinical data were prospectively abstracted from electronic health records and combined with established case report files. Baseline demographics included gender, self-reported race, decade of life at the time of surgery, cancer-related procedure and body mass

index (BMI). Self-reported race included White, Black or African American, Native American/Alaskan Native, Asian, Native Hawaiian/Pacific islander, more than one and unknown/not reported. Body Mass Index was classified according to WHO guidelines as follows: underweight (below 18.5), normal weight (18.5-24.9), pre-obesity (25-29.9) and obese (30 or above).

[0080] Inclusion criteria included patients who were to undergo one of 12 well-described, relatively standardized elective surgical procedures chosen to include a broad spectrum of anatomic locations and magnitude of systemic perturbations. These included low intensity procedures limited to superficial locations (head and neck surgery, hernia repair, mastectomy and thyroidectomy) and visceral procedures of greater intensity involving celiotomy (bariatric surgery, colectomy, cystectomy, nephrectomy, pancreatectomy, prostatectomy, thoracotomy) or major vascular or cardiovascular intervention (FIG. 5). Each incorporated general, reproducible elements of surgery, including physiologic response to wounding, nutritional deprivation, changes in activity, post-injury hemostasis and homeostasis, wound healing, and its immune correlates. Patients who were living with human immunodeficiency virus or known demonstrable immune deficiency were excluded, as were patients who were undergoing neoadjuvant chemotherapy or other frankly immunosuppressive treatments.

[0081] Peripheral blood was collected pre-operatively, and immediately upon return to the post- anesthesia care unit. Postoperative complications that occurred within 30 days after surgery were captured. Infectious complications included Superficial Surgical Site Infection (SSSI, infection that involves only skin and subcutaneous tissue of incision), Deep Surgical Site Infection (DSSI, infection involves deep tissues, such as fascial and muscle layers), organ space soft tissue infection (infection involves any part of the anatomy in organs and spaces other than the incision, which was opened or manipulated during operation), clostridium difficile colitis, central line-associated bloodstream infection (CLABSI), pneumonia, urinary tract infection (UTI) and sepsis. System dysfunction included complications arising from the cardiovascular (myocardial infarction, pulmonary embolism, deep venous thrombosis, and stroke), renal (acute kidney injury), respiratory (unplanned intubation), and/or skin/musculoskeletal (decubitus ulcer) systems. Unplanned readmission included readmission to the hospital or intensive care unit (ICU).

[0082] *Flow cytometry.* Flow cytometry was performed as previously described. Briefly, blood was collected in a Streck Cyto-Chex® BCT tube and stored at room temperature for 2 to 5 days before being processed for phenotyping into 27 distinct phenotypic subsets. Absolute counts for leukocytes and major lymphocyte subsets were first determined using BD

TrucountTM Tubes and a lyse, no wash, staining method. Innate immune cell subsets included NK Bright ($CD56^{+}$Bright) and NK Dim ($CD56^{+}$Dim). Adaptive response immune cells included markers for pan T cells ($CD3^{+}$) and their broad subsets (CD4, CD8), pan B-cells (CD19), and validated markers of progressive immune maturation including T cell memory [CD45RA, CCR7 defining naïve, central memory (CM), Effector Memory (EM), and terminal effectors (EMRA) based on the classification of Salusto, et al[7]), exhaustion (PD1), senescence (CD57), and activation (CD244). 50 ul of mixed blood was carefully added by reverse pipetting to the TruCount tube containing a lyophilized bead pellet and antibodies. Samples were incubated, and then red cells lysed by adding 1X BD PharmLyse Buffer. After another incubation, cells were run on the cytometer within half an hour of staining completion. Process control was performed by processing a CD-Chex plus sample control with each run. Absolute counts for each population were calculated as instructed in the TruCount product insert. The TruCount Gating strategy is shown in FIG. 6. Data was collected using a BD Fortessa X20 instrument running BD FACS DIVA software version 8.0.1. Data were analyzed using FlowJoTM Software FlowJo version 9.9.6 (Ashland, OR).

[0083] *Statistical Analysis.* With the exception of the exploratory analysis of baseline characteristics, all analyses adjusted for the covariates of age, sex, race (White vs non-White) and surgery type. For each analysis of a single outcome, multiple testing adjustments were made using Benjamini-Hochberg false discovery rate (FDR). For the analysis of having a complication within 30 days of the procedure, having the complication was treated as the outcome (Yes/No) and a logistic regression model was fit with baseline cell type composition (logit transformed) as the primary variable of interest. For the complication analysis, adjustments were made for the duration of the procedure as opposed to procedure type. The change from pre-Surgery to recovery-Surgery was also examined, the primary independent variable was log fold change of the cell type composition between the two time points. Further adjustments were made for the time between the two sample collections. In the analyses examining how change in cell type composition was associated with various variables, a linear regression model was fit with change in cell type composition (logit transformed) as the outcome. Finally, in the analysis testing for association between cell type composition and time between sample collection, a mixed effects model was fit where each individual had a random intercept and slope. This analysis used all baseline observations (pre-surgery, n=248) as well as the follow-up analysis. Both the random slope of time between sample collection, as well as its fixed effect (via likelihood ratio test) were tested. Time at baseline was coded as 0. Adjustments were made for the same covariates as mentioned above. Relative frequency was

calculated using the respective absolute count data, with relative frequency equal to count+1 divided by parent count+1. In instances where the parent and child count agreed, only 1 was added to the parent count. Finally, visceral vs non-visceral surgery, cancer-related surgery, laparoscopic vs open approach, and major vs minor surgery (duration in or greater than the study median) were tested. Non-visceral surgery includes any of the following types of procedures: Head and neck, Vascular, Mastectomy, Thyroidectomy or Hernia repair. Otherwise, the procedures were classified as a visceral surgery.

4. Examples

[0084] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure, and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

[0085] The present disclosure has multiple aspects, illustrated by the following non-limiting examples.

Example 1

[0086] ***Demographics and Baseline immune phenotypes***. The study population (N=248) including their demographics, procedures, and complication rates are summarized in Tables 1 and 2, respectively.

[0087] Table 1: Patient Demographics and Procedures.

	Overall (N=248)
Procedure	
Mastectomy	31 (12.5%)
Cardiothoracic	28 (11.3%)
Colorectal	28 (11.3%)
Prostatectomy	27 (10.9%)
Hernia Repair	25 (10.1%)
Nephrectomy	25 (10.1%)
Bariatric	17 (6.9%)
Thyroidectomy	17 (6.9%)
Cystectomy	15 (6.0%)

Pancreatectomy	15 (6.0%)
Vascular	12 (4.8%)
Head and Neck	8 (3.2%)
BMI category	
Underweight	5 (2.0%)
Normal Weight/Pre-Obesity	136 (54.8%)
Obese	107 (43.1%)
Sex	
Female	123 (49.6%)
Male	125 (50.4%)
Race	
White	178 (71.8%)
Black or African American	50 (20.2%)
Unknown/Not Reported	11 (4.4%)
Native American/Alaskan Native	4 (1.6%)
Asian	3 (1.2%)
More Than One	1 (0.4%)
Native Hawaiian/Pacific Islander	1 (0.4%)
Decade of Age	
(20,30]	9 (3.6%)
(30,40]	22 (8.9%)
(40,50]	34 (13.7%)
(50,60]	64 (25.8%)
(60,70]	74 (29.8%)
(70,80]	41 (16.5%)
(80,90]	4 (1.6%)
Age (years)	
Mean (SD)	58.0 (13.4)
Median [Min, Max]	60.0 [22.0, 85.0]
Cancer Related	
No	123 (49.6%)
Yes	125 (50.4%)

[0088] Table 2: Complications and Types of Surgical Procedures.

	Any Complication		**Infection**		**Readmission**		**System Dysfunction**	
	No (N=197)	**Yes (N=51)**	**No (N=228)**	**Yes (N=20)**	**No (N=212)**	**Yes (N=36)**	**No (N=225)**	**Yes (N=23)**
Procedure								
Head and Neck	8 (4.1%)	0 (0%)	8 (3.5%)	0 (0%)	8 (3.8%)	0 (0%)	8 (3.6%)	0 (0%)
Thyroidectomy	17 (8.6%)	0 (0%)	17 (7.5%)	0 (0%)	17 (8.0%)	0 (0%)	17 (7.6%)	0 (0%)

Bariatric	13 (6.6%)	4 (7.8%)	16 (7.0%)	1 (5.0%)	14 (6.6%)	3 (8.3%)	15 (6.7%)	2 (8.7%)
Hernia Repair	21 (10.7%)	4 (7.8%)	24 (10.5%)	1 (5.0%)	22 (10.4%)	3 (8.3%)	23 (10.2%)	2 (8.7%)
Mastectomy	27 (13.7%)	4 (7.8%)	30 (13.2%)	1 (5.0%)	27 (12.7%)	4 (11.1%)	31 (13.8%)	0 (0%)
Nephrectomy	21 (10.7%)	4 (7.8%)	24 (10.5%)	1 (5.0%)	23 (10.8%)	2 (5.6%)	21 (9.3%)	4 (17.4%)
Vascular	8 (4.1%)	4 (7.8%)	10 (4.4%)	2 (10%)	9 (4.2%)	3 (8.3%)	10 (4.4%)	2 (8.7%)
Colorectal	23 (11.7%)	5 (9.8%)	25 (11.0%)	3 (15.0%)	24 (11.3%)	4 (11.1%)	26 (11.6%)	2 (8.7%)
Prostatectomy	22 (11.2%)	5 (9.8%)	24 (10.5%)	3 (15.0%)	26 (12.3%)	1 (2.8%)	26 (11.6%)	1 (4.3%)
Cystectomy	9 (4.6%)	6 (11.8%)	11 (4.8%)	4 (20.0%)	10 (4.7%)	5 (13.9%)	14 (6.2%)	1 (4.3%)
Pancreatectomy	8 (4.1%)	7 (13.7%)	11 (4.8%)	4 (20.0%)	12 (5.7%)	3 (8.3%)	11 (4.9%)	4 (17.4%)
Cardiothoracic	20 (10.2%)	8 (15.7%)	28 (12.3%)	0 (0%)	20 (9.4%)	8 (22.2%)	23 (10.2%)	5 (21.7%)

[0089] Each patient's immune repertoire was defined by its relative frequency of distinct flow cytometry defined cell surface phenotypes (FIG. 7), as well as by the CD4/CD8 ratio. The pre-operative immune phenotype was diverse, characterized by wide expression ranges for parameters associated with T-cell maturation (Table 3).

[0090] Table 3: Characteristics Pre-Op of CD19+, CD3, CD4, and CD8. Data shows Median [min, max] for relative frequencies.

	CD19+ Lymphocytes	**CD3+ Lymphocytes**	**CD4+ T cells**	**CD8+ T cells**
BMI category				
Normal Weight/Pre-Obesity (N=136)	10.535 [0.53, 57.18]	73.305 [37.37, 91.62]	61.12 [24.7, 86.03]	32.72 [10.36, 72]
Obese (N=107)	10.99 [1.56, 39.46]	72.84 [51.69, 89.79]	65.23 [21.3, 89.65]	28.17 [8.78, 74.3]
Underweight (N=5)	10.64 [8.19, 17.01]	81.3 [74.15, 82.92]	59.97 [55.13, 71.3]	37.12 [26.73, 40.61]
Cancer Related				
No (N=123)	11.66 [0.53, 39.46]	73.97 [41.71, 89.79]	65.51 [24.7, 87.38]	28.43 [10.36, 69.77]
Yes (N=125)	10.07 [0.99, 57.18]	72.51 [37.37, 91.62]	60.29 [21.3, 89.65]	32.87 [8.78, 74.3]
Decade Of Age				
(20,30] (N=9)	12.66 [6.24, 17.11]	73.57 [62.12, 84.03]	57.79 [49.93, 79.76]	28.7 [17.39, 37.68]

(30,40] (N=22)	11.455 [5.97, 31.64]	73.915 [52.1, 85.81]	61.28 [41.77, 83.18]	32.28 [10.36, 51.46]
(40,50] (N=34)	13.37 [3.4, 37.66]	70.56 [41.71, 81.62]	62.37 [30.09, 81.07]	31.54 [17.67, 60.95]
(50,60] (N=64)	11.355 [1.93, 20.8]	72.88 [54.25, 89.79]	65.885 [28.52, 87.38]	28.09 [10.9, 65.62]
(60,70] (N=74)	10.425 [0.53, 39.46]	73.835 [40.88, 89.57]	63.51 [24.7, 89.65]	30.63 [8.78, 69.77]
(70,80] (N=41)	7.09 [0.99, 57.18]	72.34 [37.37, 91.62]	61.57 [21.3, 80.27]	31.64 [15.51, 74.3]
(80,90] (N=4)	4.82 [3.53, 6.28]	61.9 [54.68, 69.45]	52.705 [51.66, 55.64]	44.68 [40.81, 45.59]
Race				
Asian (N=3)	7.79 [7.41, 12.96]	65.32 [51.69, 80.72]	66.08 [39.96, 79.63]	27.77 [16.24, 52.63]
Black or African American (N=50)	13.515 [1.93, 31.58]	72.985 [41.71, 89.79]	66.59 [28.52, 87.56]	27.295 [10.9, 65.62]
More Than One (N=1)	17.53 [17.53, 17.53]	66.18 [66.18, 66.18]	65.69 [65.69, 65.69]	29.52 [29.52, 29.52]
Native American/Alaskan Native (N=4)	12.36 [10.27, 14.39]	75.77 [61.07, 77.06]	62.345 [49.97, 77.17]	32.48 [19.88, 45.66]
Native Hawaiian/Pacific Islander (N=1)	23.52 [23.52, 23.52]	54.05 [54.05, 54.05]	47.73 [47.73, 47.73]	31.09 [31.09, 31.09]
Unknown/Not Reported (N=11)	11.66 [2.84, 19.73]	73.22 [59.84, 82.81]	59.05 [42.15, 86.03]	35.76 [11.53, 46.79]
White (N=178)	9.915 [0.53, 57.18]	73.345 [37.37, 91.62]	61.775 [21.3, 89.65]	31.665 [8.78, 74.3]
Sex				
Female (N=123)	12.16 [1.93, 39.46]	72.84 [41.71, 89.57]	63.35 [24.7, 89.65]	31.5 [8.78, 69.43]
Male (N=125)	9 [0.53, 57.18]	74.78 [37.37, 91.62]	61.49 [21.3, 87.38]	30.8 [10.36, 74.3]
Overall				
Overall (N=248)	10.607 [0.529, 57.176]	73.2385 [37.368, 91.62]	62.557 [21.296, 89.65]	30.876 [8.781, 74.304]

BMI: body mass index

[0091] An analysis was performed and no significant differences were observed in lymphocyte phenotypes by sex, BMI, cancer diagnosis, race, or procedure, and no age-related changes were observed in absolute T (CD3, CD4, CD8) or relative B cell (CD19) frequencies through age 80 (Tables 4-7).

[0092] Table 4: Characteristics Pre-Op of CD4 memory cells. Data shows Median [min, max] for relative frequencies.

	CD3+ CD4+ CM	**CD3+ CD4+ CM Dim**	**CD3+ CD4+ EM**	**CD3+ CD4+ EMRA**	**CD3+ CD4+ EMRA Dim**	**CD3+ CD4+ Naïve**

BMI Category						
Normal Weight/Pre-Obesity (N=135)	27.43 [6.76, 62.83]	8.04 [1.04, 22.26]	34.33 [1.56, 77.35]	3.04 [0.25, 18.77]	3.71 [0.28, 10.04]	18.43 [0.84, 59.66]
Obese (N=107)	29.68 [3.49, 66.84]	7.07 [1.06, 22.68]	32.5 [6.96, 69.83]	2.66 [0.34, 41.2]	2.97 [0.56, 10.64]	16.76 [1.28, 60.1]
Underweight (N=5)	34.7 [21.98, 35.73]	5.23 [4.37, 5.93]	44.63 [33.95, 52.35]	3.36 [2.45, 6.1]	3.36 [2.47, 6.25]	13.94 [1.45, 29.15]
Cancer Related						
No (N=123)	27.11 [3.49, 66.84]	7.49 [1.06, 22.26]	31.97 [11.34, 77.35]	3.16 [0.3, 41.2]	3.28 [0.56, 8.54]	20.58 [1.45, 59.66]
Yes (N=124)	29.575 [6.76, 66.45]	7.325 [1.04, 22.68]	37.17 [1.56, 69.83]	2.68 [0.25, 40.14]	3.345 [0.28, 10.64]	14.135 [0.84, 60.1]
Decade of Age						
(20,30] (N=9)	23.51 [13.88,42.44]	8.75 [6.82, 16.14]	24.08 [18.63, 41.34]	2.38 [1.39, 7.26]	2.35 [1.5, 6.06]	28.58 [17.73, 48.57]
(30,40] (N=22)	28.7 [6.76, 53.74]	5.325 [1.04, 18.36]	32.8 [11.34, 55.68]	3.21 [0.94, 18.77]	2.935 [0.41, 6.12]	24.36 [2.2, 59.66]
(40,50] (N=34)	24.915 [3.49, 66.84]	8 [1.06, 22.26]	29.13 [7.53, 68.26]	3.3 [0.42, 23.26]	3.73 [0.56, 9.27]	22.1 [1.35, 54.38]
(50,60] (N=63)	27.89 [9.51, 61.11]	7.68 [1.65, 18.28]	31.97 [6.96, 71.41]	3.16 [0.3, 41.2]	3.57 [0.95, 10.64]	18.43 [1.7, 60.1]
(60,70] (N=74)	30.385 [11.18,66.45]	7.065 [1.47, 22.68]	35.48 [13.26, 77.35]	2.51 [0.34, 40.14]	3.045 [0.69, 10.04]	12.865 [0.84, 50.01]
(70,80] (N=41)	30.21 [11.87, 54.56]	8.07 [2.63, 21.49]	34.8 [1.56, 60.65]	2.68 [0.25, 15.42]	3.81 [0.28, 9.32]	13.22 [1.28, 46.45]
(80,90] (N=4)	37.87 [20.65, 41.48]	6.83 [5.47, 8.57]	43.92 [37.14, 48.19]	3.175 [1.16, 5.1]	4.11 [3.21, 4.63]	8.27 [4.33, 18.86]
Race						
Asian (N=3)	20.77 [9.54, 37.09]	3.96 [1.47, 7.75]	59.06 [34.15, 59.85]	2.98 [0.68, 4.67]	3.3 [1.03, 8.99]	15.3 [1.31, 29.76]
Black or African American (N=50)	33.4 [3.49, 66.45]	6.83 [1.06, 18.28]	32.015 [8.74, 71.41]	3.35 [0.34, 41.2]	3.06 [0.9, 8.54]	13.445 [1.28, 60.1]
More Than One (N=1)	46.66 [46.66, 46.66]	7.25 [7.25, 7.25]	20.99 [20.99, 20.99]	0.84 [0.84, 0.84]	0.95 [0.95, 0.95]	23.88 [23.88, 23.88]
Native American/Alaskan Native (N=4)	22.38 [17.57, 45.84]	10.77 [6.2, 15.97]	26.925 [17.55,38.32]	3.55 [1.36, 6.95]	3.495 [3.01, 10.04]	22.735 [14.86, 45.5]
Native Hawaiian/Pacific Islander (N=1)	32.11 [32.11, 32.11]	5.02 [5.02, 5.02]	26.22 [26.22, 26.22]	2.04 [2.04, 2.04]	1.56 [1.56, 1.56]	33.81 [33.81, 33.81]
Unknown/Not Reported (N=11)	21.91 [11.6, 30.17]	8.23 [4.3, 14.08]	36.37 [13.47, 46.89]	4.52 [1.84, 13.2]	3.71 [1.38, 9.73]	23.38 [11.31, 47.3]
White (N=177)	27.92 [6.76, 66.84]	7.88 [1.04, 22.68]	34.52 [1.56, 77.35]	2.68 [0.25, 40.14]	3.36 [0.28, 10.64]	17.9 [0.84, 59.66]
Sex						
Female (N=123)	27.07 [6.76, 66.84]	7.1 [1.04, 22.26]	33.58 [12.01, 71.41]	3.05 [0.3, 40.14]	3.73 [0.41, 10.64]	17.19 [1.31, 52.14]

Male (N=124)	29.815 [3.49, 58.65]	8.025 [1.06, 22.68]	34.23 [1.56, 77.35]	2.82 [0.25, 41.2]	3.055 [0.28, 10.04]	18.2 [0.84, 60.1]
Overall						
Overall (N=247)	28.283 [3.489,66.841]	7.494 [1.042,22.677]	33.948 [1.557,77.347]	2.935 [0.246,41.203]	3.291 [0.285,10.642]	17.734 [0.841,60.102]

CM: central memory, EM: effector memory, EMRA: terminally differentiated effector memory, BMI: body mass index

[0093] Table 5: Characteristics Pre-Op of CD4 cytokine cells. Data shows Median [min, max] for relative frequencies.

	CD3+ CD4+ CD244+	**CD3+ CD4+ CD28+**	**CD3+ CD4+ CD57+**	**CD3+ CD4+ PD1+**
BMI Category				
Normal Weight/Pre-Obesity (N=135)	3.33 [0.51, 38.55]	98.4 [56.24, 99.9]	6.44 [0.85, 50.74]	24.54 [5.85, 69.65]
Obese (N=107)	3.59 [0.23, 51.8]	97.7 [44.34, 99.93]	7.29 [0.52, 53.5]	24.85 [4.73, 68.54]
Underweight (N=5)	7.51 [0.93, 17.66]	91.32 [83.55, 99.7]	13.5 [1.49, 22.33]	25.71 [18.24, 60.42]
Cancer Related				
No (N=123)	3.16 [0.23, 51.8]	97.7 [47.8, 99.93]	6.44 [0.85, 53.5]	24.23 [4.73, 69.65]
Yes (N=124)	3.48 [0.51, 47.73]	98.105 [44.34, 99.91]	7.285 [0.52, 50.74]	25.925 [6.86, 68.54]
Decade of Age				
(20,30] (N=9)	1.67 [0.62, 2.35]	99.7 [98.5, 99.9]	3.5 [2.48, 15.08]	21.4 [8.3, 41.25]
(30,40] (N=22)	3.035 [0.59, 13.78]	98.85 [85.11, 99.9]	4.775 [1.49, 16.97]	22.68 [5.85, 47.2]
(40,50] (N=34)	2.74 [0.4, 26.59]	98.35 [70.84, 99.93]	6.455 [0.85, 35.58]	22.2 [6.52, 48.23]
(50,60] (N=63)	4.83 [0.23, 51.8]	98 [47.8, 99.91]	7.09 [0.52, 53.5]	23.33 [6.62, 68.54]
(60,70] (N=74)	3.24 [0.51, 47.73]	98.2 [44.34, 99.9]	7.03 [1.01, 40.84]	26.165 [4.73, 69.65]
(70,80] (N=41)	4.75 [0.64, 41.15]	95.21 [56.24, 99.9]	10.14 [1.96, 50.74]	27.47 [8.43, 60.42]
(80,90] (N=4)	15.6 [4.92, 22.8]	84.92 [77.53, 98.8]	19.92 [7.41, 29.6]	30.16 [19.16, 39.8]
Race				
Asian (N=3)	6.43 [1.76, 26.29]	92.81 [74.43, 98.3]	11.87 [3.95, 19]	31.36 [26.8, 36.28]
Black or African American (N=50)	4.915 [0.23, 51.8]	96.36 [47.8, 99.9]	7.705 [1.99, 53.5]	22.425 [6.62, 46.2]
More Than One (N=1)	2.53 [2.53, 2.53]	98.3 [98.3, 98.3]	4.78 [4.78, 4.78]	25.38 [25.38, 25.38]

Native American/Alaskan Native (N=4)	4.86 [1.17, 17.82]	98.505 [82.72, 99.93]	7.09 [1.59, 19.42]	27.67 [14.26, 34.53]
Native Hawaiian/Pacific Islander (N=1)	2.2 [2.2, 2.2]	99.7 [99.7, 99.7]	3.82 [3.82, 3.82]	18.63 [18.63, 18.63]
Unknown/Not Reported (N=11)	4.89 [0.51, 26.7]	96.2 [73.34, 99.9]	6.1 [2.05, 25.3]	22.89 [5.85, 41.78]
White (N=177)	3.19 [0.4, 47.73]	98.4 [44.34, 99.91]	7.04 [0.52, 50.74]	24.95 [4.73, 69.65]
Sex				
Female (N=123)	4.84 [0.23, 47.73]	97.1 [44.34, 99.93]	7.56 [0.85, 45.05]	25.69 [4.73, 69.65]
Male (N=124)	2.84 [0.51, 51.8]	98.65 [47.8, 99.91]	6.385 [0.52, 53.5]	22.94 [6.62, 68.54]
Overall				
Overall (N=247)	3.357 [0.231, 51.802]	98.104 [44.336, 99.928]	7.092 [0.518, 53.502]	24.848 [4.729, 69.65]

BMI: body mass index

[0094] Table 6: Characteristics Pre-Op of CD8 Memory cells. Data shows Median [min, max] for relative frequencies.

	CD3+ CD8+ CM	CD3+ CD8+ CM Dim	CD3+ CD8+ EM	CD3+ CD8+ EMRA	CD3+ CD8+ EMRA Dim	CD3+ CD8+ Naive
BMI Category						
Normal Weight/Pre-Obesity (N=135)	5.72 [0.24, 51.41]	3.54 [0.24, 22.03]	35.61 [2.49, 70.75]	21.84 [1.24, 80.63]	12.16 [1.26, 33.19]	12.39 [0.41, 69.77]
Obese (N=107)	6.3 [0.63, 33.55]	3.7 [0.42, 23.1]	31.73 [5.78, 76.94]	20.9 [3.61, 77.72]	11.44 [3.72, 42.66]	13.49 [0.8, 56.38]
Underweight (N=5)	2.81 [1.9, 20.59]	3.03 [1.41, 4.51]	36.5 [15.59, 37.81]	33.21 [14.06, 63.48]	15.1 [7.5, 21.35]	8.73 [1.57, 21.84]
Cancer Related						
No (N=123)	5.77 [0.63, 21.21]	3.66 [0.42, 23.1]	32.68 [5.78, 59.55]	20.94 [2.07, 77.72]	11.72 [3.02, 26.27]	14.74 [0.8, 69.77]
Yes (N=124)	5.7 [0.24, 51.41]	3.485 [0.24, 22.03]	34.945 [2.49, 76.94]	22.7 [1.24, 80.63]	11.64 [1.26, 42.66]	10.955 [0.41, 54.02]
Decade of Age						
(20,30] (N=9)	6.74 [2.1, 14.56]	5.84 [1.43, 8.7]	32.21 [11.96, 56.45]	15.78 [6.2, 19.04]	10.7 [4.44, 18.59]	30.35 [13.49, 56.38]
(30,40] (N=22)	4.57 [0.24, 22.41]	3.61 [0.24, 7.73]	35.485 [2.49, 58.96]	21.515 [5.28, 80.63]	13.145 [1.26, 22.07]	22.105 [7.46, 46.08]
(40,50] (N=34)	4.765 [0.71, 20.86]	4.49 [0.44, 15.71]	30.88 [6.04, 55.1]	20.825 [4.88, 47.77]	12.99 [3.87, 23.43]	21.905 [2.48, 69.77]
(50,60] (N=63)	7.38 [0.63, 33.55]	3.92 [0.42, 14.28]	31.28 [5.78, 60.05]	19.49 [2.07, 59.5]	11.09 [3.02, 26.27]	17.06 [0.8, 51.62]

(60,70] (N=74)	7.01 [0.82, 51.41]	3.165 [0.62, 23.1]	37.81 [4.97, 74.44]	22.735 [1.3, 77.72]	11.14 [3.81, 33.19]	8.24 [0.41, 54.02]
(70,80] (N=41)	4.78 [0.53, 47.92]	2.86 [0.41, 22.03]	34.3 [2.87, 58.15]	33.38 [1.24, 79.52]	13.03 [1.53, 25.54]	7.42 [1.16, 26.61]
(80,90] (N=4)	4.815 [2.53, 13.27]	2.535 [1.22, 4.49]	42.055 [33.36, 76.94]	18.255 [6.64, 27.07]	20.57 [9.8, 42.66]	3.355 [1.35, 5.73]
Race						
Asian (N=3)	7.81 [0.77, 10.06]	2.55 [0.97, 5.41]	30.24 [26.87, 40.31]	32.63 [22.33, 40.65]	16.97 [8.2, 22.97]	8.28 [8.21, 19.56]
Black or African American (N=50)	6.045 [0.71, 33.55]	4.025 [0.44, 14.28]	24.205 [4.97, 58.15]	27.85 [3.88, 77.72]	9.1 [3.72, 25.48]	19.735 [2.48, 69.77]
More Than One (N=1)	20.9 [20.9, 20.9]	4.92 [4.92, 4.92]	18.8 [18.8, 18.8]	2.07 [2.07, 2.07]	3.02 [3.02, 3.02]	51.62 [51.62, 51.62]
Native American/Alaskan Native (N=4)	8.55 [1.45, 20.27]	4.625 [0.94, 8.4]	42.71 [5.78, 48.67]	24.375 [4.88, 59.5]	8.91 [5.19, 12.73]	16.565 [2.87, 24.49]
Native Hawaiian/Pacific Islander (N=1)	3.67 [3.67, 3.67]	2.59 [2.59, 2.59]	35.36 [35.36, 35.36]	22.09 [22.09, 22.09]	20.6 [20.6, 20.6]	16.8 [16.8, 16.8]
Unknown/Not Reported (N=11)	5.06 [1.47, 14.44]	2.73 [0.76, 7.73]	35.61 [13.63, 48.46]	20.94 [8.39, 63.6]	10.48 [7.12, 25.1]	17.62 [2.68, 35.36]
White (N=177)	5.77 [0.24, 51.41]	3.51 [0.24, 23.1]	37.74 [2.49, 76.94]	20.65 [1.24, 80.63]	12.59 [1.26, 42.66]	10.51 [0.41, 54.02]
Sex						
Female (N=123)	5.45 [0.24, 51.41]	3.39 [0.24, 23.1]	31.71 [2.49, 60.05]	21.73 [2.07, 80.63]	12.06 [1.26, 26.27]	13.54 [1.16, 56.38]
Male (N=124)	6.14 [0.59, 47.92]	3.6 [0.42, 22.03]	37.175 [2.87, 76.94]	21.87 [1.24, 67.54]	11.46 [1.53, 42.66]	10.865 [0.41, 69.77]
Overall						
Overall (N=247)	5.731 [0.244, 51.415]	3.544 [0.244, 23.099]	34.298 [2.492, 76.937]	21.728 [1.236, 80.629]	11.717 [1.264, 42.655]	12.62 [0.413, 69.772]

CM: central memory, EM: effector memory, EMRA: terminally differentiated effector memory, BMI: body mass index

[0095] Table 7: Characteristics Pre-Op of CD8 Cytokine cells. Data shows Median [min, max] for relative frequencies.

	CD3+ CD8+ CD244+	CD3+ CD8+ CD28+	CD3+ CD8+ CD57+	CD3+ CD8+ PD1+
BMI Category				
Normal Weight/Pre-Obesity (N=135)	60.07 [8.61, 96.7]	62.99 [4.98, 96.21]	42.33 [7.87, 91.71]	31.47 [4.67, 68.64]
Obese (N=107)	62.14 [20.33, 91.3]	61.16 [11.55, 93.81]	47.27 [9.55, 87.71]	28.93 [6.55, 59.47]

Underweight (N=5)	53.69 [0.76, 77.47]	40.89 [20.68, 83.33]	35.98 [18.45, 78.55]	34.72 [12.1, 58.33]
Cancer Related				
No (N=123)	60.01 [0.76, 96.7]	62.82 [11.99, 92.82]	39.28 [7.87, 90.51]	31.57 [5.39, 66.22]
Yes (N=124)	65.475 [15.4, 96.7]	61.745 [4.98, 96.21]	45.795 [14.4, 91.71]	30.745 [4.67, 68.64]
Decade of Age				
(20,30] (N=9)	51.26 [20.33, 63.29]	73.36 [65.22, 92.23]	25.53 [9.55, 36.43]	26.98 [11.65, 49.37]
(30,40] (N=22)	55.52 [23.14, 79.45]	70.145 [47.8, 88.63]	28.33 [10.66, 57.88]	31.955 [15.45, 59.26]
(40,50] (N=34)	56.78 [16, 82.01]	71.675 [22.99, 92.82]	35.465 [7.87, 76.33]	29.375 [9.45, 67.39]
(50,60] (N=63)	59.58 [27.05, 94.91]	62.42 [8.05, 90.93]	41.02 [10.17, 91.71]	31.07 [9.24, 63.67]
(60,70] (N=74)	66.89 [0.76, 94]	62.095 [20.52, 96.21]	42.86 [13.08, 84.25]	29.005 [4.67, 60.33]
(70,80] (N=41)	75.35 [8.61, 96.7]	46.62 [4.98, 86.21]	57.88 [18.94, 91.7]	31.95 [5.39, 68.64]
(80,90] (N=4)	86.395 [80.52, 90.57]	31.74 [22.4, 68.9]	74.585 [33.43, 79.56]	45.29 [14.64, 60.95]
Race				
Asian (N=3)	68.56 [48.75, 78.42]	48.55 [45.61, 51.83]	48.95 [37.23, 64.47]	30.08 [16.08, 35.13]
Black or African American (N=50)	57.76 [0.76, 96.7]	62.22 [8.05, 92.82]	45.975 [9.55, 91.71]	21.415 [4.67, 53.77]
More Than One (N=1)	29.27 [29.27, 29.27]	90.52 [90.52, 90.52]	19.6 [19.6, 19.6]	31.07 [31.07, 31.07]
Native American/Alaskan Native (N=4)	51.385 [41.91, 61.9]	79.09 [50.03, 87.02]	27.43 [17.29, 58.6]	37.055 [31.95, 42.94]
Native Hawaiian/Pacific Islander (N=1)	68.18 [68.18, 68.18]	55.21 [55.21, 55.21]	51.12 [51.12, 51.12]	27.28 [27.28, 27.28]
Unknown/Not Reported (N=11)	55.3 [23.14, 86.44]	61.36 [32.18, 85.03]	42.33 [10.66, 64.69]	31 [15.75, 53.45]
White (N=177)	64.08 [8.61, 96.7]	62.36 [4.98, 96.21]	42.84 [7.87, 91.7]	33.16 [6.55, 68.64]
Sex				
Female (N=123)	61.11 [15.4, 94]	61.36 [11.55, 96.21]	42.64 [7.87, 87.71]	28.63 [6.55, 67.39]
Male (N=124)	60.425 [0.76, 96.7]	62.48 [4.98, 92.82]	44.655 [10.61, 91.71]	31.83 [4.67, 68.64]
Overall				
Overall (N=247)	61.069 [0.762, 96.703]	62.183 [4.98, 96.209]	42.878 [7.873, 91.714]	30.816 [4.669, 68.644]

BMI: body mass index

[0096] However, substantial age-related patterns were observed in the relative maturation phenotypes within individuals' T-cell populations, consistent with an anticipated accumulation of immune experience over the lifespan. Most prominently, there was a progressive and marked loss of naïve CD8 T cells over time. In addition, there was a generalized accumulation of phenotypes indicative of prior antigen experience (FIG. 1B), including accumulated senescent (CD57+) and exhausted (PD1+) T cells, an increase in CD244 expressing T cells, and a progressive loss of CD28, a prominent costimulation checkpoint molecule required for naïve cell activation. Thus, patients presented for elective surgery with relatively similar gross numbers of lymphocytes but with widely disparate degrees of T-cell maturation and phenotypic changes suggestive of unique, prior antigen exposure histories.

Example 2

[0097] ***Preoperative immune phenotypes and postoperative outcomes.*** As this was an elective surgical cohort, outcomes were generally favorable. With a median follow-up of 6 months, there were no deaths, and overall survival at 30 days was 100%. However, 20.5% of the cohort experienced a postoperative complication (infection, readmission, or system dysfunction). The most common complication was 30-day readmission (14.5%), and 5% of all patients required an unplanned admission to the ICU. Complications varied by case in an anticipatable way with more complicated and technically intense procedures having more complications. There were no complications in the head and neck surgery or thyroidectomy groups, while cardiothoracic and pancreatectomy cases had the highest overall complication rates (15.7 and 13.7%, respectively). Pancreatectomy and cystectomy accounted for 40% of the identified infections (20% each).

[0098] After correcting for age, sex, race, and duration of procedure (as a proxy for procedure intensity), two pre-surgery flow cytometry phenotypes were observed that were significantly associated with postoperative infection (FIG. 1C). A propensity towards infection was seen in patients with fewer CD4+ naïve T cells ($p=0.002$, $pFDR=0.03$) or more accumulated CD4 T_{EM} cells ($p=0.001$, $pFDR=0.030$), both indicative of more extensive prior immune responses. A significant association was also observed between systemic complications (Systems Dysfunction) and lower pre-operative double negative (CD4-CD8-) T cells ($p=0.024$, $pFDR=0.070$), cells with known association with chronic inflammatory and autoimmune conditions.

Example 3

[0099] ***Postoperative changes in immune phenotypes and their association with outcomes***. Surgery induced immediate changes in peripheral cell phenotypes, which were evident on arrival to the post-anesthesia care unit. When examining the change in relative frequency of cell phenotypes from preoperative to immediate postoperative timepoint, four significant associations were observed with Systems Dysfunction. Interestingly, an increase in the CD4-CD8- T cells was associated with increased odds of any complication (p=0.01, pFDR=0.094), as was an increase in CD4+CD57+ (p=0.013, pFDR=0.094) T cells, typically associated with immune senescence. Decreased CD4+ T cells (p=0.011, pFDR=0.094) and CD8+PD1+ (p=0.013, pFDR=0.094) were also associated with increased odds of any complication (FIG. 2A). While not significant after correction for multiple testing, several other signals related to T-cell maturation, senescence, and exhaustion trended toward association with surgical complications (p-value <0.05). Increased odds of infection associated with pre-operative CD8 exhaustion (CD57+, p=0.029, pFDR=0.174), CD8 loss of the costimulation molecule CD28 (p=0.031, pFDR=0.174), and increased inhibitory molecule CD244 expression (p=0.016, pFDR=0.144) were observed. It was also observed that patients with fewer CD4+ naïve (p=0.021) T cells, or more accumulated CD4 T_{EM} or CD8 T_{EMRA} had increased odds of developing any postoperative complications (p=0.025 and 0.031 respectively). Similarly, prior accumulation of CD57+ T cells was marginally associated with the occurrence of any complication for either CD4+CD57+ cells (p=0.027) or CD8+CD57+ cells (p=0.013). Loss of CD28 on CD8+ cells also correlated with development of any complications (p=0.045). Readmission was more common in patients with fewer CD4+ naïve cells (p=0.018), more accumulated CD8+CD57+ cells (p=0.023), or more CD4+ T_{EM} (p=0.030) or CD8+ T_{EMRA} (p=0.012). Examining change in relative frequency, results indicated that a larger change in the ratio of CD4/CD8 T cells was associated with a decrease in the odds of Systems Dysfunction (p=0.029, pFDR=0.140). A similar association was observed for CD8+ CM Dim cells (p=0.029, pFDR=0.140, FIG. 2B). In aggregate, all associations encountered trended toward a positive relationship between advanced T-cell differentiation and worse surgical outcomes. Moreover, surgery led to marked perturbations in peripheral T-cell phenotypes during the immediate postoperative period, presumably the result of cell mobilization and redistribution. These changes were substantially influenced by the physiologic magnitude of the procedure, as measured by the time from incision to the time of collection in the recovery room. Notable was a generalized T cell lymphopenia apparent in all T cells (CD3+) including CD4 and CD8 populations, and the concomitant emergence of an innate NK response (CD56+), particularly

evident in the $CD56^{dim}$ population, which significantly increased in prevalence postoperatively (FIG. 2C). Most T-cell phenotypes were generally depressed immediately postoperatively, with the marked exception of cells that expressed CD57 (CD4 and CD8), or CD244, or lacked CD28. Importantly, the surgical experience was highly dynamic with regard to peripheral lymphocyte phenotype distribution.

Example 4

[0100] ***Types of surgery and their effect on immune phenotypes***. Visceral surgery was associated with smaller logit change in relative frequency of CD19+ lymphocytes (p=0.003, pFDR=0.103, FIG. 3A) compared to non-visceral procedures. Laparoscopic surgery was associated with smaller logit change in relative frequency of CD3+ lymphocytes (p=0.013, pFDR=0.155), CD8+CD28+ T cells (p=0.040, pFDR=0.155) and greater change in relative frequency of CD4+EM (p=0.028, pFDR=0.155), CD4+EMRA (p=0.029, pFDR=0.155), CD8+CD57+ (p=0.037, pFDR=0.155), CD8+EMRA (p=0.038, pFDR=0.155) and CD56+Dim lymphocytes (p=0.023, pFDR=0.155, FIG. 3B) compared to open surgery. Finally, major (prolonged) surgery was associated with logit changes in relative frequency of CD3+ lymphocytes (p=0.0007, pFDR=0.0104), CD4+ T cells (p=0.026, pFDR=0.129), CD8+ T cells (p=0.012, pFDR=0.082), CD8+CD28+ T cells (p=0.006, pFDR=0.0613), CD8+CD57+ (p=0.037, pFDR=0.144), CD8+EM (p=0.028, pFDR=0.129) and CD56+Dim lymphocytes (p=0.0002, pFDR=0.006, FIG. 4) compared to minor (shorter) procedures. The presence or absence of cancer, considered across surgical procedures was not associated with stereotypical changes in immune phenotype (data not shown but can be made available upon request).

[0101] Table 8: Characteristics Pre-Op of CD56 Bright/Dim Lymphocytes/ CD4-CD8- T cells. Data shows Median [min, max] for relative frequencies.

	CD4- CD8- T cells	**CD56 Bright Lymphocytes**	**CD56 Dim Lymphocytes**
BMI Category			
Normal Weight/Pre-Obesity (N=136)	3.9 [0.29, 23.53]	0.645 [0.05, 3.67]	11.375 [0.84, 46.56]
Obese (N=107)	3.18 [0.55, 22.67]	0.5 [0.07, 2.73]	11.15 [1.06, 40.56]
Underweight (N=5)	1.6 [1.11, 5.69]	0.49 [0.07, 0.66]	7.56 [5.14, 11.87]
Cancer Related			
No (N=123)	3.36 [0.59, 22.67]	0.57 [0.07, 2.73]	10.2 [1.06, 46.56]
Yes (N=125)	3.64 [0.29, 23.53]	0.59 [0.05, 3.67]	11.92 [0.84, 42.7]
Decade of Age			
(20,30] (N=9)	8.28 [2.7, 17.59]	0.81 [0.4, 1.06]	8.46 [5.27, 27.82]
(30,40] (N=22)	4.435 [0.97, 20.81]	0.495 [0.2, 2.13]	9.475 [2.78, 29.39]

(40,50] (N=34)	4.255 [0.79, 7.97]	0.625 [0.15, 2.73]	9.515 [1.45, 42.58]
(50,60] (N=64)	3.4 [0.61, 16.36]	0.57 [0.05, 2.69]	12.075 [1.06, 40.56]
(60,70] (N=74)	3 [0.29, 23.53]	0.58 [0.1, 3.67]	10.53 [0.84, 42.7]
(70,80] (N=41)	3.39 [0.36, 17.09]	0.48 [0.13, 1.92]	12.67 [1.36, 46.56]
(80,90] (N=4)	1.93 [1.66, 2.16]	1.4 [0.58, 1.8]	27.03 [16.28, 37.71]
Race			
Asian (N=3)	5.58 [3.54, 6.11]	0.71 [0.59, 0.83]	23.47 [6.51, 30.23]
Black or African American (N=50)	2.865 [0.29, 15.61]	0.46 [0.05, 1.08]	10.275 [1.06, 42.58]
More Than One (N=1)	4.23 [4.23, 4.23]	0.85 [0.85, 0.85]	12.44 [12.44, 12.44]
Native American/Alaskan Native (N=4)	3.685 [2.14, 4.48]	0.935 [0.43, 1.82]	8.485 [7.87, 21.94]
Native Hawaiian/Pacific Islander (N=1)	20.81 [20.81, 20.81]	0.2 [0.2, 0.2]	19.1 [19.1, 19.1]
Unknown/Not Reported (N=11)	4.53 [2.05, 11.45]	0.68 [0.1, 1.56]	10.52 [5.28, 27.27]
White (N=178)	3.425 [0.36, 23.53]	0.595 [0.07, 3.67]	11.385 [0.84, 46.56]
Sex			
Female (N=123)	3.36 [0.55, 23.53]	0.63 [0.14, 2.73]	11.28 [0.84, 42.58]
Male (N=125)	3.66 [0.29, 22.67]	0.56 [0.05, 3.67]	11.15 [1.36, 46.56]
Overall			
Overall (N=248)	3.4235 [0.292, 23.529]	0.5825 [0.052, 3.673]	11.1775 [0.835, 46.555]

BMI: body mass index

[0102] Table 9: Results from Modeling change from Incision to Recovery. Association with time.

Cell Population	Fixed Effect	Fixed P-value	Random P-value	Overall P-value	Overall P-value FDR
CD3+ Lymphocytes	-0.129	1.12e-35	2.11e-10	8.44e-44	2.28e-42
CD56 Dim Lymphocytes	0.16	3.57e-28	0.00526	2.38e-30	3.21e-29
CD56 Bright Lymphocytes	0.073	1.86e-14	0.408	1.5e-13	1.35e-12
CD3+ CD8+ CD28+	-0.0568	4.55e-11	0.0196	1.41e-11	9.5e-11
CD3+ CD8+ Naive	0.0292	0.0119	2.91e-10	2.04e-11	1.1e-10
CD3+ CD8+ CD57+	0.0468	7.33e-09	0.000454	2.96e-10	1.33e-09
CD3+ CD8+ PD1+	-0.0375	4.6e-07	3.53e-05	6.8e-10	2.62e-09
CD8+ T cells	0.0242	8.17e-05	2.65e-09	1.57e-09	5.3e-09
CD3+ CD4+ EMRA	0.0593	8.38e-09	0.364	1.23e-08	3.68e-08
CD4+ T cells	-0.0257	0.000111	1.02e-07	8.3e-08	2.24e-07
CD3+ CD8+ EMRA	0.0425	1.02e-06	0.048	9.45e-07	2.32e-06
CD3+ CD8+ EM	-0.0378	2.12e-07	1	1.38e-06	3.09e-06
CD3+ CD4+ CD244+	0.051	2.54e-06	0.76	1.86e-06	3.87e-06
CD3+ CD4+ EM	-0.0226	0.00758	0.00116	7.96e-06	1.54e-05

CD19+ Lymphocytes	0.0274	4.99e-06	0.849	1.67e-05	3.01e-05
CD3+ CD4+ EMRA Dim	0.0375	0.000158	0.484	0.000645	0.00109
CD3+ CD4+ CM	-0.0241	0.0128	0.00851	0.001	0.00159
CD3+ CD4+ Naïve	0.0315	0.000365	1	0.00169	0.00253
CD3+ CD4+ CD28+	-0.0362	0.00401	0.029	0.00199	0.00282
CD3+ CD4+ CD57+	0.0217	0.00171	0.393	0.00458	0.00618
CD4- CD8- T cells	0.0195	0.00259	0.233	0.011	0.0142
CD3+ CD8+ CM	-0.0225	0.0651	0.0239	0.0159	0.0195
CD3+ CD8+ CM Dim	-0.00868	0.426	0.00573	0.021	0.0246
CD3+ CD8+ CD244+	0.00989	0.286	0.00684	0.0225	0.0253
CD3+ CD4+ CM Dim	0.016	0.0711	0.735	0.176	0.19
CD3+ CD8+ EMRA Dim	0.0132	0.0811	1	0.218	0.226
CD3+ CD4+ PD1+	-0.00606	0.434	0.314	0.475	0.475
Modeling Relative frequency (logit transformed) and its association with time from incision to recovery sample. Pre-surgery observation recorded as time point 0 even if collected prior or slightly after incision. Time (in minutes) was modeled as both a fixed effect and a random component. Overall P-value is the test for either fixed or random being 0. Fixed effect relates to the impact of time on mean, while the random effect is the impact of time on the variance (spread) of the data. Random P-value of 1 indicates that the model estimated that time related variance related as 0					

CLAIMS

What is claimed is:

1. A method of assessing a subject's immune fitness for a physiological stressor, the method comprising:

determining a frequency of CD4+ lymphocytes and a frequency CD8+ lymphocytes in population of cells isolated from a sample from a subject; and

determining a frequency of at least one lymphocyte maturation marker in the population of cells isolated from the sample from the subject.

2. The method of claim 1, wherein determining the frequency of CD4+ lymphocytes, the frequency CD8+ lymphocytes, and/or the frequency of the at least one lymphocyte maturation marker comprises using flow cytometry to determine a relative frequency based on absolute count data.

3. The method of claim 1 or claim 2, wherein the at least one lymphocyte maturation marker comprises CD45RA, CCR7, CD27, CD28 CD57, CD244, and/or PD1.

4. The method of any one of claims 1 to 3, wherein the method further comprises determining a ratio of CD4+ lymphocytes to CD8+ lymphocytes.

5. The method of any one of claims 1 to 4, wherein the at least one lymphocyte comprises a T lymphocyte.

6. The method of any one of claims 1 to 4, wherein the at least one lymphocyte comprises a B lymphocyte.

7. The method of any one of claims 1 to 6, wherein the method further comprises determining a frequency of CD3+ lymphocytes in the population of cells isolated from the sample from the subject, and wherein the at least one lymphocyte maturation marker is determined from the population of CD3+ lymphocytes.

8. The method of any one of claims 1 to 7, wherein the frequency of the at least one lymphocyte maturation marker is determined from the population of the CD4+ and/or CD8+ lymphocytes in the cells isolated from the sample from the subject.

9. The method of any one of claims 1 to 8, wherein the sample is a blood sample, a serum sample, a tissue sample, a bone marrow sample, a lavage fluid sample, a cerebral spinal fluid sample, a urine sample, or a fecal sample.

10. The method of any one of claims 1 to 9, wherein the subject is a non-human mammal.

11. The method of any one of claims 1 to 9, wherein the subject is a human.

12. The method of any one of claims 1 to 11, wherein the physiological stressor comprises a surgical procedure performed on the subject.

13. The method of claim 12, wherein the surgical procedure comprises head and neck surgery, hernia repair surgery, a mastectomy, or a thyroidectomy.

14. The method of claim 12, wherein the surgical procedure comprises cardiovascular surgery, bariatric surgery, a colectomy, a cystectomy, a nephrectomy, a pancreatectomy, a prostatectomy, or a thoracotomy.

15. The method of any one of claims 1 to 11, wherein the physiological stressor comprises a non-surgical medical procedure.

16. The method of any one of claims 1 to 11, wherein the physiological stressor comprises prolonged physical exertion.

17. The method of any one of claims 1 to 16, wherein the method further comprises reassessing the subject's immune fitness after exposure to the physiological stressor.

18. The method of any one of claims 1 to 16, wherein the method further comprises assessing the subject's immune fitness before and after exposure to the physiological stressor.

19. The method of any one of claims 1 to 18, wherein an increased frequency of CD57+ lymphocytes is indicative of immune system senescence and decreased immune fitness in the subject.

20. The method of any one of claims 1 to 19, wherein an increased frequency of PD1+ lymphocytes is indicative of immune system exhaustion and decreased immune fitness in the subject.

21. The method of any one of claims 1 to 20, wherein an increased frequency of CD244+ lymphocytes is indicative of immune system activation and decreased immune fitness in the subject.

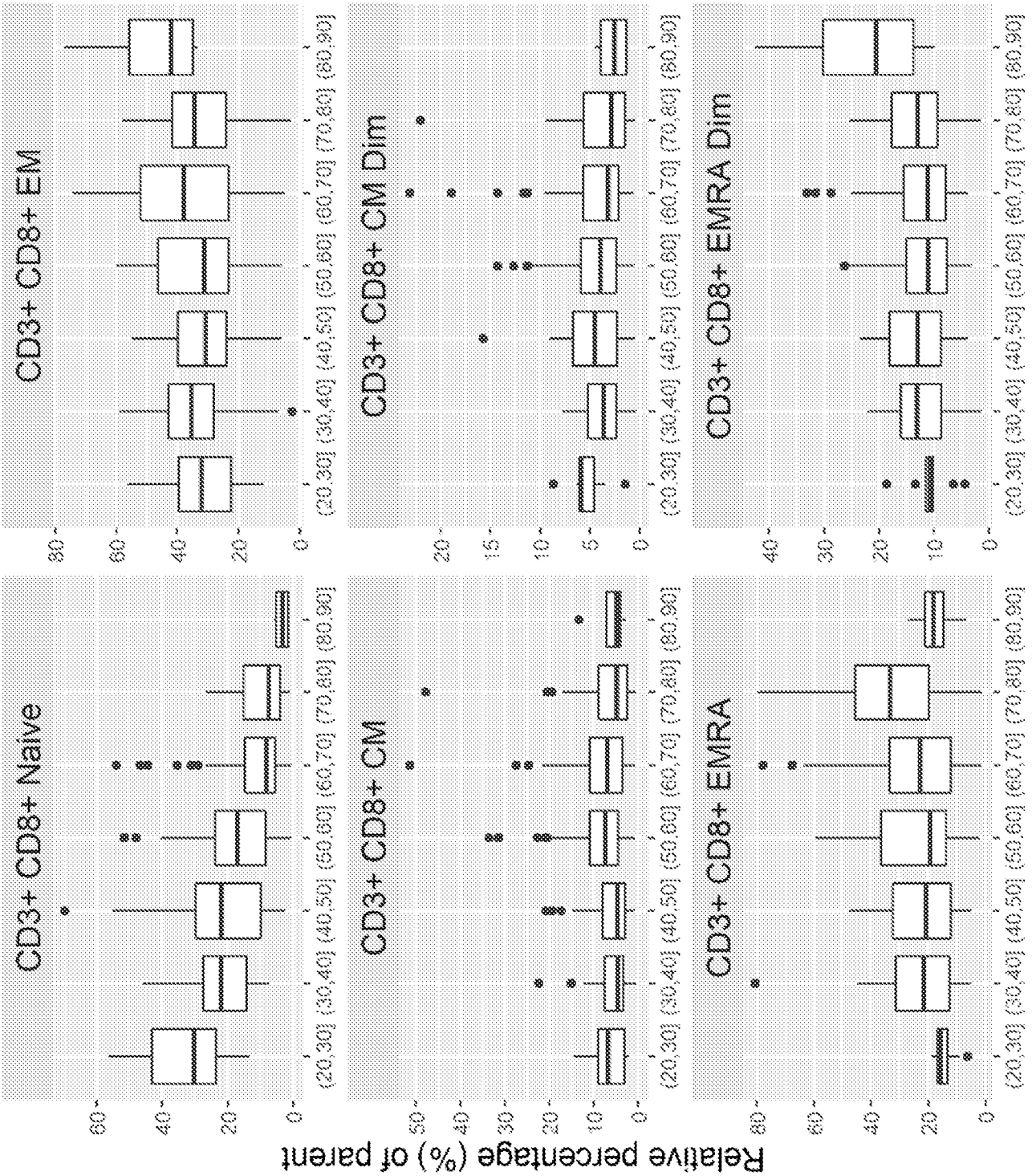
22. The method of any one of claims 1 to 20, wherein a decreased frequency of CD28+ lymphocytes is indicative of reduced naïve T cell activation and decreased immune fitness in the subject.

23. The method of any one of claims 1 to 20, wherein a decreased frequency of CD45RA+ lymphocytes and/or CCR7+ lymphocytes is indicative of increased T cell maturation and decreased immune fitness in the subject.

24. The method of any one of claims 19 to 23, wherein decreased immune fitness comprises an increased likelihood of at least one of hospital readmission, infection, system dysfunction, and/or post-surgical complications.

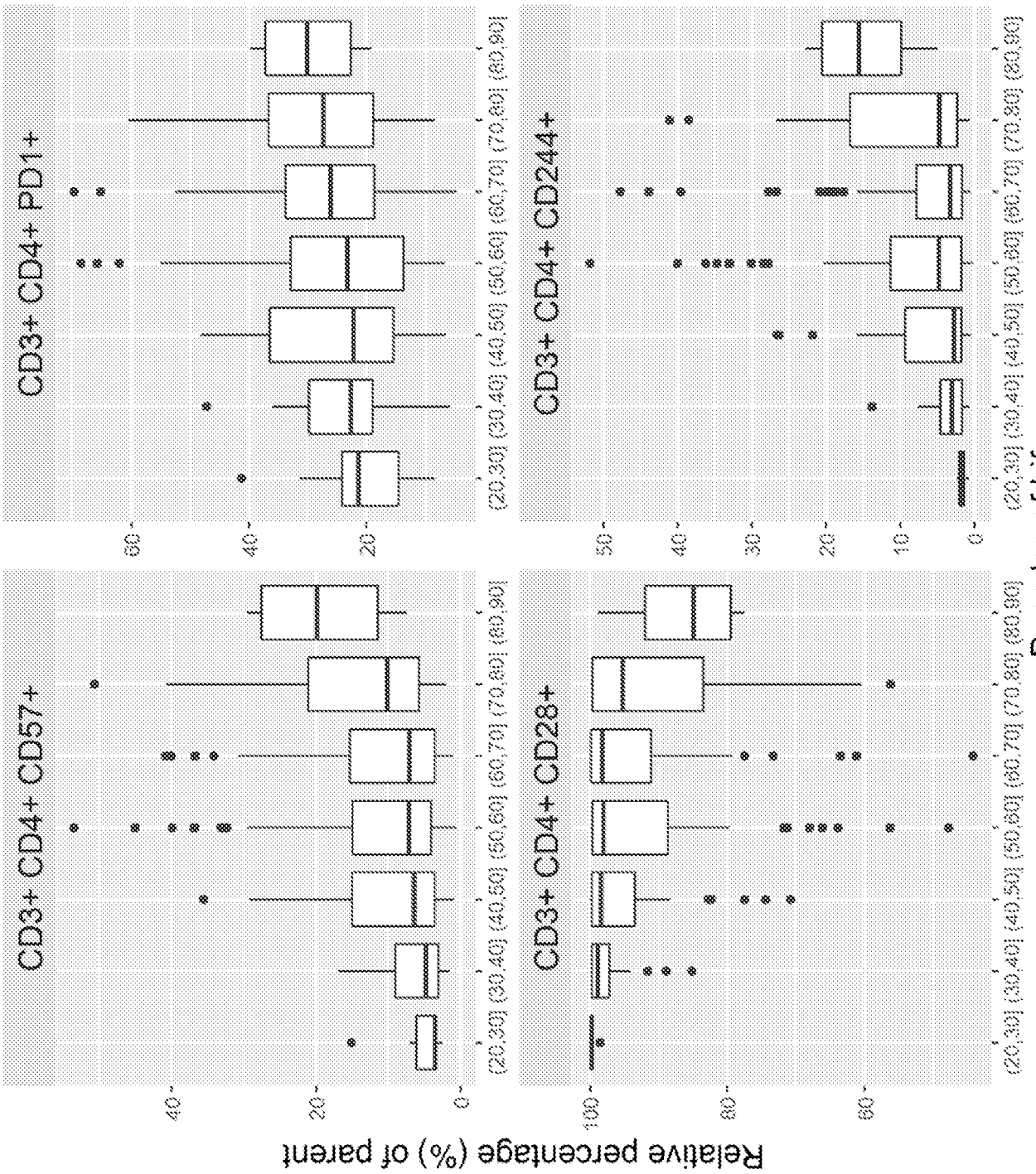
25. The method of claim 24, wherein the method further comprises administering a treatment to the subject to increase immune fitness.

FIG. 1A:



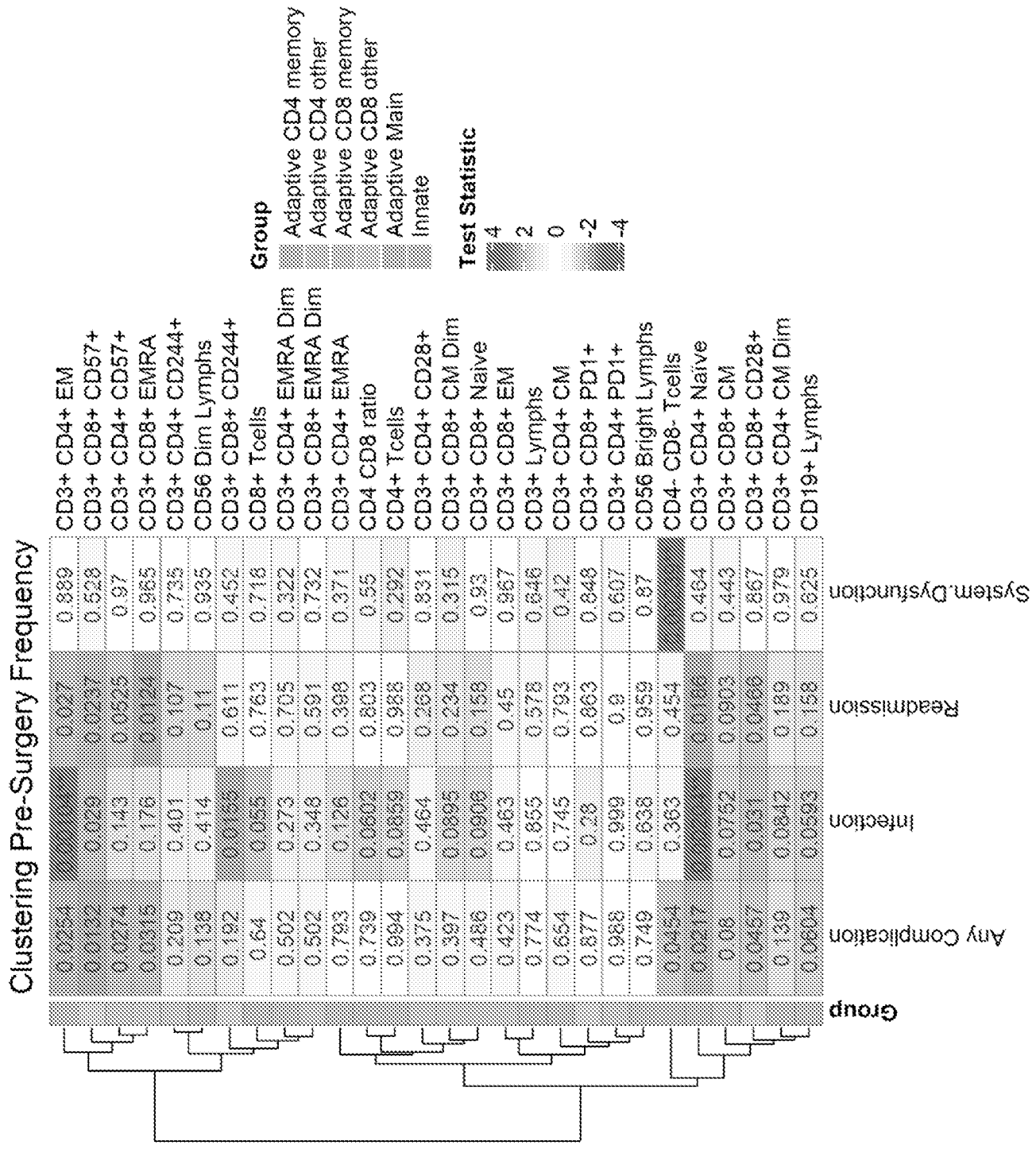
FIGS. 1A-1C

FIG. 1B:



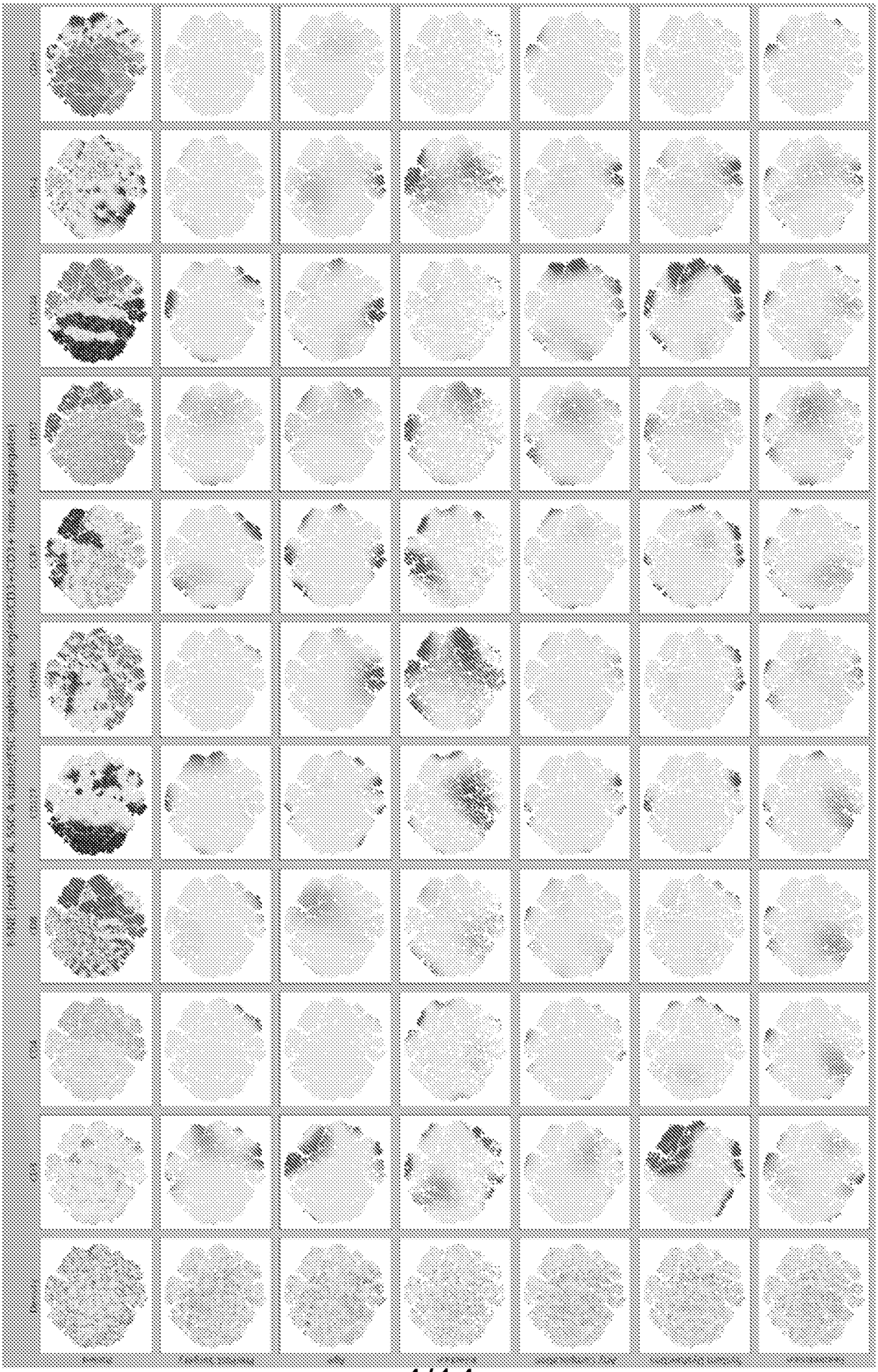
FIGS. 1A-1C (cont'd)

FIG. 1C:



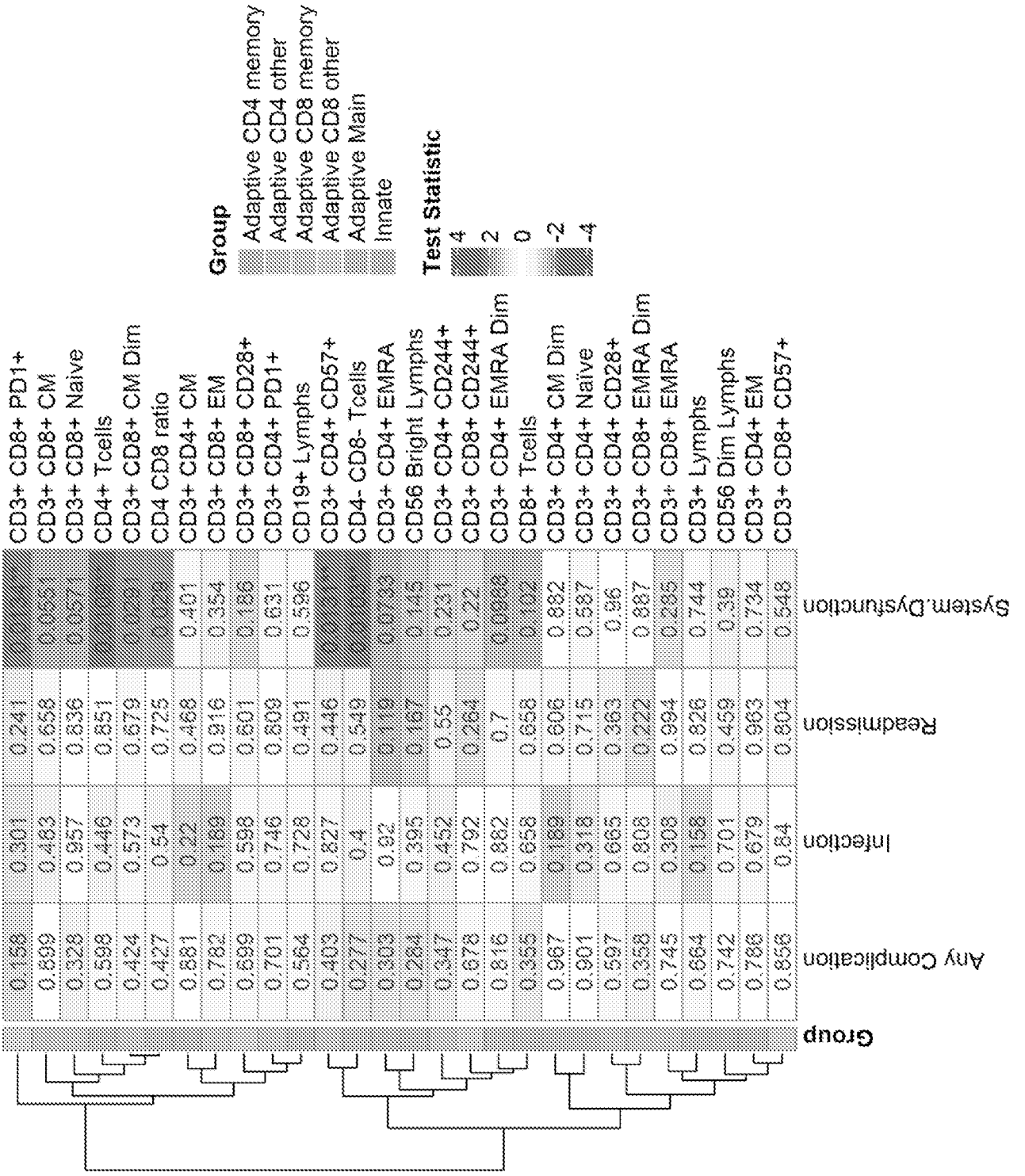
FIGS. 1A-1C (cont'd)

FIG. 2A:



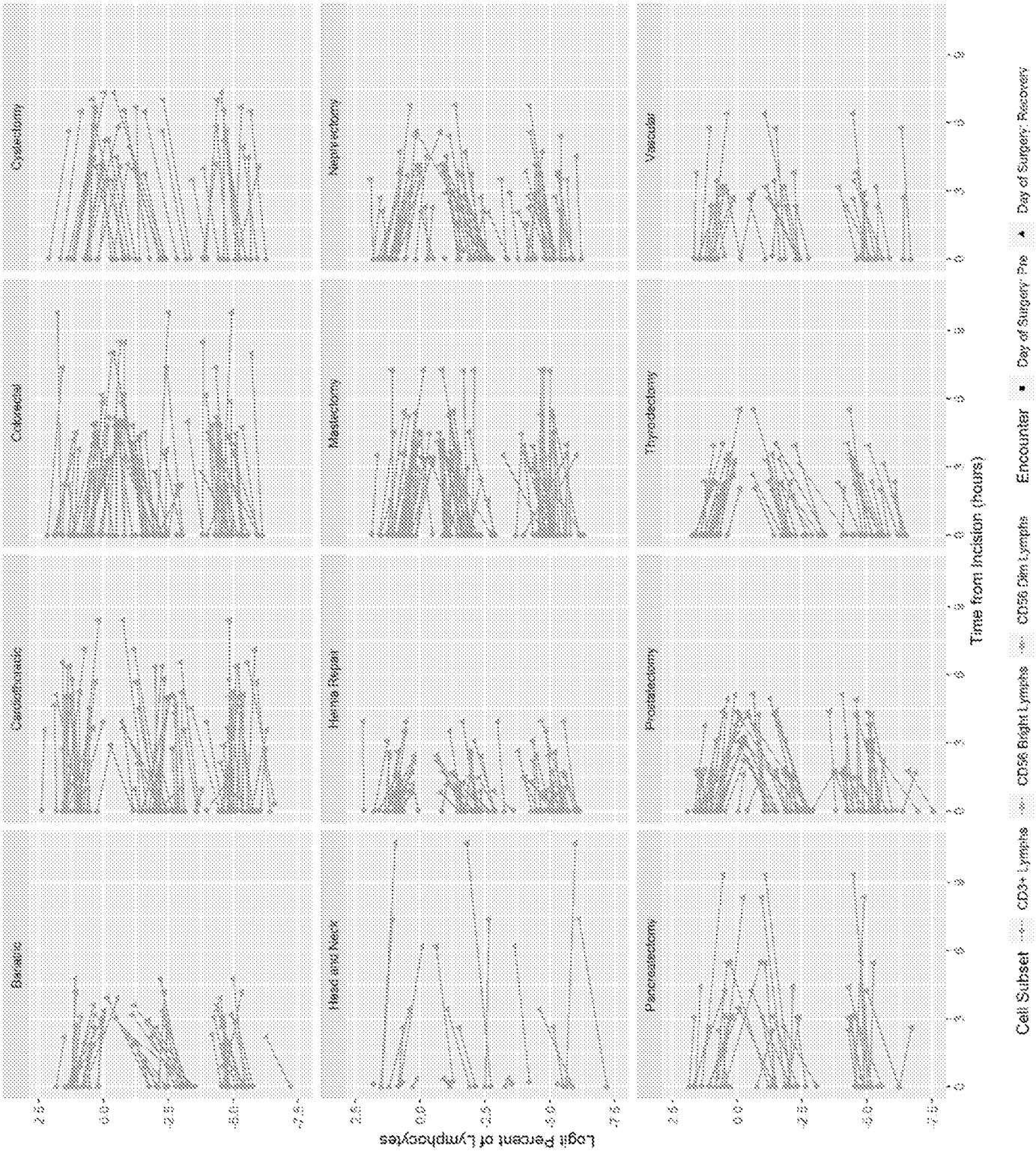
FIGS. 2A-2C

FIG. 2B:



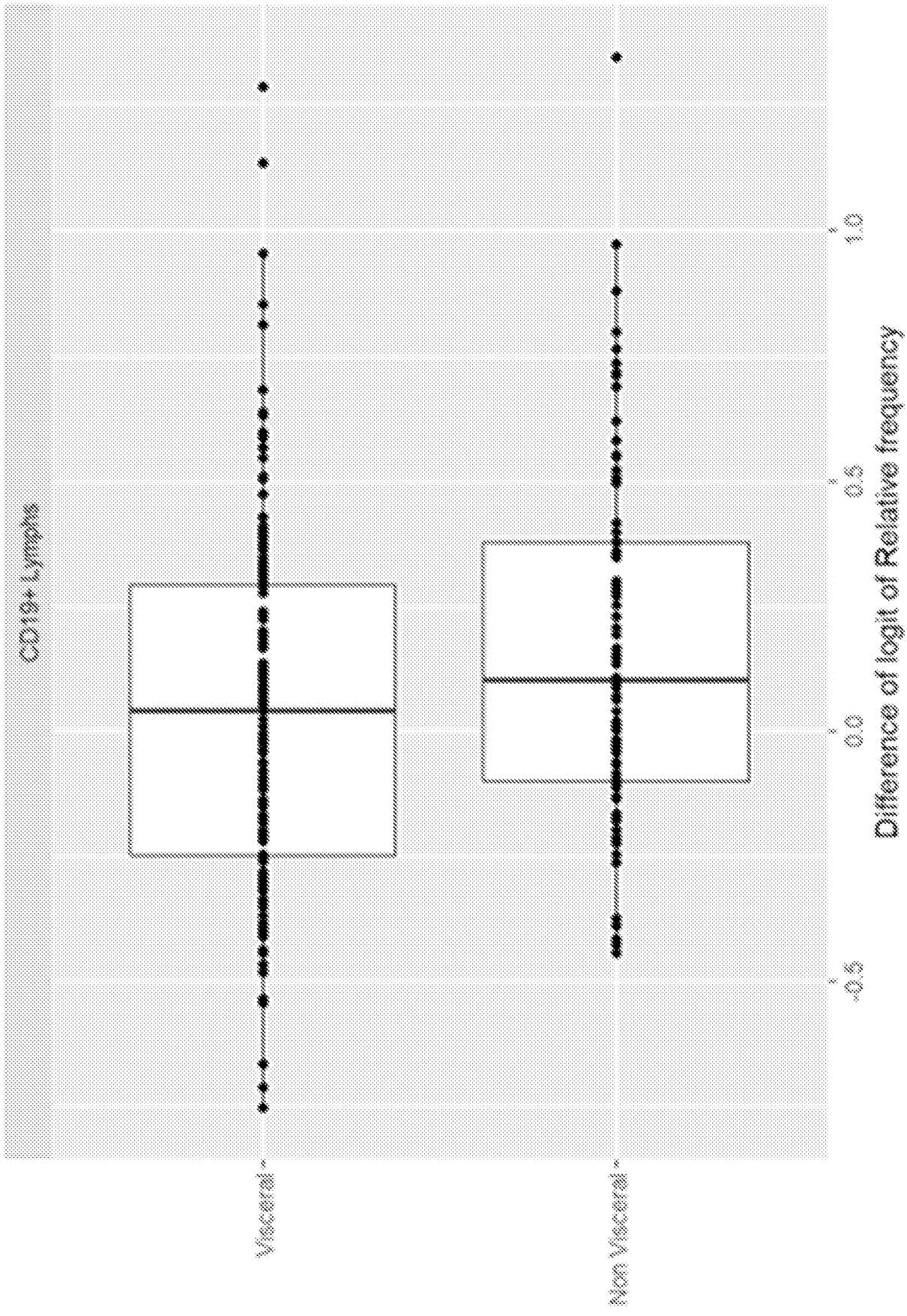
FIGS. 2A-2C (cont'd)

FIG. 2C:



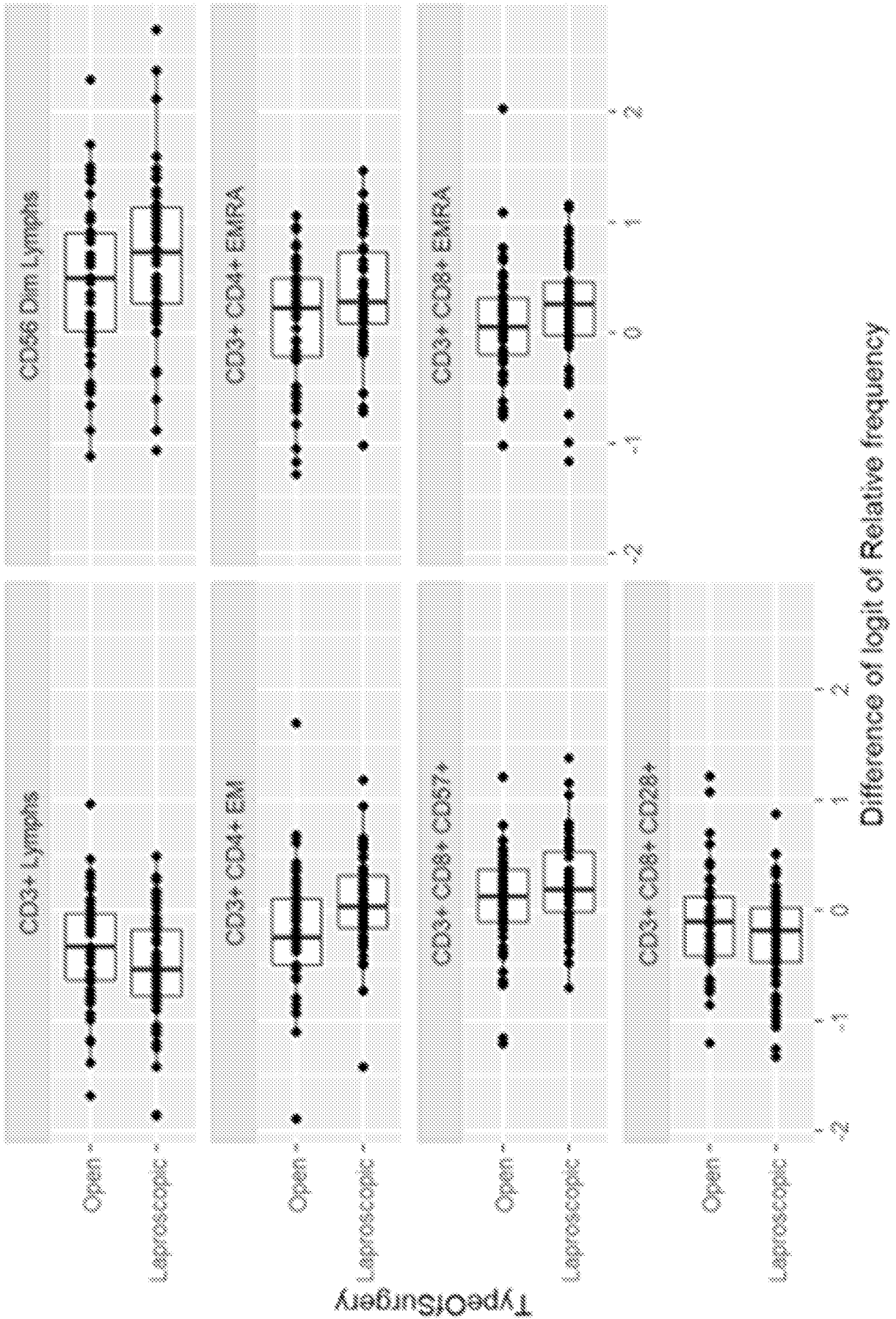
FIGS. 2A-2C (cont'd)

FIG. 3A:



FIGS. 3A-3B

FIG. 3B:



FIGS. 3A-3B (cont'd)

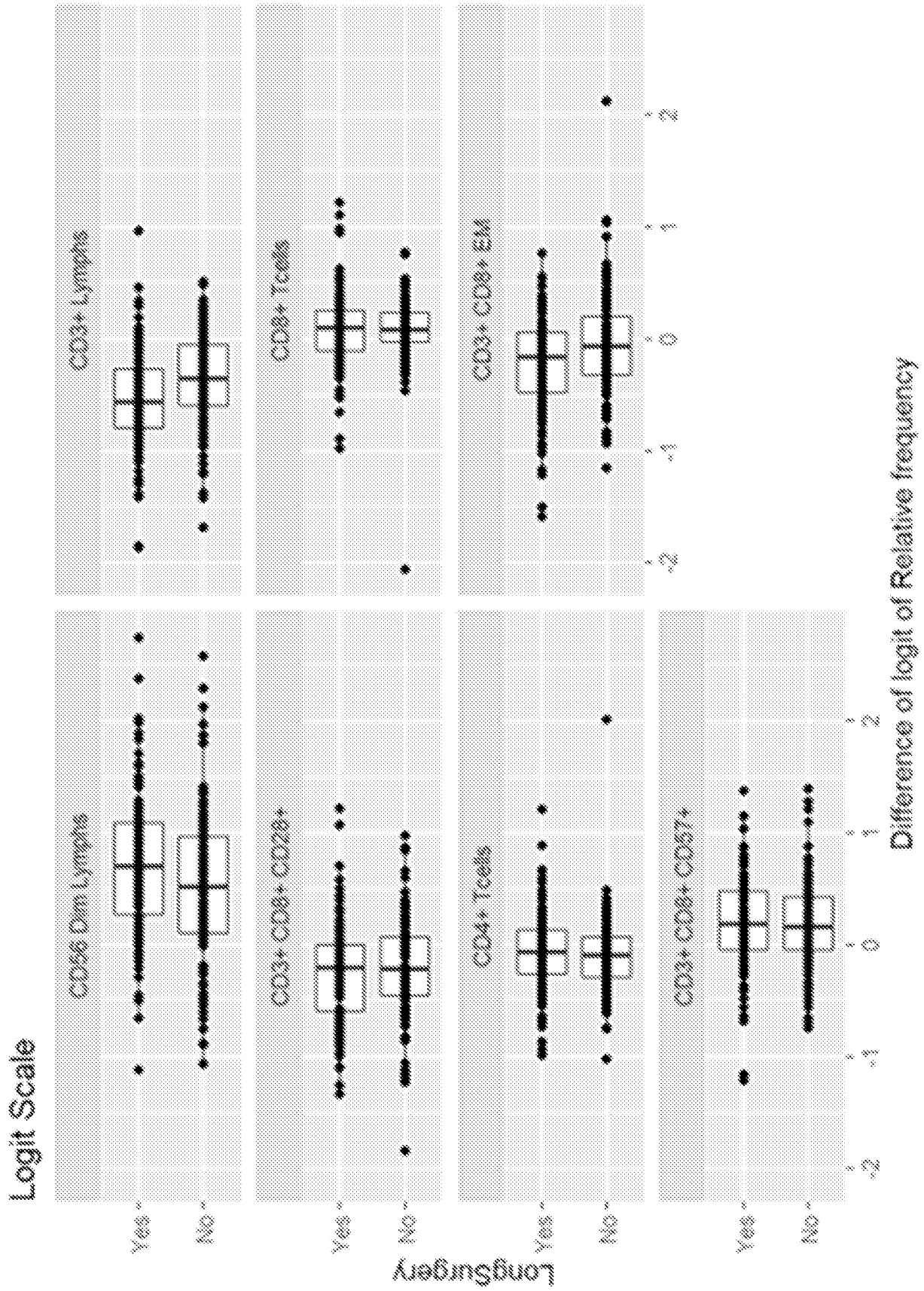

Logit Scale
CD3+ Lymphs
CD8+ Tcells
CD3+ CD8+ EM
CD56 Dim Lymphs
CD3+ CD8+ CD28+
CD4+ Tcells
CD3+ CD8+ CD57+
Yes
No
Longsurgery
-2
-1
0
1
2
Difference of logit of Relative frequency

FIG. 4

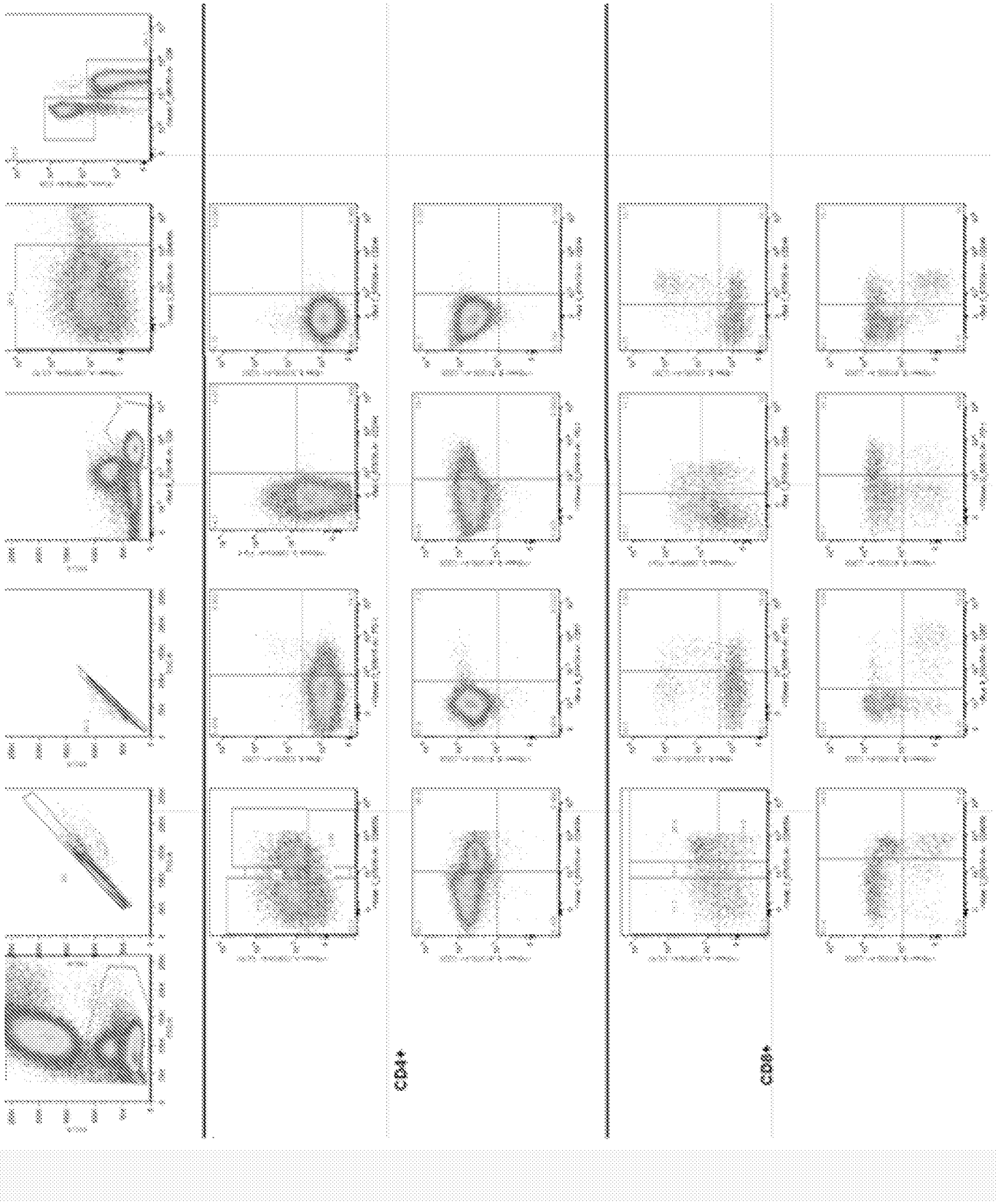


FIG. 5

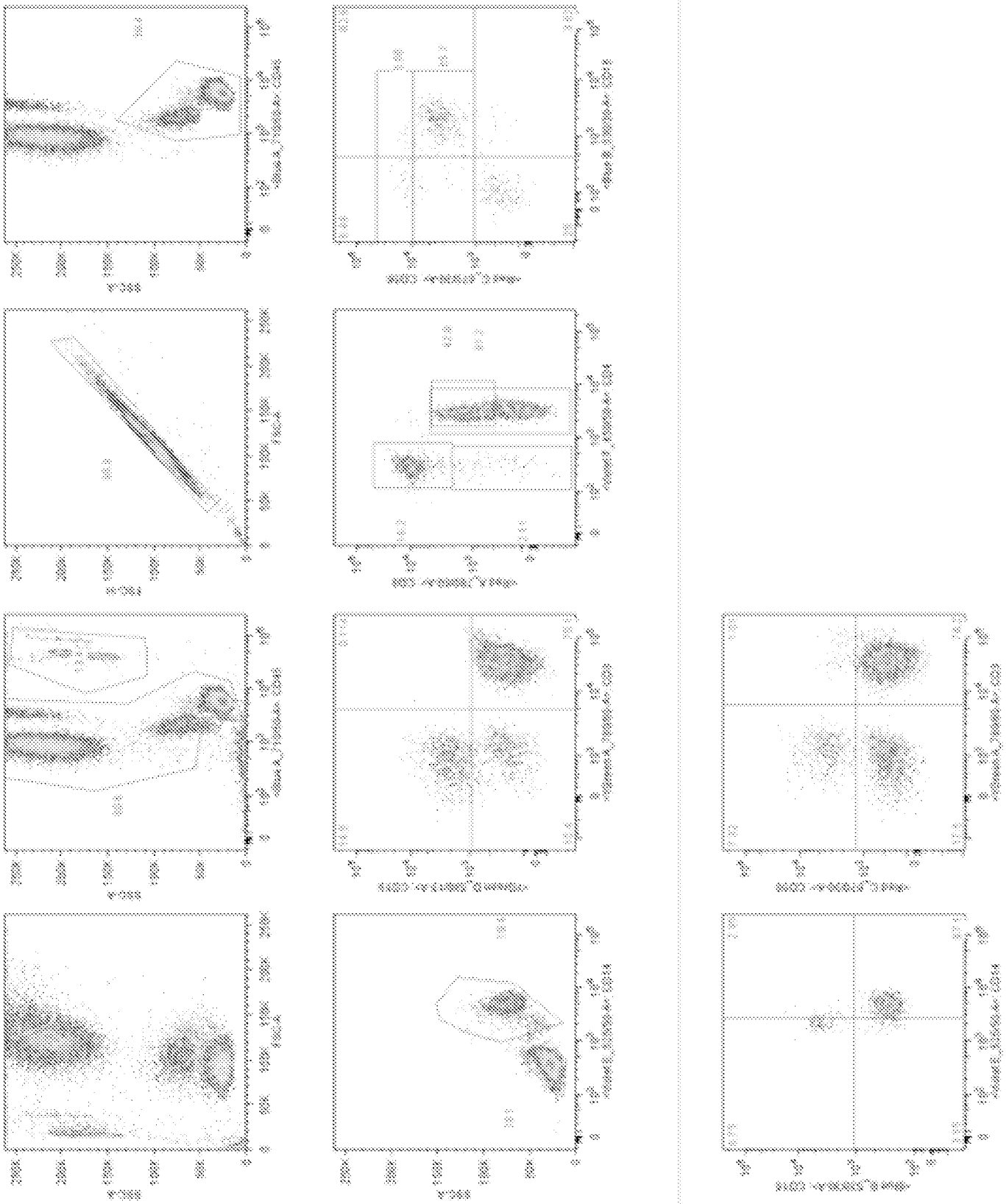


FIG. 5 (cont'd)

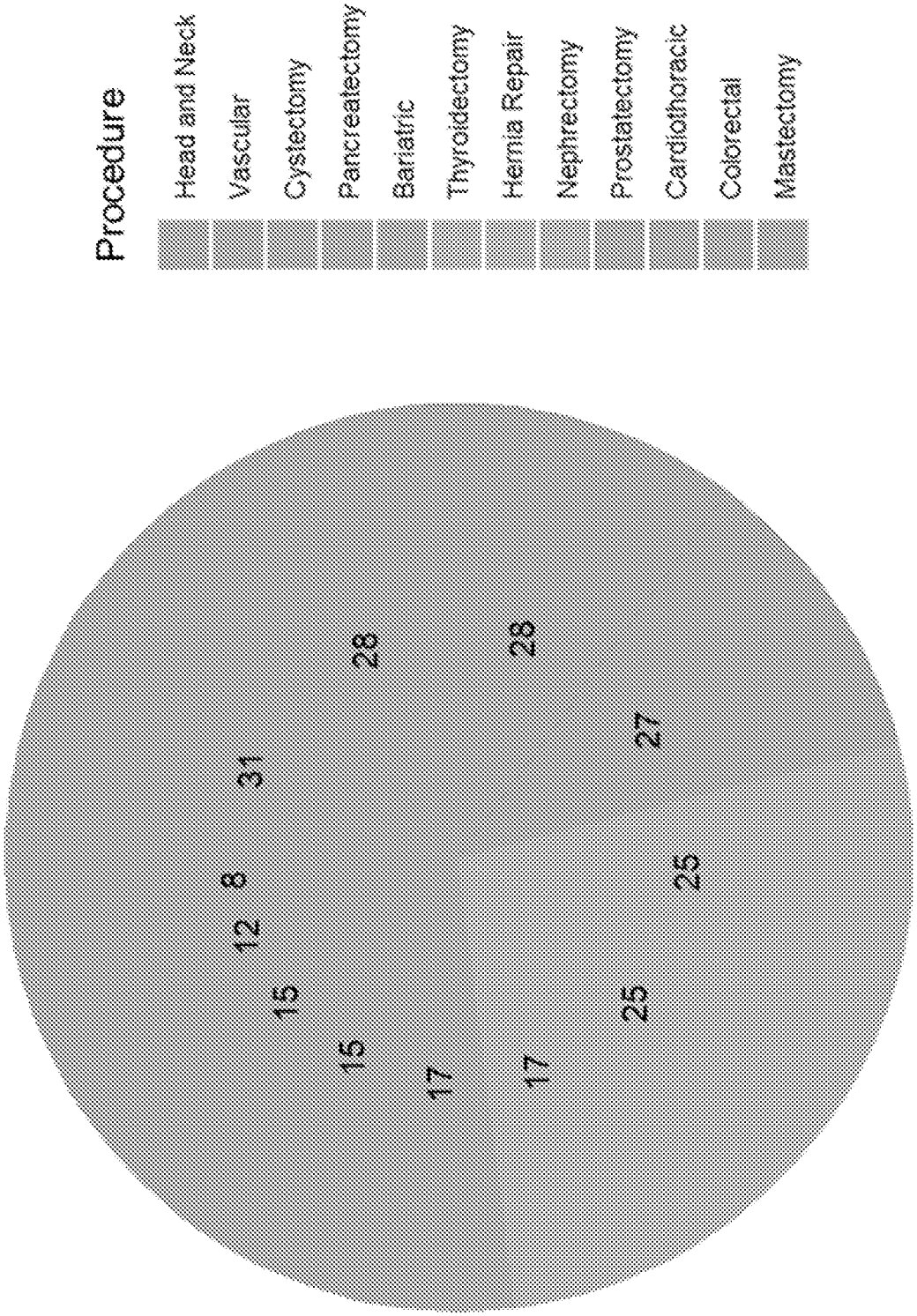
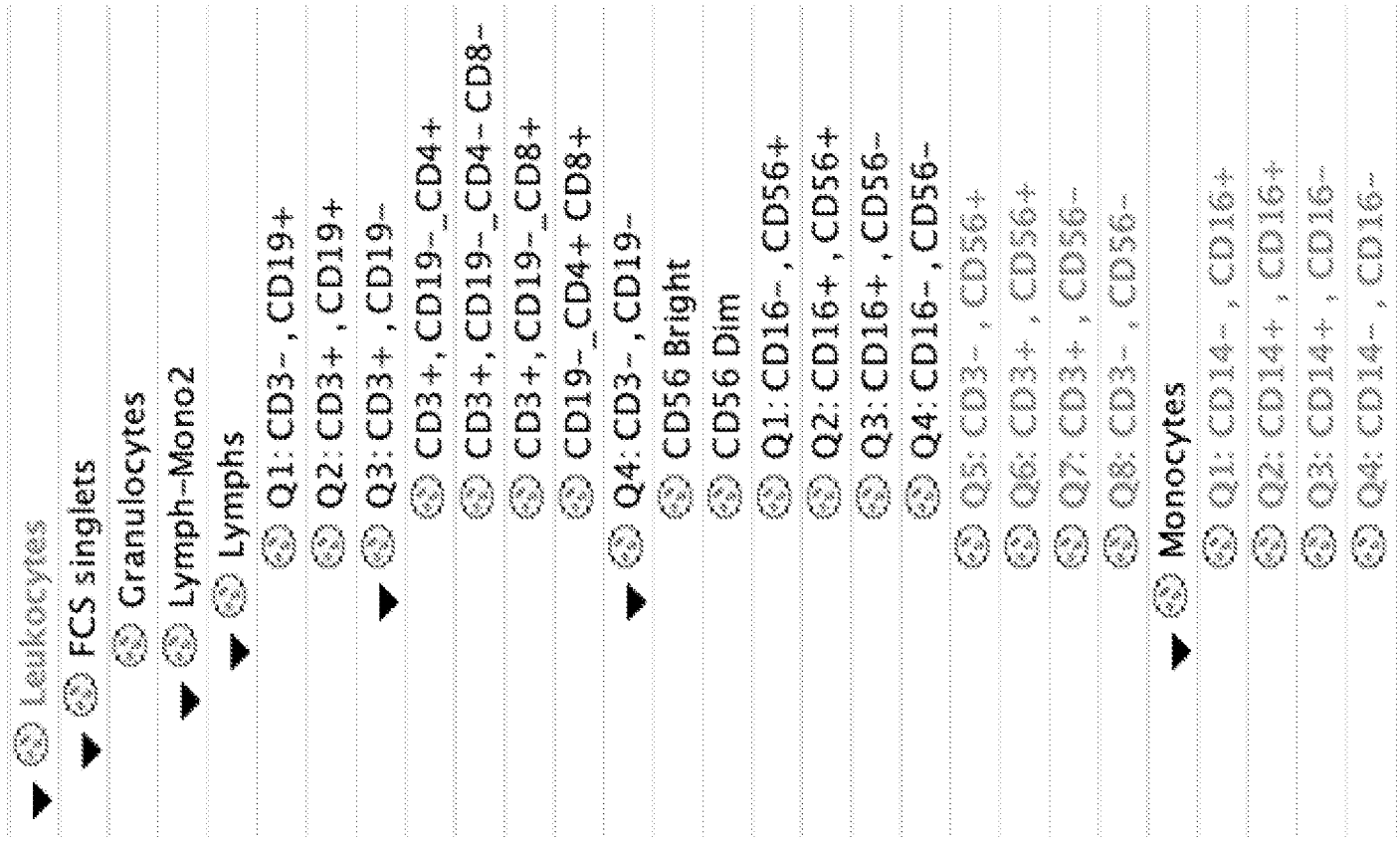

Procedure
Head and Neck
Vascular
Cystectomy
Pancreatectomy
Bariatric
Thyroidectomy
Hernia Repair
Nephrectomy
Prostatectomy
Cardiothoracic
Colorectal
Mastectomy
28
28
31
27
8
25
12
15
25
15
17
17

FIG. 6



FIG. 7

FIG. 7
(cont'd)


Leukocytes
FCS singlets
Granulocytes
Lymph-Mono2
Lymphs
Q1: CD3-, CD19+
Q2: CD3+, CD19+
Q3: CD3+, CD19-
CD3+, CD19-_CD4+
CD3+, CD19-_CD4- CD8-
CD3+, CD19-_CD8+
CD19-_CD4+ CD8+
Q4: CD3-, CD19-
CD56 Bright
CD56 Dim
Q1: CD16-, CD56+
Q2: CD16+, CD56+
Q3: CD16+, CD56-
Q4: CD16-, CD56-
Q5: CD3-, CD56+
Q6: CD3+, CD56+
Q7: CD3+, CD56-
Q8: CD3-, CD56-
Monocytes
Q1: CD14-, CD16+
Q2: CD14+, CD16+
Q3: CD14+, CD16-
Q4: CD14-, CD16-