inhibitor (TIMP1), Heat Shock protein 90 (HSP90) , Cathepsin B (CATB), Ceruloplasmin (CERU), Calprotectin, 14-3-3 sigma (1433S), alpha-2-hs-glycoprotein (FETUA), alpha-2-macroglobulin (A2MG), Transthyretin (TTHY). The subject is likely to have cancer if the levels of these markers change in comparison to the levels in a control sample from a subject lacking cancer. The sample is optimally an ocular sample, such as an isolated tear sample or ocular wash, but can also be from saliva, or other bodily fluid. An ocular sample indicates a tear sample encompassing secretions from the lacrimal gland and other tissues that connect with the lymphatic system.

In one aspect, the present disclosure refers to a method of determining whether a subject has breast cancer comprising: performing steps of detecting levels in a tear sample encompassing secretions from the lacrimal gland that has been obtained from the subject of at least two of the markers provided in Table 1; and determining the subject has, or is likely to have, breast cancer if the levels of the at least two markers change as compared to the levels in a non-cancer control sample, wherein the at least two markers comprise S100A9 and LG3BP, and wherein the levels of S100A9 are increased and the levels of LG3BP are decreased.

Kits for performing methods described herein are also provided. Kits can contain a sample collection platform, a tube for collection and extraction that can comprise a protease inhibitor or other protein-stabilizing agent, sample extraction reagents and testing apparatus.

In another aspect, the present disclosure refers to an immunoassay system, comprising

(a) a tear sample encompassing secretions from the lacrimal gland from a human subject, wherein the subject is suspected of having breast cancer, or has a palpable lump suspected of being cancerous, wherein the tear sample encompassing secretions from the lacrimal gland comprises at least two protein markers comprising S100A9 and LG3BP; and

(b) binding agents and reagents that accommodate a binding interaction specific to the at least two protein markers in (a) to produce a reaction which can be quantitated based on production of a detectable signal,

wherein the levels of S100A9 are increased and the levels of LG3BP are decreased relative to a non-cancer control sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: ANOVA of spectral intensities for the following proteins identified in Experiment 1: (A) Ig Heavy Chain V-IV region HiL, (B) Ig Heavy Chain region V-III region BRO, (C) Ig Heavy Chain V-III region VH26, (D) Antileukoproteinase, (E) β2 Microglobulin, (F) Calmodulin like protein 5, (G) Lipocalin 1, (H) Cystatin B, (I) Galectin 3, (J) Zinc-α 2 glycoprotein.

Figure 2: ROC curves for cancer, benign, and control, from Experiment 1, using Ig Heavy Chain V-IV region HiL, Ig Heavy Chain region V-III region BRO, Ig Heavy Chain V-III region VH26, Antileukoproteinase, β2 Microglobulin, Calmodulin like protein 5, Lipocalin 1, Cystatin B, Galectin 3, Zinc-α 2 glycoprotein.

Figure 3: Mosaic plot of the distribution of breast density for 66 of the 75 samples tested in Experiment 1.

Figure 4: ANOVA of spectral intensities for proteins identified in Experiment 2. Proteins shown are: (A) 14-3-3 protein sigma (B) 6-phosphogluconate dehydrogenase, decarboxylating (C) Alpha-actinin-4 (D) Retinal Dehydrogenase (E) Argininosuccinate synthase (F) Beta-2-microglobulin (G) Calmodulin (H) Ceruloplasmin (I) Cofilin (J) Cystatin-SN (K) Enolase 1 (L) Gelsolin (M) Heat shock protein beta-1 (N) Ig alpha-1 chain C region (O) Lacritin (P) Lysozyme (Q) Basement membrane-specific heparin

CLAIMS

What is claimed is:

1. A method of determining whether a subject has breast cancer comprising: performing steps of detecting levels in a tear sample encompassing secretions from the lacrimal gland that has been obtained from the subject of at least two of the markers provided in Table 1; and determining the subject has, or is likely to have, breast cancer if the levels of the at least two markers change as compared to the levels in a non-cancer control sample, wherein the at least two markers comprise S100A9 and LG3BP, and wherein the levels of S100A9 are increased and the levels of LG3BP are decreased.
2. The method of claim 1, wherein the subject is a human.
3. The method of claim 1 or 2, wherein the LG3BP marker is decreased at least, 1.5 fold, 2 fold, 4 fold or more relative to the level of the marker in the control sample.
4. The method of claim 1 or 2, wherein the S100A9 marker is increased at least, 1.5 fold, 2 fold, 4 fold or more relative to the level of the marker in the control sample.
5. The method of any one of claims 1-4, comprising detecting the levels of a third marker from the list of markers provided in Table 1, and determining the subject has, or is likely to have, breast cancer if the levels of the third marker change as compared to the levels in a control sample lacking cancer, wherein the third marker comprises S100A8, and wherein the levels of S100A8 are increased.
6. The method of claim 5, wherein the S100A8 marker is increased at least, 1.5 fold, 2 fold, 4 fold or more relative to the level of the marker in the control sample.
7. The method of any one of claims 1-6, wherein the levels of the markers are detected by liquid chromatography-mass spectroscopy (LC-MS).
8. The method of any one of claims 1-6, wherein the levels of the markers are detected by an antibody-based detection method.
9. The method of any one of claims 1-6, wherein the levels of the markers are detected by multiplex protein detection method.
10. The method of any one of claims 1-6, wherein the levels of the markers are detected by an mRNA detection method.
11. The method of any one of claims 1-10, wherein the subject is suspected of having breast cancer.
12. The method of any one of claims 1-10, wherein the subject is at increased risk for developing breast cancer.
13. The method of any one of claims 1-10, wherein the subject has a palpable lump

suspected of being cancerous.

14. The method of claim 1, wherein the breast density of the subject is category 1, category 2, category 3 or category 4.
15. The method of claim 14, wherein the breast cancer is detected as a stage of breast cancer.
16. An immunoassay system, comprising

    (a) a tear sample encompassing secretions from the lacrimal gland from a human subject, wherein the subject is suspected of having breast cancer, or has a palpable lump suspected of being cancerous, wherein the tear sample encompassing secretions from the lacrimal gland comprises at least two protein markers comprising S100A9 and LG3BP; and

    (b) binding agents and reagents that accommodate a binding interaction specific to the at least two protein markers in (a) to produce a reaction which can be quantitated based on production of a detectable signal,

    wherein the levels of S100A9 are increased and the levels of LG3BP are decreased relative to a non-cancer control sample.
17. The immunoassay system of claim 16, wherein the tear sample encompassing secretions from the lacrimal gland comprises a third protein marker comprising S100A8, and wherein the binding agents comprise antibodies that specifically bind to the S100A8 protein marker, and wherein the levels S100A8 are increased relative to the non-cancer control sample.
18. The immunoassay system of claim 16 or 17, wherein the tear sample encompassing secretions from the lacrimal gland is an ocular wash sample.
19. The immunoassay system of any one of claims 16-18, wherein the breast density of the subject is category 1, category 2, category 3, or category 4.