# Proteomics: Clinical Applications

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The word proteomics was coined in 1997 to describe the changes in all proteins expressed by a genome. Several sophisticated techniques including two-dimensional electrophoresis, imaging, mass spectrometry, and bioinformatics are used in proteomics to identify, quantify, and characterize proteins. Clinical proteomics is the application of proteomics techniques to the medical field. The main aim of this methodology is to identify proteins involved in pathological processes and to understand how illness can lead to altered protein expression. Clinical proteomics offers the opportunity and the potential to develop new diagnostic and prognostic tests, to identify new therapeutic targets, and eventually to allow the design of individualized patient treatment. Here we present an overview of proteomics applications to the study of disease and its potential to improve diagnosis and prognosis.

**ABREVIATIONS:** 2-DE = two-dimensional electrophoresis; DCM = dilated cardiomyopathy; ELISA = enzyme-linked immuno-absorbent assay; HCC = hepatocellular carcinoma; IL-6 = interleukin-6; LC/MS/MS = liquid chromatography mass spectrometry; MS = mass spectrometry; PSA = prostate-specific antigen; SELDI-MS = surface-enhanced laser desorption/ionization time-of-flight.

**INDEX TERMS:** cancer; clinical proteomics; protein expression; two-dimensional electrophoresis.

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## LEARNING OBJECTIVES

1. Present an overview of proteomics methodology.
2. Comprehend how proteomic methodology is applied in the medical field.
3. Describe the potential application of clinical proteomics in the development of new biomarkers in diagnostic and prognostic tests.

Clinical proteomics aims to identify proteins involved in pathological processes and to evaluate changes in protein expression during illness. Moreover, clinical proteomics offers technical capabilities to develop biomarkers for diagnosis and therapeutic interventions. Proteomics analysis utilizes multiple methodologies to characterize and identify altered proteins as a result of disease. One of the most common techniques is two-dimensional electrophoresis (2-DE), which is used to compare the protein expression among healthy individuals and diseased subjects. In addition, a combination of immunochemical analyses of proteins, bioinformatics (the use of computational techniques to extract meaning from biological data), and different mass spectrometry techniques are used for protein detection and identification.

The diagnosis of various disease states such as cancers and cardiomyopathies is currently based on the detection of single proteins such as troponin, prostate specific antigen (PSA), CA-125, and others. Proteomic analysis examines thousands of proteins at one time allowing the detection of specific protein patterns expressed as a consequence of abnormal cellular function or cellular interactions.[1] Therefore, poten-

tial biomarkers developed as a result of proteomics analysis will have higher sensitivity and specificity since multiplexed panels of clinical tests will measure the altered proteins.

As often happens when new technology is introduced there are many expectations and hopes. There is no exception for clinical proteomics, however, major challenges still remain. One major challenge is to optimize the detection of low abundance proteins (cytokines, transcription factors, and cell-signaling proteins) in tissue, plasma, and body fluids, which are found in the nanogram and picogram range.[2] Moreover, further improvement in software development for data acquisition and interpretation is needed. A good understanding of data management, correlation, interpretation, and validation is crucial to obtain accurate and meaningful results.

Here we present some examples of how clinical proteomics is being used to study disease, and its potential applications in biomarker discovery.

## CANCER DIAGNOSTICS

No standardized screening test is available to reliably detect ovarian cancer. Most women with ovarian cancer are diagnosed at the latest stages of the disease with a five year survival rate of 35%.[3] However, if this cancer could be detected earlier the chances of survival would increase dramatically to >90%.[4] The tumor marker CA-125 is often used to screen for ovarian cancer. However, this test lacks sensitivity and specificity as Ca-125 may be elevated in other physiologic and pathologic conditions. A recent proteomics study has shown promising results. Serum samples of 50 patients with ovarian cancer and 50 unaffected patients were analyzed by SELDI-TOF mass spectrometry. Results showed different protein patterns in cancer patients compared to unaffected individuals with 100% sensitivity and 95% specificity.[3,5] However, the protein profile observed in the cancer group was not identified. In addition, the population tested was small and there were reproducibility problems.[6] Identification of aberrant proteins expressed as a result of cancer is of primary importance for the development of immunoassays and future markers.

Another cancer that has been screened for biomarkers using a proteomic approach is hepatocellular carcinoma (HCC). Protein expression of patients with HCC has been analyzed using serum auto-antibodies that showed cross-reactivity with proteins from the patient's tumor. Three proteins were found to be overexpressed in this analysis: hsp 70, peroxidoxin, and manganese-superoxide dismutase (Mn-SOD). These proteins have been considered potential markers for HCC.[7]

Lung cancer causes more deaths than the combination of the three most common cancers: colon, breast, and prostate. Patients affected by this disease have a five year survival rate when the disease is still localized. However, only 24% of lung cancer cases are diagnosed at an early stage.[8] Proteomics analysis has shown that Napsin A protein was only expressed in patients with primary lung adenocarcinoma.[9] Therefore, this protein has been used as a potential biomarker to differentiate the primary form of lung adenocarcinoma from its metastatic form.[9,10]

## CARDIOVASCULAR DISORDERS

Among several heart diseases, cardiomyopathies have received special attention from proteomics researchers. In 1998, a novel finding by Corbett and others was the decreased expression of 88 myocardial proteins in humans with dilated cardiomyopathy (DCM). These results were confirmed later by other investigators.[11] Some of these low abundant proteins might be considered as useful diagnostic and/or prognostic markers for DCM.

Proteomics can be a key tool for the prognosis of cardiac allograft rejection. Tissue rejection is one of the major problems after cardiac transplantation. Post-transplant endomyocardial biopsies have shown that 100 proteins were overexpressed after cardiac transplantation; however, only thirteen proteins had cardiac-tissue specificity. Of those, two proteins (alpha beta-crystallin and tropomyosin) could be measured in patient's serum presenting cardiac rejection after three months.[12] This is an example of how powerful proteomics techniques can be and their applications in biomarker discovery.

## MICROBIOLOGY

In order to simplify proteomics methodology microbiologists have used bacterial protein-enriched fractions instead of the whole bacterial proteome. This approach examines a specific component of the bacterial cell.[13] For example, examining the cell membrane and/or extracellular proteins of an organism is of great interest since these proteins are often involved in host-pathogen interactions. Blonder and others used liquid chromatography coupled with mass spectrometry to analyze the *Pseudomonas aeuriginosa* membrane subproteome (selected fraction of proteins from an organism).[14] This study identified 786 proteins. Some of these proteins were integral

and outer membrane proteins involved in adaptation and antibiotic resistance. Although the results of this study are subject to further confirmation, many proteins identified may be targets for development of novel drug therapies.

In an effort to improve immunodiagnostics and vaccines for *Mycobacterium tuberculosis,* Bahk and others examined *M. tuberculosis* proteins secreted in culture by 2-DE.[15] Eight proteins were identified by liquid chromatography and tandem mass spectrometry (LC/MS/MS). All proteins were cloned and over-expresssed in *E. coli* cells. Three proteins (rRV3369, rRv3874, and rRv0566c) were selected for pre-screening and potential serodiagnostic antigens. Sera from 100 tuberculosis patients and 100 sera from healthy controls were analyzed by ELISA. Results from the analysis showed that the antigens rRV3369 and rRv3874 had 60% and 74% sensitivity and 96% and 97% specificity, respectively. These proteins have the potential to be used in the serodiagnosis of tuberculosis.

Proteomic analysis has been used to study protein expression in virulent and avirulent strains, interaction of bacteria with eukaryotic cells grown *in vitro*, the host immune response to infection, and drug resistance of microorganisms.[16]

Based on the information presented, it is clear that the application of proteomics to the medical field has a great potential for the improvement of diagnostics and therapeutics. Nevertheless, there are challenges that need to be overcome. Blood is perhaps one of the most complex proteomes because in addition to proteins normally found in plasma other proteins that are not normally present may be found. These additional proteins may be released from tissue in response to injury or a disease state.[6] Protein concentration in plasma varies dramatically. Among the most abundant proteins in plasma are albumin and the globulins, which are present in the milligram per milliliter range, in contrast to cytokines (IL-6), which are found in picograms per milliliter.[2,6] This extreme difference poses a problem during proteomic analysis. Therefore, in proteomic studies elimination of highly abundant proteins is required to facilitate analysis, however, during elimination of the abundant proteins, such as albumin, low abundance proteins that bind albumin and that may be of interest for biomarker discovery may also be removed.

It is necessary to have a good understanding of the variability sources that may contribute to error such as pre-analytical, analytical, and biological variation. Pre-analytical variability may be introduced during specimen collection and manipulation, pipetting, and dilution of samples. Careful consideration should be given to specimen collection using different tube type (whole blood/plasma/serum), coagulation times, and storage conditions. Analytical variability may occur in inaccurate calibration of instruments (MS/2-DE), standardization of output, and appropriate controls and proper bioinformatics methodology. In addition, it is important to account the biological variability due to gender, age, race, and fluctuations that may occur daily within an individual (biorhythm, fasting, time of the day). All these variables may induce changes in that are not pathological in nature but that have to be differentiated from a pathological-induced process.

## CONCLUSION

Clinical proteomics offers the promise of biomarker discovery and early detection, diagnosis and prognosis of disease, but major challenges still remain. Further advances in technology are needed to eliminate proteomics deficiencies and augment its contributions to the medical field.

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