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Biomarkers in prostate cancer: defining ‘pussycat versus tiger’ phenotype by proteomic modeling

Bandar Alghanem

A Project undertaken at the
Interdisciplinary Centre for Biomedical Research,
Nottingham Trent University

**A Dissertation submitted in partial fulfillment of the requirements for the
degree of Master of Research in Advanced Genomic and Proteomic
Sciences at the University of Nottingham**

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Abstract

Prostate cancer is the one of the major causes of morbidity and mortality in the western world. It affects the prostate gland of males with a significant increase in the disease incidence every year. Current diagnostic and prognostic markers, such as prostate specific antigen (PSA), rectal examination and Gleason grades have their own limitations in a wider context of disease treatment and prediction. There is therefore a pressing need for novel and powerful biomarkers at protein or metabolite level. This study attempts to profile and identify candidate prostate cancer stage specific markers, within a defined population of samples. The samples were classified, based on the pathological information as “aggressive” (Gleason grade > 7) and “nonaggressive” (Gleason grade < 7). The proteomic protocols standardised at the John van Geest Cancer Research Centre, were used for the initial characterisation of the samples. The MS spectra obtained from the samples were used applied to an artificial neural network (ANN) based algorithm to generate predictive ions able to classify the samples. Three ions (m/z 1268.8, 998.6, 910.4) were able to predict and classify with high specificity and sensitivity. 24 samples were immunodepleted and subjected to nano-LC fractionation and MALDI-TOF analysis, generating 80-120 protein identities per sample. The three ions predicted previously by the ANN identified as Haemopexin, Gelsolin and Apolipoprotein B 100. Using ProfileAnalysis software, this study identified Apolipoprotein isoforms, including Apolipoprotein B 100, and Afamin as the proteins which showed differential expression in between the groups. This study identifies Apolipoprotein B 100 as a potential marker using two different modeling approaches suggesting this protein as the potential biomarker candidate. The utility of high throughput proteomic platforms such as Robotic liquid handling, MALDI-TOF and LC-MALDI for serum biomarker identification in PCa has been shown during this investigation.

Biomarkers in prostate cancer: defining ‘pussycat versus tiger’ phenotype by proteomic modeling

Bandar Alghanem

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Abbreviations

1-D SDS PAGE/1DGE - 1-Dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis.

2-D SDS PAGE/2DGE - 2-Dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

ACN – Acetonitrile

ANN – Artificial Neural Network

APCI-Atmospheric Pressure Ionisation

BPH – Benign Prostate Hyperplasia

BSA – Bovine Serum Albumin

CID – Collision Induced Dissociation

CHCA – α -Cyano-4-Hydroxy-Cinamic Acid

Da- Dalton

DER-Digital Rectal Exam

ESI – Electrospray Ionisation

FPSA-Free Prostate Specific Antigen

GC-Gas Chromatography

HGPIN-High grade Prostatic intraepithelial Neoplasia

HPLC – High Performance Liquid Chromatography

LC-Liquid Chromatography

LGPIN-Low Grade Prostatic intraepithelial Neoplasia

m/z- Mass to charge ratio

MARS – Multiple Affinity Removal System

MALDI – Matrix assisted Laser Desorption/Ionisation

MS – Mass Spectrometry

MSMS – Tandem Mass Spectrometry

MTP – MALDI Target Plate

PMF – Peptide Mass Fingerprint

PCa – Prostate Cancer

PCA-Principle Component Analysis

PSA – Prostate Specific Antigen

PIN- Prostatic intraepithelial Neoplasia

QC – Quality Control

RF-Radio Frequency

ROC – Response Operator Curve

TOF – Time of Flight

TFA – Trifluoroacetic Acid

TRUS-Transrectal ultrasound

WHO-World Health Organisation

1-Introduction

1.1 Cancer

Cancer can be defined as an abnormal growth of cells caused by various different changes at the genetic and epigenetic level which ultimately leads to uncontrolled cell replication, growth and proliferation. This abnormal growth eventually invades other adjacent tissues and can metastasise to distant sites causing morbidity or mortality (Ruddon 2007). According to the World Health Organisation (WHO) there were around 12 million new cases of cancer in 2008, and 7 million deaths from cancer in the same year. Among the known cancer types, lung cancer is the most common cause of death in both genders, followed by breast cancer in females and prostate cancer in males. These statistics are obtained from developed and developing countries which are hugely different in many factors such as the lifestyle, dietary, pollution and health care efficiency. Along with molecular changes, these factors also might contribute towards the development of cancer in many parts of the world.

In the past few decades there have been significant scientific advancements in the field of cancer biology, diagnosis and therapeutic intervention, though we still need more research effort to be undertaken in different cellular physiologic and developmental context to fully understand prostate cancer aetiology. Even though many cancers share a common root of disease development, a common strategy of treatment is yet to be devised. Different cancers and individuals respond to the chemo- and immuno-therapy differently depending on the stage of the cancer, so it is necessary to identify and define each of the patient situations more accurately, if possible at the molecular level prior to devising a suitable treatment regime.

1.1.1 Prostate cancer

Prostate cancer is the one of the major causes of death in the western world; in UK there were around 35,000 cases in 2007, and 10,200 of men died from prostate cancer in the same year (UK Research, Cancer). Like other cancers Prostate cancer is also an anomalous growth of cells in prostate gland which leads to abnormally forming tumours. The prostate is a walnut sized and shaped gland found in men under the bladder (figure 1.1) which secretes prostatic fluid which is released during ejaculation. Normally the size of prostate gland increases with age and the condition is commonly referred to as benign prostatic hypertrophy (BPH) (Evans et al., 2008). The enlargement in the gland causes difficulty with urination, with common symptoms of lack and delay in stream, non-contiguous running, dribbling, and burning during streaming. However, in some cases the need to urinate frequently, particularly during sleep which results in lack of sleep and poor health in general. In the UK there are 78.000 new cases with these symptoms every year (UK Research Cancer). These symptoms do not necessarily indicate prostate cancer, because the patients with BPH or infections in their prostate gland have the same symptoms. Therefore it is hard for the physicians to diagnose localised cancer with these symptoms alone.

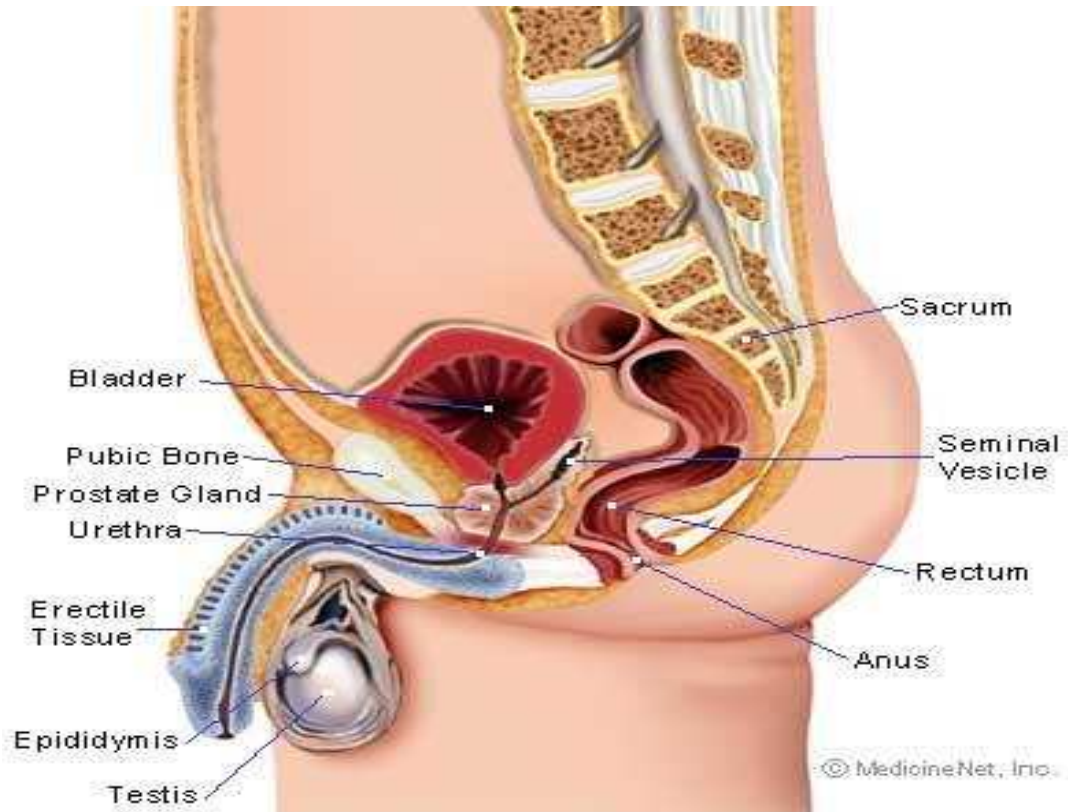


Figure 1.1. This shows the location and the shape of the prostate gland. (figure was taken from: <http://lowerbloodpressurecheap.com/>)

There are several diagnostic procedures adopted by different clinics around the world. Traditional Prostate Specific Antigen (PSA) screening in conjunction with Digital Rectal Exam (DRE) has been used for the last two decades (Damber and Aus 2008). Moreover the combination of these two tests is more efficient than when they are used individually. In DRE the clinician or nurse insert a gloved finger into the rectum to examine if there is any enlargement or abnormality in the prostate gland. The efficiency of this test is entirely dependent on the examiner who is conducting the examination. In PSA screening, patient blood is extracted and the serum tested for the presence and levels of PSA in a clinical biochemistry laboratory.

1.1.2 Prostate Specific Antigen (PSA)

PSA is an enzyme of the human glandular kallikrein family, which is formed in the prostate gland. PSA is an important compound in the seminal fluid, which causes proteolysis in the gel-forming proteins that are found to trap and cleave spermatozoa into small fragments, therefore releasing spermatozoa during ejaculation. PSA enters the serum at low concentrations by leaking from luminal cells through the epithelial membranes. Blood samples from the patient are analysed using an immunoassay system based on the reaction between the antigen and the antibody and detect and quantify the PSA concentration in the blood. The concentration of PSA measured is at the nanogram per millilitre (ng/mL) levels, and the normal range is considered to be less than 4 ng/mL (UK Research Cancer). Unfortunately the lack of specificity and sensitivity for PSA leads to improper diagnoses which lead to increased risk of false negatives and positives (Hellstrom et al., 2007). However most urologists will request a “free PSA” test if the result of PSA is between the ranges of 4-10 ng/ml. “Free PSA” is the concentration of PSA that is not bound to any other proteins. Free PSA percentage in the serum can give more specificity for the PSA test and determine whether patients need to go for further diagnostic procedures such as biopsy examinations. The decreased percentage of free PSA is most likely to be a risk factor of prostate cancer, however not all laboratories around the world have free PSA test, and most clinicians not satisfied with DRE and PSA tests will suggest a biopsy examination.

1.1.3 Biopsy test

All the prostate cancer screening methods mentioned above support the urologist's decisions for urgent biopsy study or to repeat these tests (DRE, PSA and FPSA) after a period of time. When the clinician recommends a biopsy test, the patient will be asked to follow some instructions before the study day. Some medications will be stopped for a week prior the biopsy such as aspirin or any non-steroidal anti-inflammatory drugs in order to minimise any bleeding during the biopsy. Patients are also asked to take some antibiotics before and after the study for minimising any infections. In biopsy operations urologists will take some cells specimen from the different places in the prostate gland by needle to analyse cancerous cells or tissues under microscope by a pathologist.

There are three types of prostate biopsies: the transrectal, the transurethral, and the transperineal. The transrectal prostate biopsy is guided by the transrectal ultrasound (TRUS) through the anus and into the rectum. The transurethral biopsy is tested by a lighted cystoscope up through the urethra so it will allow the urologist to see deeply to the prostate. The transperineal biopsy collects the cells through a small incision in perineum, which is located between the anal sphincter and the scrotum. In most cases TRUS will be performed as the primary biopsy, in which ultrasound probe will be inserted through the rectum and get waves back from prostate gland then biopsies will be obtained. Usually the biopsy collection is carried out several times with 8-12 cores taken from different areas in the prostate which depends on the size of prostate gland. The biopsies will be analysed by two pathologists for inter-observer agreement. It is rare to have different interpretations, in which one pathologist states cancer and the other not, However, there is a chance of getting both the diagnoses wrong if the biopsies missed the

area of cancerous growth, thus urologists will ask for re-biopsy if they doubt there is possibility of prostate cancers based on DRE and PSA screening or even with high family history of this cancer. In this case there is 50% possibility to detect prostate cancer with repeating biopsy. The common measure of the aggressiveness and invasion of the cancerous growth in PC is interpreted by the pathologist by the Gleason grade.

1.1.4 Gleason grade

Gleason grade was invented in 1987 by the physician and pathologist Dr Donald F. Gleason, who was studying the prognostics of prostate cancer. He obtained this score to differentiate aggressive from non-aggressive cancer based on the appearance of cancer cells and the degree of difference in shape from normal cells. Basically the grade takes score from 1 to 5. Score 1 is considered to be very similar to normal cells, and becoming more differentiated going up until score 5 which is very different from normal cells (figure 1.2). There are two grads, the first one represents the most common pattern of the tissue and the second is the next most common pattern of the gland and the sum of two grades will give the Gleason score (sum) which is from two to ten. Since the final grade is the cumulative of the two most predominant patterns in the tumour section the interpretation of the score is also slightly different. For example when Gleason sum is 7, it can be represented in two ways (3+4) and (4+3, both give the same sum, but with pattern and the completely of the two cases are entirely different. Gleason 4+3 considered to be behave more aggressively than Gleason 3+4, while the primary pattern (grade) can give more significant indication of the behaving of the tumour than the secondary pattern. However there are many studies still trying to prove this significant difference in tumour

interpretation between Gleason grade 3+4 and 4+3 like situations (Lopez-Beltran et al., 2006).

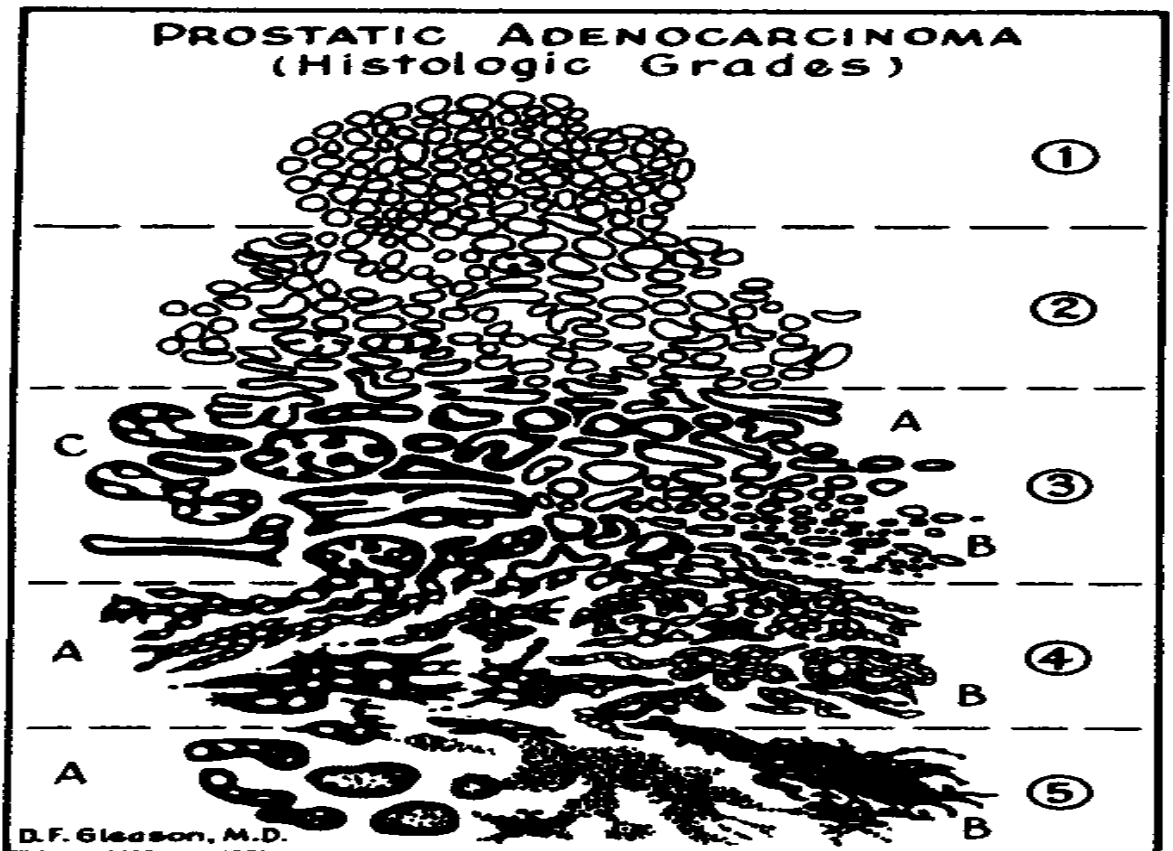


Figure 1.2. Histologic grades been obtained by Dr.Gleason illustrated the differences of the cancers cells shape from score 1-5. (The figure was taken from: www.malecare.com/gleason-score_58.htm)

However, once the pathologist has obtained the Gleason score in his report the other test for the biopsy slides which might be included in the report is the presence of prostatic

intraepithelial neoplasia (PIN). In this examination the pathologist will examine how the cells look and based on it will decide whether the biopsies specimens have high grade prostatic intraepithelial neoplasia (HGPIN), or low grade prostatic intraepithelial neoplasia (LGPIN). In LGPIN patients have low risk of cancer, whereas patients with HGPIN have high risk of prostate cancer. So usually the urologist will recommended for re-biopsy for who those with HGPIN, so the possibility of finding prostate cancer cells is between 35-45%. However, a comprehensive report will be delivered to the urologist including whether prostate cancer present or not, with Gleason scores and the area of the cancers cells if they have been found, then if patient has HGPIN or not. The urologist will take his final diagnosis based on the pathologist report and with his findings from previous tests (PSA, DRE) for any further follow-up or treatments.

1.1.5 Prostate cancer treatment

Prostate cancer treatment remains a big challenge for all the surgeons and the urologists. For patients with metastatic cancer, there is no cure even with the advancement in chemotherapy treatments. However, there are many areas that should be considered before taking the decision for any particular treatments such as pattern of the cancer, spread of the cancer, age, patient health, sexual function of the patient and other factors. Furthermore; there should be consideration of the benefit and the risk of each type of treatment. Fortunately, prostate cancer cells grow slowly in many cases, in which the physician will just monitor the patient and will not go for any treatments (watchful waiting) unless the tumor starts to grow significantly. In some cases patients will undergo

a surgical option and if the patient is not too old and also has a high grade in Gleason score. The surgeon will remove the prostate gland by radical prostatectomy using laparoscopic methods to monitor the prostate gland by small camera inserted into the body. In this type of treatment the patient might be cured completely if the cancer has not already spread. Another type of surgical intervention is removing both testicles (orchidectomy) so testosterone will not cause growth of the prostate because most testosterone is produced from the testicles, and in this is type of hormone therapy that will be followed when the cancer spreads out from the prostate gland such to the bone. There is different hormone therapy without surgery, using only medications which inhibit testosterone in the body. Chemotherapy is a good option for those who have metastatic cancer, in these treatments there is no cure but the spread might be slowed.

1.2 Proteomics

The enormous advancements in genomics technology in the past decade after the human genome sequencing in 2001 paved the way to understanding the molecular framework at the genomic sequence level. However, the functional understanding of each gene requires the interpretation of these genes at the functional level in the context of various genetic physiological and environmental conditions. Post genomic era of modern molecular biology such as micro arrays has enabled the scientific community to understand the gene expression and its regulation at the transcriptional level. But most of the functional understanding of each gene requires its definition at the protein level since proteins are

the far end of gene expression which carry hundreds or even thousands of post translational modifications.

Proteomics is the study of all the proteins expressed in a cell, tissue, or organ at any given time or condition often using high throughput technologies such as mass spectrometry combined with appropriate bioinformatics tools to identify and typify proteins. The protein expression studies have been well documented even before the recent technological advancement as evidenced by the work of Anderson and colleague in an attempt to identify all the proteins in human plasma in 1977. For many years polyacrylamide gel based one dimensional size fractionation were widely used for the characterisation of differentially expressed proteins. However, this technique was superseded by much more informative two dimensional electrophoresis (2-DE) which separates and fractionates the total proteome in two dimensions based on the charge (PI) and the mass (mw). The consequence is dramatic, the power of 2D gel electrophoresis and mass spectrometry has enabled the identification and characterisation of hundreds of proteins differentially expressed in various biological conditions. Despite of all these advancements, proteomics has its own inherent problems such as the lack of high throughput nature, the information content from each experiments and the reproducibility. Even though, importance of protein studies still remains high on the agenda because of the post translational complexities which cannot be accounted by transcriptional interpretation alone. There are around 30,000 genes in the human genome which encode approximately 500,000-1000000 proteins. This disparity indicates one gene does not necessarily encode one protein in the cell. Several factors such as the

mRNA splicing, phosphorylation, glycosylation and other protein folding mechanisms contribute towards the functional diversity of the total proteome.

Alternative splicing is one of the reasons that make one gene produce different proteins, and this is transcriptional level diversification of the protein. Genes contain many exons (the coding DNA) and introns (the non-coding DNA) at the DNA sequence level. If a gene contains 6 exon, one transcribed mRNA can contain 1-5 exons and another mRNA can contain 1-4 and 6 exons by a process called alternate splicing (Domingues et al., 2007) (figure 1.3)

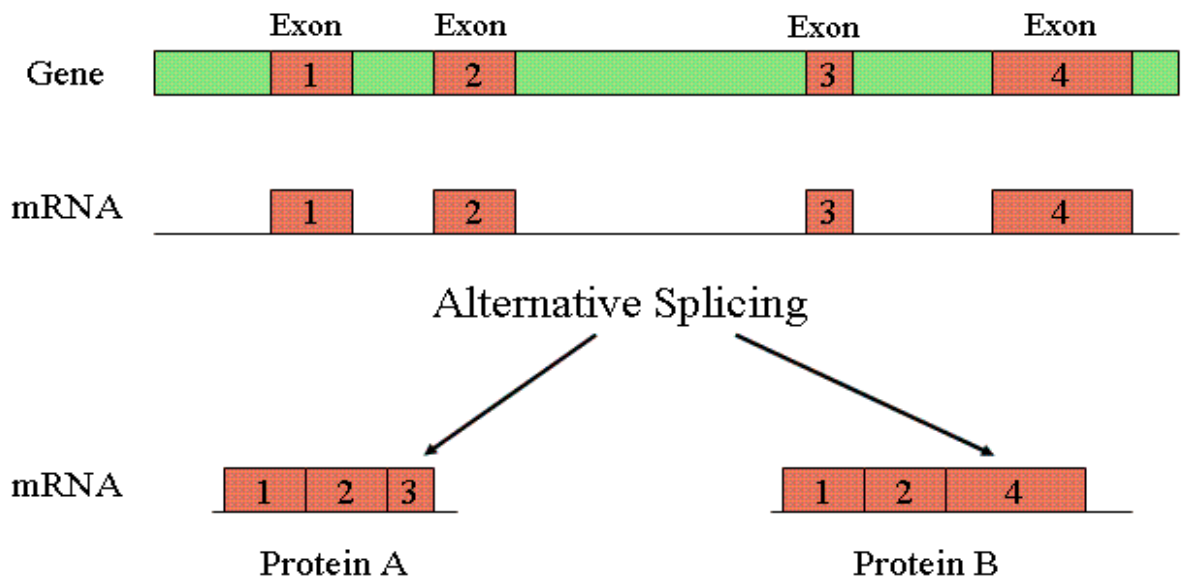


Figure 1. 3 illustration showing alternative splicing.
(http://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/alternative_splicing.html)

Another reason lead to the huge variability of proteins from certain gene is post-translational modifications. Basically these modifications occur after the translation level by proteolytic cleavage or by chemical modifications such as phosphorylation, methylation, and acetylation to one or more amino acids. Therefore, these modifications lead to more diversity of proteins from the essential synthesised proteins. The importance of post-translations modifications becomes clear when determining the activity status, protein localizations, protein turnover, and the protein-protein interactions. Thus, the analysis of the expression of mRNA poorly indicates the protein function, whereas the direct analysis of proteins enhances our understanding of their function. Another limitation of proteomics is the low abundance of the proteins; some of the proteins exist at very low concentrations so detection of these proteins is very difficult with conventional gel based approaches. Lack of a similar technique like polymerase chain reaction which increases the mRNA transcript levels is lacking in proteomics which makes the scenario more difficult for protein studies in comparison to the mRNA studies.

There are two approaches in the field of proteomics, “expression proteomics” which is the analysis of the protein patterns in different conditions (health/illness), and functional proteomics which deals with the protein - protein interactions and their activities in the cells. These approaches have achieved considerable momentum recently with the massive improvement in the instrumentation and fractionation techniques in mass spectrometry and chromatography. The data generated in proteomic studies are considerably larger and massively parallel compared with the traditional gel based approaches. The revolution in technology has generated highly multidimensional data sets and the bioinformatics tools

which allow us to increase our understanding of proteomics have made it possible to investigate the proteome in a system biology approach.

1.2.1 Mass spectrometry

During the past decade Mass spectrometry (MS) has been emerged as the mainstay instrumentation in all proteomics laboratories around the world. The sensitivity and reproducibility allows this technology to identify proteins rapidly, so that it can be used in highthroughput screening method to study different biological complexities. All the mass spectrometry analysers are composed of three main parts; 1- ionizations source, 2- mass analyser, and 3- detector (figure 1.4)

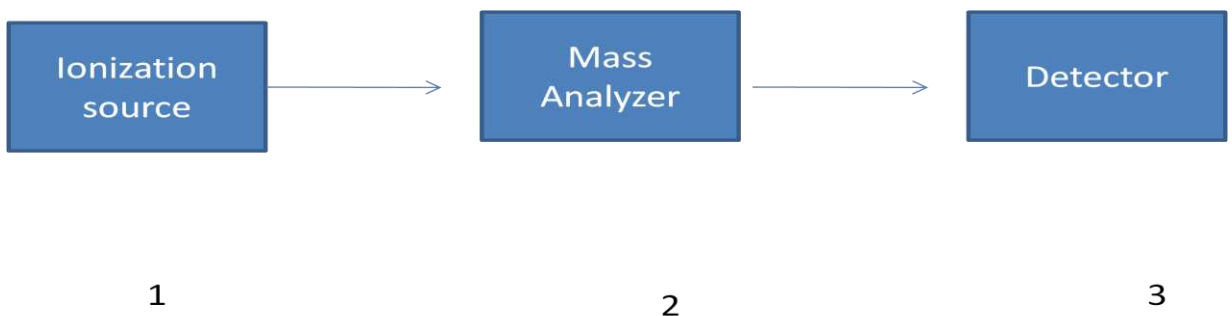


Figure 1.4. The Basics of mass spectrometry

The ionisation source is a device which ionises the protein\peptide molecule which is then transported by magnetic or electric fields to the mass analyser. There are many types of ionisations such as protonation $[M + H]^+$ deprotonation, $[M - H]^+$ and cationisation $[M + \text{Cation}]^+$. There are different types of the ionisation sources are available such as Electrospray ionisation (ESI), Atmospheric pressure chemical ionisation (APCI), Matrix-assisted laser desorption/ionisation (MALDI), and Desorption/ionisation on silicon (DIOS). The major differences among these types of source are the way the analyte is ionised, for example, in ESI the ionisation is facilitated by the evaporation of the charged droplets by the high voltage electric field, whereas in MALDI the ionisation is mainly by photon absorption and proton transfer. After the ionisation, the analyte it is guided to the mass analyser. There are many types of these analysers are used in various mass spectrometers such as Quadruple, time of flight (TOF), and ion-trap. A Quadrupole analyser has four steel rods that are connected to an radio frequency (RF) generator, which allows ions with specific m/z to pass through it to the detector. Quadrupoles have a significant mass rang with the capability of the analyzing up to an m/z of 4000, which is useful because ESI of proteins mostly produces charged distributions from m/z 1000 to 3500. In ion-trap analyser, the ions are passing through a quadrupole analyser and trapped in a radio frequency quadrupole field. The radio frequency is scanned to resonantly excite and therefore eject ions through small holes in the endcap to a detector. As the RF is scanned to high frequencies, higher m/z are excited, ejected, and detected.

Another type of mass analyser commonly used is time of flight (TOF). TOF is basically a long tube with high vacuum, which has a pulse device used to accelerate the ions into the flight tube and all ions enter at the same time. Ions with low m/z travel faster, whereas

ions with high m/z travel slower. Once all molecules are ionised and guided by the mass analyser to a detector. When the ions hits the detector spectra (peaks) will be obtained which shows the intensity at different masses (m/z values). MALDI- TOF is the most widely used method for the characterization of the peptides and the proteins. In peptide mass fingerprint (PMF) the peptide mass obtained from MS is matched with abstract peptide mass generated from known sequence of proteins or genome using computer software like MASCOT to search the database such as SWISSPROT for proteins identification. The limitation of this method is that PMF algorithms suggest all peptides which come from one protein, so to overcome this problem when we have number of peptides from a mixture of proteins is to fragment each peak by different methods such as collision induced dissociation (CID) which allow for tandem MS (MSMS) in order for more specificity protein identifications.

1.2.2 MALDI-TOF/TOF mass spectrometry

It is crucial to choose the appropriate type of MS for any given study, considering all the advantages and disadvantages for each type of MS instrumentations and which one of them will promise for more accuracy. The new MALDI-TOF/TOF-MS is guarantee with high sensitivity and reproducibility. As described in the previous section MALDI-TOF/TOF is analyser measure the mass to charge ratio (m/z) for protein/peptide ions.

In MALDI-TOF the sample (protein/peptide) will be mixed with organic acid “matrix” (such as α -Cyano-4-hydroxycinnamic acid CHCA), in which will accelerate the ionization of the protein/peptide molecules then a dry spot will be fixed in target plate

(ground steel plate or Anchorchip plate) and then the laser power will be applied which will fire at specific spot and ionise the protein/peptide molecule. Then the ions will be guided into mass analyser TOF/TOF. Rather than the liner TOF as mention above, TOF reflecton has two flight tubes and reflectron (ion mirror) take place in end of the first flight tube and reflect the ions into the second time flight tube which allow for more length of the flight tube, and thus enhance the resolution (ions separations). End of the second flight tube there is a detector. Graphical peaks (spectra) will be generated shows intensity for all the peptide mass. One of the advantages of MALDI-TOF is its operational simplicity, easy maintenance, and its ability to detect high mass range (200-500,000 Da).

1.2.3 Proteomics biomarkers discovery

One of the challenging demands of all the clinicians around the world is to find reliable biomarkers for specific diseases (cancer) or disease staging which enhance early diagnosis and prognosis thereby increasing the chances of better management of the disease. Biomarker can be defined as any biological substance which can indicate the state of disease or the treatment efficacy. These biomarkers can be used to predict, diagnose, and prognoses disease and detect the response of particular drug or the stage of the disease, these markers are genes, metabolites (peptide, amino acids, and carbohydrates), or proteins. Although genetic and metabolites markers are good indicators, it is highly necessary to identify protein biomarkers because most of the disease states are well linked to abnormal proteins mainly modified at the post translational level. Some of the protein biomarkers currently identified such as CA 125

(cancer antigen 125) for ovarian cancer (Hanash et al., 2008), CA 15-3 (cancer antigen 15-3) for breast cancer, CEA (Carcinoembryonic Antigen) (Xue et al., 2008), PSA (prostate specific antigen) for prostate cancers to monitor the treatments for certain cancers, and troponin I (cTnI) as cardiac marker (myocardial infection) (Lescuyer et al., 2007) are emphasising the importance of novel biomarkers. Several factors decide the quality of an ideal biomarker. Primarily, the efficiency of any biomarker relies upon its specificity and sensitivity with low false positive and negative rates. Thus, they must be robust. Secondly, reproducibility, Candidate biomarkers identified in the clinical laboratory settings do not necessarily reproduce their utility when they are taken in to a real life scenario. Thirdly, the requirement of biological sampling such as bio-fluid (serum, plasma, and urine) markers avoids the need for an invasive sampling and clinicians can then investigate rapidly. In the current biomarker discovery scenario, serum and plasma are widely used by many laboratories despite their complexities. Apart from its easy accessibility, serum is considered to a goldmine for biomarkers because serum has the ability to connect with all the cells of the body. In addition to this, serum contains many low abundant proteins that leak from diseased tissues, especially during illness (cancers). These low abundant proteins promise to be significant biomarkers, in particular cancer-related biomarkers. Although it is important to identify proteomics biomarkers in serum and other biological fluids, the discovery is particularly challenging in the serum due to its complexity. The changes in protein state from one patient to another one due to many factors such as environmental, diet, lifestyle, and stress, making protein expression altered even though they have the same disease. Another factor is the high dynamic range of protein concentration in serum, in which the serum albumin

concentration is 50g/l compared with some other proteins with very low concentration such as PSA at ~4ng/ml (more than 10 orders of magnitude for protein concentrations reported in human serum) (Jacobs et al., 2005). Appropriate pre-fractionation methods which will selectively eliminate high abundant proteins prior to mass spectrometry analysis will enhance the ability to identify potential biomarkers.

1.2.4 Fractionation techniques

Serum is considered as the greatest source of proteomics markers in human, on other hand it is highly dynamic which means it undergo many biological changes in a very short period of time in patients. Unfortunately these complexities and the high dynamic range in serum make it difficult as a starting material for biomarker discovery. However the advancement in separation and fractionation methods prior to MS analysis reduce the complexity of the spectra obtained from MS especially for the low abundance proteins which is has the power for significant biomarkers. So the biggest challenge facing proteomics analysis is the way to separate the low abundance proteome from high abundance proteins (such as ALB, and IgG).

In present day, there are two approaches followed in most proteomics laboratories; first bottom-up, in which the mixture of proteins in sample will be enzyme cleaved into small proteins/peptides, then these peptides, will be further separated by GC or LC technology (Righetti et al., 2005). This method is highly used and shows a high separations of digested peptide and with many proteins been identified. However there are limitations in this method also such as the limited number of peptides which is relying

on to identify a given protein might not be sufficient especially for protein isoforms. Second approach is top-up, which is usually applied by ESI-MS. The ion source (ESI) generated proteins ions are fragmented by gas-phase, then be introduced by MS. This approach, compared with bottom-up, can give a complete sequence for a given protein. On the other hand this approach suffers in isolating proteins because of the complex spectra obtained from multiple charged proteins.

The depletion of high abundance proteins and enrich the low abundance proteins is become one of the technique been used recently used as a primary method prior to downstream analysis, which shows high ability of detecting proteins with ng/mL concentration (Tang et al., 2006). The use of Cibracon blue dye is one of the most basic method applied to serum sample which has a specific affinity for ALB (steel et al., 2003). Recently, the multiple affinity removal system (MARS) immunodepleted for more than one protein has been widely used as an effective protein fractionation technique. This immunodepleted column can be found commercially targeting 6 or 14 for the high abundance proteins in the serum such as Albumin, Immunoglobulin G, Transferrin, Haptoglobin, α -1-Antitrypsin, Immunoglobulin A, Fibrinogen, α -2-Macroglobulin, α -1-Acid Glycoprotein, Immunoglobulin M, Apolipoprotein AI, Apolipoprotein AII, Complement C3 and Transthyretin (figure 1.5 shows the 12 most abundance proteins in serum). Despite the importance of this method, the risk of losing some of low abundance proteins during the preferential binding of the high abundant proteins is still considered to be a big challenge. Even though many kits are available commercially, the complete removal of the high abundant fractions yet to be achieved. Other fractionation techniques for separating proteins are based on their specific properties using electrophoresis or

chromatography techniques. In this technique the separation is achieved based on three property of protein; (1) the interaction of the protein with stationary phase and the mobile phase, then the ability to control this mobile phase by changing of gradient time or pH or changing of salt concentration (2) the type of column (for example strong ion exchange, reversed phase (based on hydrophobicity interactions) or normal phase (silica) (3) gel properties (Issaq et al., 2002). Recently most of the fractionation/separation methods for proteins in proteomics laboratories are followed the multidimensional separation approach, in which more than one separation method will be applied prior to MS analysis (Lee et al., 2006). For example the sample will be separated by 2-DE, then each fraction (protein spot) in the gel will be enzymatically digested (trypsin) followed by LC-MS/MS analysis (Meng and Veenstra, 2007).

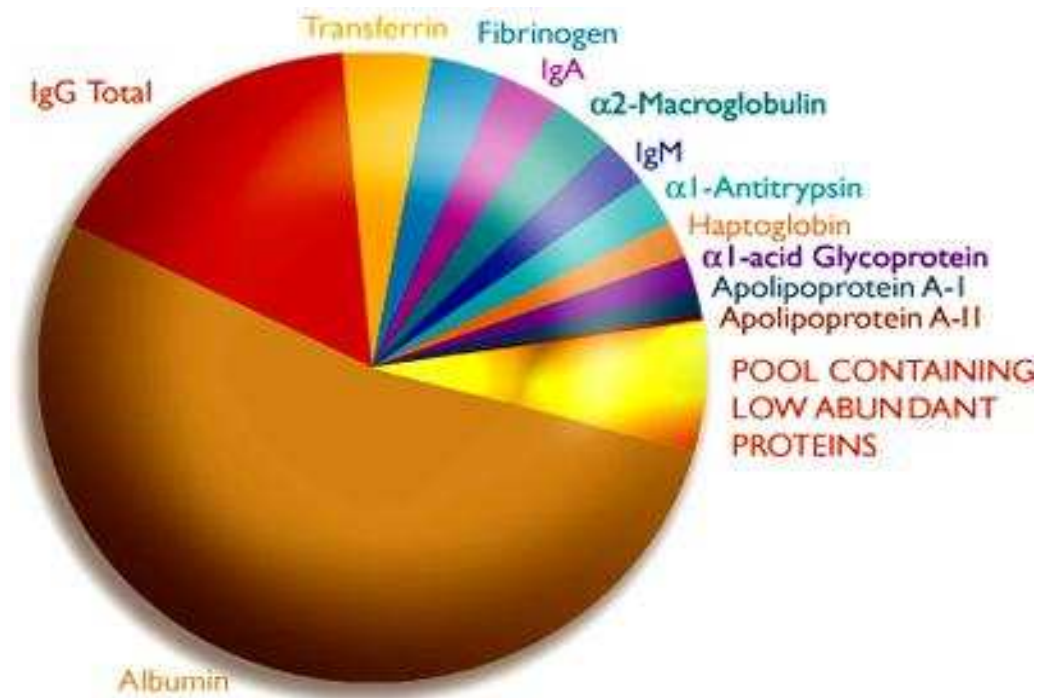


Figure 1.5. The most 12 abundant proteins in serum, in which ALB represent more than 50% of total protein in the serum (Appliedbiomics.com image)

1.2.5 The application of bioinformatics in proteomics

The amount of data generated from modern MS analysers is huge and multidimensional which make analysis a big challenge for biologists. Appropriate analysis of the data needs the integration of computational and statistical tools (Gu and Wong 2008). Fortunately the advancement in bioinformatics allows us to overcome the majority of this problem or at least help us to gain a satisfactory results with some biological explanations. However, the use of bioinformatics in proteomics it's not only lie on the characterisation and identification of proteins from the already available sequence databases such as Sequest and Mascot (Perkins et al., 1998), in which the outcome of the peptide sequence is matched with a known peptide generated from proteins or even from genome by some of the predefined algorithms. So the experimental peptide will be compared with theoretical peptide using this database search, which results in protein identification with some indication about the significance of the match provided from the search. The search result includes the sequence coverage and other statistics generated with each match.

Another application of bioinformatics by proteomics is generating a predictive model which suitably addresses the question of the study. But before using any bioinformatics tool we should address one question; what the purpose of this study? In other words if we are looking for a prediction specific site (such as glycosylation) among complex proteins, the use of support vector machine classifier shows a good result (Caragea et al., 2007), and another approach which shows a good for prediction capability of new biomarkers is artificial neural networks (ANNs) (Wang et al., 2010).

ANNs basically is an algorithm learning network aims to predicatively classify two groups with prior knowledge. This network contain three layers ; 1- input layer, 2- hidden layer , 3- and output layer. The data (m/z values) in the two groups enter as the first step forming the input layer, and in this step the system will analysis all the nodes and train each nods (m/z values) many cycles based on biological relationship between this node and all the other node which can discriminate the two groups. The model is trained for each node many times resulting in low error and best predictive percentage. The step-wise method is then applied by the system which allow for new nodes doing the same which in this case gain new nodes have high score predictive value and less error, which is in hidden layer. This process will continue form less number of nodes hidden layer for less number of nodes have the potential to discriminate between the two investigated groups as output (figure 1.6).

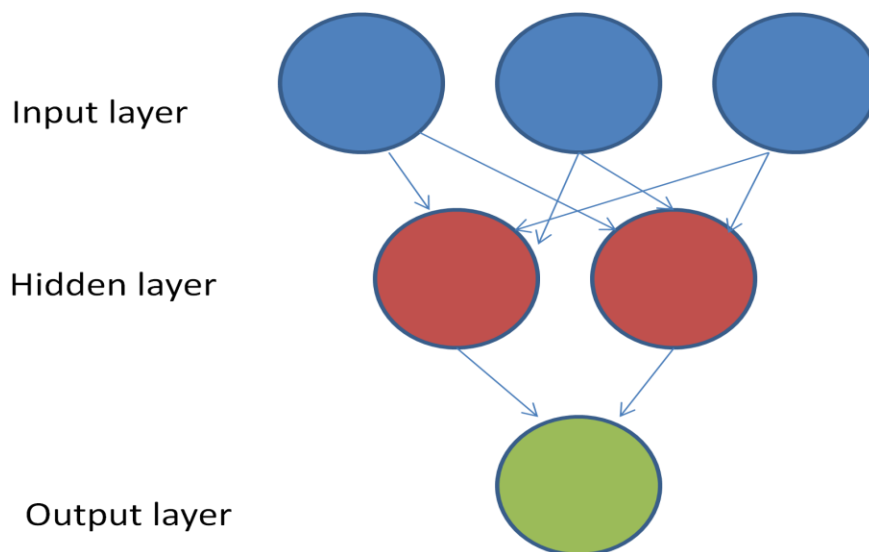


Figure 1.6. Illustration showing how the ANN model works. The ANN contains three layers: input, hidden, and output layers.

The training system in an ANN can generate a huge number of cycles which can lead to “overfitting”, in which some of the nodes will be missed. There is a development in ANNs modeling which shows the ability to overcome of this problem. By dividing the input data into three groups; the first one containing 60% of the population which serve as a trained model. Second one contain 20% which is asses the first group to avoid overfitting. The last group hold 20% which is responsible of testing unseen data from previous steps (Matharoo-Ball et al., 2007)

1.3 Objectives

The aim of this thesis is to use proteomic and biostatistical methods to identify prostate cancer specific prognostic biomarkers that associate with “tiger” versus “pussycat” (aggressive and non-aggressive) phenotypes of prostate cancer and to validate their performance in an independent geographical heterogeneous population. The surrey study samples will be used to identify serum prostate cancer biomarkers predictive of nonaggressive “pussycat “versus aggressive “tiger” phenotypes. A variety of sample fractionation and deconvolution techniques and mass-spectrometry based proteomic protocols combined with bioinformatic (Artificial Neural Network- ANN) and other biostatistical methods for the discovery and identification of protein/peptide biomarkers for prostate cancer will be used. A protein/peptide library will be created which will allow for further identification of the key proteins associating with cancer progression and treatment outcome. The identification of candidate biomarkers will allow the

extension of the study to determine whether a second patient cohort (Nottingham) stratifies according to the predictive models.

2- Materials and methods

2.1 Reagents list

Table 2.1. Proteomic Reagents

Reagent	Company
Acetonitrile (HPLC grade)	Fisher Scientific, Loughborough UK
Ammonium Bicarbonate	Sigma-Aldrich, Gillingham UK
Trifluoroacetic Acid (HPLC grade)	Fisher Scientific, Loughborough UK
Dichloromethane (HPLC grade)	Fisher Scientific, Loughborough UK
Acetone (HPLC grade)	Fisher Scientific, Loughborough UK
Methanol (HPLC grade)	Fisher Scientific, Loughborough UK
Ethanol (HPLC grade)	Fisher Scientific, Loughborough UK
α -Cyano-4-Hydroxy-Cinnamic Acid	Laser Bio Labs, Cedex FR
Sinapinic Acid	Laser Bio Labs, Cedex FR
2,5 Dihydroxybenzoic Acid	Sigma-Aldrich, Gillingham UK
Peptide Calibration Standard	Bruker Daltonics, Coventry UK
Protein Calibration Standard	Bruker Daltonics, Coventry UK
Trypsin (MS grade) 15,000 u/mg	Promega, Southampton UK
MARS equilibration/wash Buffer A	Agilent Technologies, Wokingham UK
MARS elution Buffer B	Agilent Technologies, Wokingham UK
Bovine Serum Albumin	Sigma-Aldrich, Gillingham UK
Acrylamide	National Diagnostics, Loughborough UK
Coomassie Blue	Sigma-Aldrich, Gillingham UK
Sodium Dodecyl Sulphate	Sigma-Aldrich, Gillingham UK
Propanol	Fisher Scientific, Loughborough UK
Glycerol	BioRad, Hemel Hempstead UK
Temed	Fisher Scientific, Loughborough UK
Ammonium Persulphate	Sigma-Aldrich, Gillingham UK
Resolving Gel (x10)	National Diagnostics, Loughborough UK
Protogel	National Diagnostics, Loughborough UK
Running Buffer (x10)	National Diagnostics, Loughborough UK
Stacking Gel (x10)	National Diagnostics, Loughborough UK
SDS	Sigma-Aldrich, Gillingham UK
DDT	Sigma-Aldrich, Gillingham UK

2.2 Reagents and buffers

Table 2.2. Reagent solutions and buffers

Solution	Composition
1% TFA Solution	1 mL TFA Stock 99 mL ddH ₂ O
0.1% TFA Solution	100 µL TFA Stock 99.9 mL ddH ₂ O
50% Acetonitrile (ACN) Solution	25 mL ACN Stock 25 mL 0.1% TFA
80% ACN Solution	40 mL ACN Stock 10 mL 0.1% TFA
100 mM Ammonium Bicarbonate Solution	0.395 g NH ₄ CHO ₃ Powder 50 mL ddH ₂ O
5 mg/mL CHCA Matrix	0.05 g CHCA Powder 10 mL 50% Acetonitrile
0.5 mg/mL Trypsin Protease	100 mg Trypsin Powder 200 mL 100 mM NH ₄ CHO ₃
0.01% BSA Solution	1 mg Stock BSA 10 mL ddH ₂ O
0.01% AAG Solution	1 mg Stock AAG 10 mL ddH ₂ O
Sample Reducing Buffer	2.5 mL 0.5M Tris HCl buffer (pH 6.8) 400 mg SDS 2 mL Glycerol 200 mg DTT A few grains of bromophenol blue made up to 20 mL ddH ₂ O
Running Buffer	100 mL 10x Tris/glycine/SDS- electrophoresis grade 900 mL ddH ₂ O
(Resolving Gel Buffer) (pH 8.8)	18.16 g Trizma base 0.4 g SDS make up to 100 mL ddH ₂ O adjust pH to 8.8 with HCl
(Stacking Gel Buffer) (pH 6.8)	6 g Trizma base 0.4 g SDS make up to 100 mL ddH ₂ O adjust pH to 6.8 with HCl
Silver stain	
Fixation solution	100 mL Ethanol, 25 mL Acetic acid, Make up to 250 mL ddH ₂ O(using 250 mL glass bottle) 25 mL silver nitrate solution, make up to 250 mL ddH ₂ O
Silver reaction	Sodium carbonate (6.25g) 1 packet, 100 µL of formaldehyde (87%), make up to 250 mL ddH ₂ O 75 mL Ethanol,10 mL sodium thiosulphate (5% w/v)
Developing solution	1 packet sodium acetate, make up to 250 mL ddH ₂ O
Sensitizing solution	25 mL glycerol(87%), make up to 250 mL ddH ₂ O

2.3 Equipment and software

Table 2.3

Equipment	Company
1D Electrophoresis Gel Tank	GeneFlow, Staffordshire UK
1D Electrophoresis Power Supply	Consort E122, GeneFlow, Staffordshire UK
Microcentrifuge	Minispin ,Eppendorf
Ultra Low Temperature Freezer (-80°C) Freezer (-20)	New Brunswick Scientific
Incubator	D-63450, Heraeus Instruments
Vortex	Whirlimixer, Fisher Brand
Sonicator	Ultrasonic Cleaner, VWR
C ₁₈ ZipTips	Millipore
Xcise liquid handling robot	Shimadzu/Proteome Systems, UK
MARS Hu-14 Immunodepletion Column	Agilent Technologies, Wokingham UK
MALDI Mass Spectrometer	UltraFlex III, Bruker Daltonics, Germany
MALDI Mass Spectrometer	UltrafleXtreme, Bruker Daltonics, Germany
Proxeon Easy-nLC	Bruker Daltonics, Germany

Software

Table 2.4

Flex Control	Bruker Daltonics, Germany
Flex analysis	Bruker Daltonics, Germany
ClinProTools	Bruker Daltonics, Germany
BioTools	Bruker Daltonics, Germany
Profile Analysis	Bruker Daltonics, Germany
Statistica v7	Statsoft Ltd.
Step-wise launcher v2	NTU Bioinformatics, UK
Mascot/Mascot Daemon 2.1	Matrix Science, UK
Microsoft Office 2003	Microsoft Corporation (provide by NTU,UK)

2.4 Procedures and protocols

This section illustrates the protocols that have been followed during the project starting from sample collection and storage through preparing the samples for analysis by

MALDI-MS using C₁₈ ZipTip (clean up) then digestion of proteins using enzymatic technique (trypsinisation) to generate m/z values. These data were applied to ANN for generating a list of peptides that have the ability to discriminate between two groups (aggressive vs nonaggressive prostate cancer). Moreover, this section will have an overview of our procedures for the next part of our study which is deep proteomic analysis using the immunodepletion technique followed by fractionation (LC/MALDI) and as a result protein identification. I want to state here that some of the protocols been used here were taken from previous work (Neil Devenport's MRes thesis 2009).

2.5 Samples collection and storage

The PCa samples in our study were obtained from Surrey hospital, which they take 15-20 mL of blood from patients before entering theatre for surgery. All samples were left for at least 30 min to allow time for the blood in the serum tube to clot. Then the samples left for 1 h on ice in order to prevent protein degradation before processing. The samples then centrifuged at 22 °C for 15 min and at 2000 rpm. Then the samples were pipetted into trays of micronic V tube aliquots. The volume of the serum samples was 150 µL and was stored in -80 °C freezer until the day we were given them. Once we had the samples we split them into three aliquots to avoid the freeze-thaw cycles and stored them at -80°C freezer until required for use.

QCs and BSA samples were used in our study in order to check the reproducibility of MALDI-MS and their spectra were compared with previous data either visually or using PCA analysis. Along with PCa samples, QCs, and BSA we included blank samples (0.1

TFA %) to our MALDI-MS analysis to determine whether there are contaminating background peaks or excessive instrument noise.

2.6 Patient details

The samples obtained in our study were approved by the Nottingham Research and Ethics Committee. Surrey hospital provided us with 118 PCa serum samples. Patient information was provided which included the PSA results and Gleason grade. 49 patients with Gleason grade <7 (3+3), 32 patients samples with Gleason grade >7 (17 with Gleason grade (3+4) and 15 with (4+3)), and 41 patients samples without Gleason grade (unknown). Anyway in regard to the PSA results the range was from 3.1 to 128 ng/mL.

2.7 Integrated protocol for identifying tryptic peptide biomarker ions and sequences by MALDI-MS and ANNs

The first part of our study followed a previously optimised method developed in the John van Geest Cancer Research Centre proteomics group to generate tryptic peptide biomarker ions from MALDI-MS and ANNs (figure 2.1).

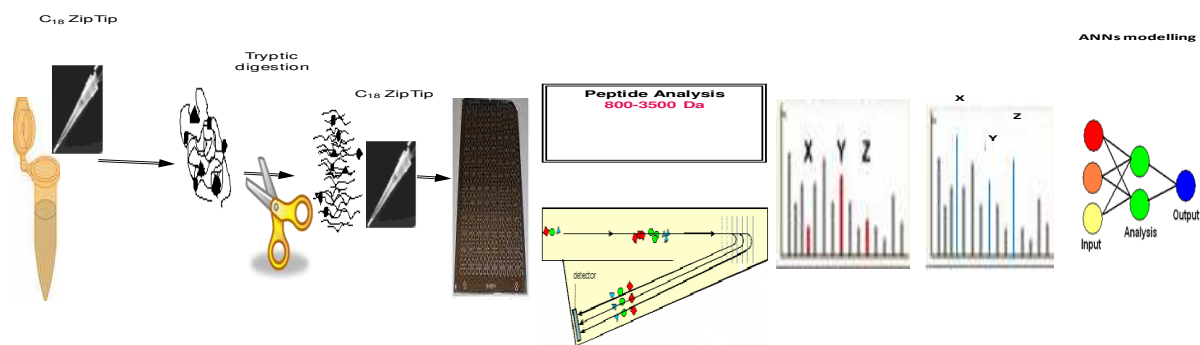


Figure 2.1. Optimised methods for predictive tryptic peptide ions markers that were followed in our study.

2.7.1 ZipTip processing, tryptic digestion and spotting on the MIP.

Initially PCa and QCs serum samples were diluted 1:20 with 0.1 TFA% (11 μ L of the serum samples, and 209 μ L of 0.1% TFA) prior to ZipTip processing the samples automatically. C₁₈ ZipTip solid phase extraction was performed. The samples were ZipTipped automatically using the Xcise robotic liquid handling system (Shimadzu, Manchester, UK). Moreover 30 μ L of all the serum samples were placed in two 96 well plates including (10 QCs, 10 Blank, and 9 BSA randomly positioned using Microsoft Office Excel 2003 to limit the bias that might occurs due to the position on the target plate ground steel MTP 384) were used to analyse the samples by MALDI-MS. The ZipTips were conditioned by wetting twice with 10 μ L with 80 % ACN, and then equilibrated using 2 x 10 μ L 0.1 % TFA. Next the sample binding step, in which the sample was bound to the ZipTip using 15 cycles (aspirate and dispense). The Tip was then washed with 10 μ L 0.1 % TFA for 2 times, and eluted in 8 μ L of 80% ACN using 15 aspiration and dispensing cycles. 16.6 μ L NH₄HCO₃ and 7.6 μ L dH₂O were

automatically added to all the samples in preparation for adding the trypsin for overnight digestion at 37° C.

After the first clean up step all samples were manually digested with 0.7 µL Trypsin gold and incubated at 37° C overnight. The next day digestion was terminated by adding 0.5 µL 1 % TFA, then the samples were ZipTipped again (second clean up step) and duplicate spotted automatically by mixing 1.1 µL of sample with 1.1 µL CHCA matrix solution directly onto MTP plate.

2.7.2 MALDI-MS analysis

Prior to MALDI-MS analysis we perform external calibration to our MTP plate by spotting calibration mixture (Bruker Daltonics, Germany) manually with one centre spot for every nine spots. The MTP plate was analysed on the Ultraflex III TOF/TOF Mass spectrometer (Bruker Daltonics, Germany) operated in reflectron mode. The laser power was optimised and the mass range applied was 800-3500 Da. Furthermore the spectra (m/z values) were generated and visually checked in order to assess the final profile for all the patients and compare the duplicate spots to each other and remove and bad spectra. The data was then pre-processed and used to generate the ANN model.

2.7.3 MS data pre-processing for ANNs modelling

Initially the MS data for all 118 PCa samples generated from MALDI-TOF/TOF analyser was pre-processed prior to submit to the ANN. In house methodology optimised by

Proteomics laboratory team protocol (John van Geest Cancer Research Centre) was followed to extract only peaks with signal to noise above 2.5 and baseline subtraction was applied in the FlexAnalysis software. The program creates a txt file contain all the peaks with their charge to ratio and intensity. Using software provided it by our bioinformatics team to bin our data to 0.1 Da. Then the file was being opened in Statistica and the file transposed in order to open it in Excel. Once in Excel the average intensity was taken for all the duplicate samples and reopened this file in Statistica again. In Statistica software another variable was added, which 0, 1 for nonaggressive (<7 in Gleason sum) and aggressive (>7 in Gleason sum) respectively based upon prior information. Finally the data was saved as a txt file, and this file is be submitted to the ANN software.

2.7.4 MS data and the ANN analysis

The idea in ANNs is to find a panel of ions that have the ability to discriminate between our two study groups using step-wise algorithm. After submitting the data to the ANN, step-wise analysis starts, to assess all the ions to see if they have the power to predict one group from the other based on their intensity. The process starts with first loop in which the step-wise choose one ion with high performance prediction and low error value and selects this ion as the top one and then starts another loop to find the second one which will be assessed cumulatively to to the first one. These loops continue until a panel of icons is created that is able to predict as much the population as possible.

Once we get this panel of predictive ions for all our sample groups, 44 PCa samples (22 with Gleason sum <7 , and 22 with Gleason sum >7) were selected based on their high intensities and applied the model to them in order to predict between them and population chart was obtained. These 44 PCa samples were used for further proteomic analysis.

2.8 Proteomic analysis

This section covers the protocols that were followed for proteomic analysis, starting with immunodepletion of the abundant proteins followed by LC-MALDI MS/MS and protein identifications. Previously established protocols for deep proteomic analysis were used (figure 2.2)

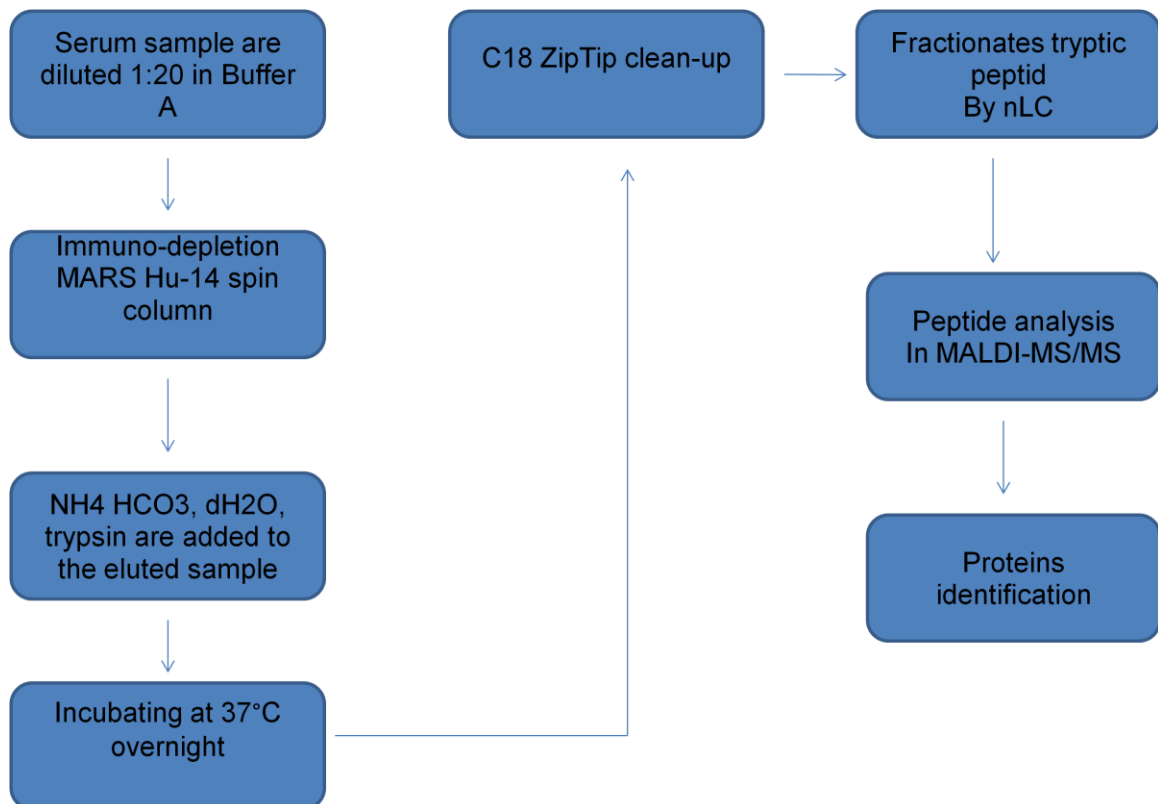


Figure 2.2 Flow chart for well established protocols for proteomics analysis

2.8.1 Immunodepletion of high abundant proteins

44 PCa patient samples (22 with Gleason sum < 7, and 22 with Gleason >7) were selected. and these 44 samples were the same samples which we had been identified from the ANN model using for population chart for the top three ions (1268.6, 998.6, and 910.4) from stepwise ANN analysis. 14 MARS Hu-14 spin column (Agilent technologies) was used for all the 44 samples, which is deplete the top 14 serum proteins(Albumin, Immunoglobulin G, Transferrin, Haptoglobin, α -1-Antitrypsin, Immunoglobulin A, Fibrinogen, α -2-Macroglobulin, α -1-Acid Glycoprotein, Immunoglobulin M, Apolipoprotein AI, Apolipoprotein AII, Complement C3 and Transthyretin) for specific polyclonal immunoglobulin.

Before each run the column was removed from the refrigerator and left for 5 minutes for equilibration to room temperature. 2×50 mL falcon tubes were labelled as buffer A and B. The Buffer A tube was filled with 6 mL and the buffer B tube with 3 mL. The two syringes provided with the kit were labelled as A and B. Dilute the serum sample (1:20) with buffer A (8 μ L serum sample with 192 μ L of buffer A). The diluted sample (200 μ L) was placed in a 0.22 μ m filter and spun in a microcentrifuge (make/model) for 90 s at 2000 rpm. Screw-top collection tubes were labelled F1 and F2. The spin cartridge was then prepared by removing the top cap and bottom cap. The luer lock adaptor was placed on the top of the spin column. Using the syringe labelled A and draw 4 mL of buffer A and attach to luer lock adaptor spin cartridge. Buffer A was dispensed through spin cartridge, and checked to see if there were any bubbles to remove. Then syringe A and luer lock adaptor was removed from column. The spin column was placed into the F1 tube an 200 μ L of diluted sample added to the top of the spin column and cap column

was capped loosely. The column was spun in a microcentrifuge for 2 min at 2000 rpm. The column was removed from the F1 tube (containing the low abundance proteins) and the F1 tube capped.

The column was incubated for 5 min at room temperature. 400 μ L of buffer A was added to the column which was placed into F1 tube) and spun for 2 min at 2000 rpm. Fraction 1 contained 600 μ L of low abundance proteins. 400 μ L of buffer A was added to the column which was placed in tube F2, spun for 2 min at 2000 rpm. Fraction F2 contained the higher abundance proteins (400 μ L).

Fractions F1 and F2 were combined and stored at -80 C for further analysis, and then the column was eluted with buffer B (2.5 mL of buffer B) using syringe B to waste. The column was washed with 4 mL buffer A using syringe A, so the column was ready for next use.

2.8.2 MARS Hu-14 spin column efficiency assessment

During the depletion of the 44 samples that were applied to the MARS Hu-14 spin column, the column was assessed twice by running a human serum QC sample and analysing it using 1D gel electrophoresis (1D-SDS-PAGE), stained with silver stain and a digitally photographed.

The resolving gel was prepared by adding 3 mL of protogel with 1.87 mL of resolving gel and 2.54 mL of ddH₂O into a 50 mL tube. Mix them and add 75 μ L of ammonium persulphate (10%) and 7.5 μ L of TEMED, and the resolving gel poured between the plates. Some drops of hydrated-butanol were added to remove any bubbles. The gel was left for 30 min to polymerise.

The stacking gel was prepared by adding (0.52 mL protogel, 1 mL of stacking gel, and 2.44 mL ddH₂O) mix and add 20 µL of ammonium persulphate, and 4 µL of TEMED. This was added it to the top of resolving gel and we drops of hydrated-butanol added to avoid any bubble formation. Combs with 10 wells were placed in the gel until it was completely polymerised, then the combs were removed.

In first lane from the left we add 8 µL of protein standard ladder was added (Bio-Rad WesternC precision plus™ protein standards). For the next 3 lanes 20 µL of diluted QC (1:20) with MARS buffer A was added. Then the next the three lanes, 20 µL depleted QC and the gel was stained using silver stain see the table below.

Table 2.5 shows the procedure of silver stain for the 1D gel electrophoresis. For the reagents used for this protocol please refers to table 2.2.

Step	Solutions	Time
Fixation	Ethanol Acetic acid glacial H ₂ O	30 min
Sensitizing	Ethanol Sodium thiosulphate (5% w/v) Sodium acetate (17 g) H ₂ O	30 min
Washing	ddH ₂ O	3 x 5 min
Silver reaction	Silver nitrate solution (2.5% w/v) H ₂ O	20 min
Washing	ddH ₂ O	2 x 1 min
Developing	Sodium carbonate (6.25 g) Formaldehyde (37% w/v) H ₂ O	2-5 min
Stopping	10 % Acetic acid 10 % Ethanol	10 min
Washing	ddH ₂ O	3 x 5 min
Preserving	Ethanol Glycerol (87 w/w) H ₂ O	2 x 30 min

2.8.3 Cleaning AnchorChip™ targets

Prior to LC-MALDI MS/MS analysis the 384 AnchorChip MALDI target plate (MTP) was cleaned using three chemical compounds Acetone (HPLC grade), Acetonitrile (HPLC grade), and Methanol (HPLC grade). The purpose of this step is to avoid any contamination that might occur due to the frequent use of the MTP. The procedure starts by removing the previous sample from the MTP by rinsing it with acetone (20-30mL using squeeze bottle HPLC grade). One thing to take into account is that rinsing should go cover the entire MTP surface. It was then rinsed with Acetonitrile (HPLC grade) followed by Methanol (HPLC grade). The MTP was placed glass in class jar containing 50% Methanol in 0.1 %TFA, and Sonicated at 20° C for 15 min. After sonication it was rinsed with 100% Methanol (HPLC grade) and air dried following rinsing with Acetonitrile (HPLC grade).

2.9 LC-MALDI-MS/MS and proteins mapping of PCa depleted samples.

24 depleted PCa samples (12 with Gleason sum <7, and 12 with Gleason sum >7) were analysed by LC-MALDI-TOF/TOF (UltrafleXtreme, Bruker Daltonics, Germany).

2.9.1 nanoLC Fractionation of depleted serum samples

Depleted serum samples were injected onto a nano-LC system (Bruker badged Proxeon Easy nLC) and eluted with an increasing gradient of organic solvent and spotted directly onto a Bruker 800-384 Anchorchip™ MTP in 10 second fractions using a Proteineer fcII

fraction collector (Bruker Daltonics). The nano-LC system was set up to run at a flow rate of 300 nL/min; mobile phase A 0.1 % TFA in LCMS grade water; mobile phase B 0.1 % TFA in LCMS grade acetonitrile. Mobile phase A was 0.1% aqueous TFA and mobile phase B was 0.1% TFA in ACN. Gradient elution conditions were as follows: linear gradient 2–45% B, 0–64 min; 100% B, 64–76 min, column conditioning at 2% B, 76–86 min. Sample loading onto the trap/pre-column was 5 min at 8 μ L/min following injection of 18 μ L of sample. The LC analytical column was a C₁₈ PepMap-100 (75 μ m i.d. \times 15 cm, 3 μ m, 100 A; Dionex, UK). The eluant from the nano-LC was mixed, prior to spotting, with a MALDI matrix solution of α -Cyano-4-hydroxycinnamic acid (CHCA) (Bruker Daltonics, Germany) containing 748 μ L of 95%ACN/0.1%TFA, 36 μ L of CHCA stock solution (CHCA saturated in 90%ACN/0.1%TFA), 8 μ L of 10%TFA in water and 8 μ L of 100mM NH₄H₂PO₄ in water). Prior to analysis by MS 96 calibrant spots were manually loaded with 0.5 μ L of calibrant (1:300 dilution of Bruker peptide standard II in the MALDI matrix described above).

2.9.2 MALDI-TOF/TOF analysis of LC-fractionated samples

Mass spectra were acquired using a Bruker UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) operating in reflectron positive ion mode. FlexControl (version 3.3, Bruker Daltonics) software was used to control the analysis. Mass range detected was set to 500-5000 Da with the sampling rate set at 4 Gs/s. Automated acquisition of mass spectra (MS and MSMS) of LC-fractions was controlled using WARP-LC software (version 3.2, Bruker Daltonics) interfaced with FlexControl.

MALDI laser intensity was selected by the operator to provide optimal intensity and resolution of acquired mass spectra. Peaks in the MS spectra were detected using FlexAnalysis (Version 3.3, Bruker Daltonics) using the SNAP algorithm and the top 14 MS peaks on each spot were sent for MSMS analysis. MSMS (LIFT) spectra were produced using post-source decay following precursor ion selection induced by increasing the laser power by 43 % for the fragment ions.

2.9.2.1 MASCOT search parameters

MASCOT (ver 2.3 server, Matrix Science) is computer software providing a search tool to identify proteins from MS and MSMS data. The parameters used in the study were optimised by the proteomics laboratory group (John van Geest center, NTU) and was as follow, the taxonomy searched for the all the samples was for human (homo sapiens). Variable modification was oxidation (M) with MS mass tolerance 100 ppm; MSMS tolerance 0.8 Da; Trypsin enzyme; Swissprot database (Jul 2010); +1 charge; MALDI-TOFTOF selected as the instrument.

2.10 Protein expression differences between nonaggressive Vs aggressive

Following analysis of all the 24 PCa samples a model was generated using ProfileAnalysis™1.1 (Bruker Daltonics, Coventry UK). The ProfileAnalysis is software used for data evaluation of LC-MS analysis, which is based on principle component analysis (PCA) technique. When we analyse the LC-MS data, the ProfileAnalysis

software generates a model for a component list contain mass to charge ratio and intensity for significant peaks ($P < 0.05$). This model can submit to WARP-LC prior to MS-MS analysis. Anyway this software looks for a complete folds change between two our group study, which can provide peptide expression differences for nonaggressive Vs aggressive PCa patient's samples.

3- Results

3.1 Prostate cancer (PCa) samples and MALDI-MS results

3.1.1 BSA spectra and scores

The PCa samples (118 samples; 49 with Gleason sum <7, 32 with Gleason sum >7, and 41 with unknown Gleason sum) were spotted (MTP, Bruker Daltonics, Germany) automatically using the Xcise robotic liquid handling system (Shimadzu, Manchester, UK) to avoid any variation that might occur if we did it manually. Moreover, the spots on the MTP were randomised in order to avoid the risk of biased positioning on the MTP. 9 BSA digest samples were added to the plate and were spotted automatically and randomly among all the serum samples (PCa, and QCs samples) to assess the MALDI-MS spectra (and including the trypsinisation), in which the BSA score were obtained by using MASCOT search as peptide mapping fingerprint PMF. The MOWSE scores were positive and above 60 for the nine BSA samples. Furthermore the spectra generated from MALDI-MS were assessed visually (figure 3.1).

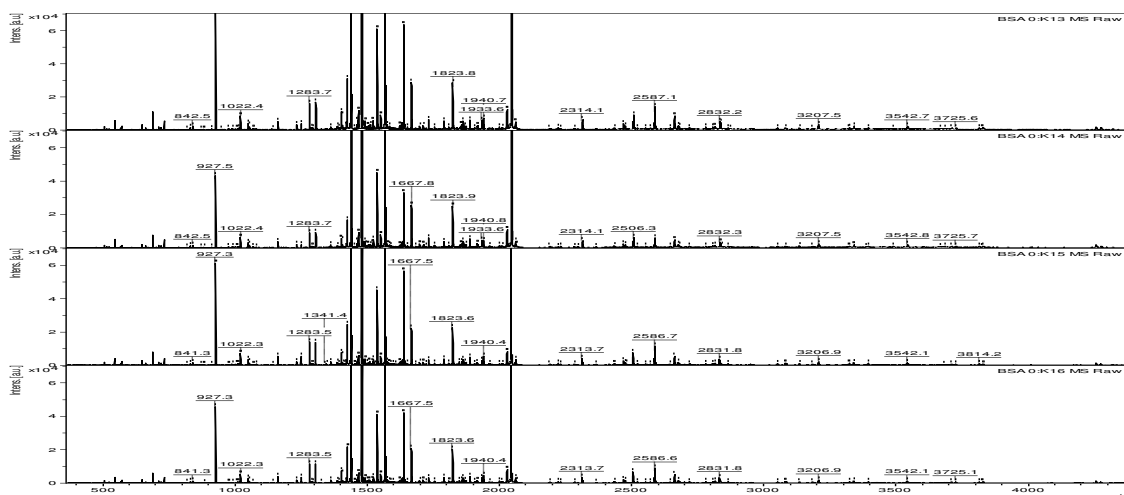


Figure 3.1 Four MALDI-TOF/TOF spectra generated from MALDI-MS and shows identical peaks for different BSA samples.

3.1.2 Reproducibility of MALDI-MS data

The reproducibility of MALDI-MS data was assessed during the analysis of the PCa samples which included 10 QCs samples. The spectra generated from MALDI-MS show identical peaks among these samples and are similar to other QCs data from last year (figure 3.2).

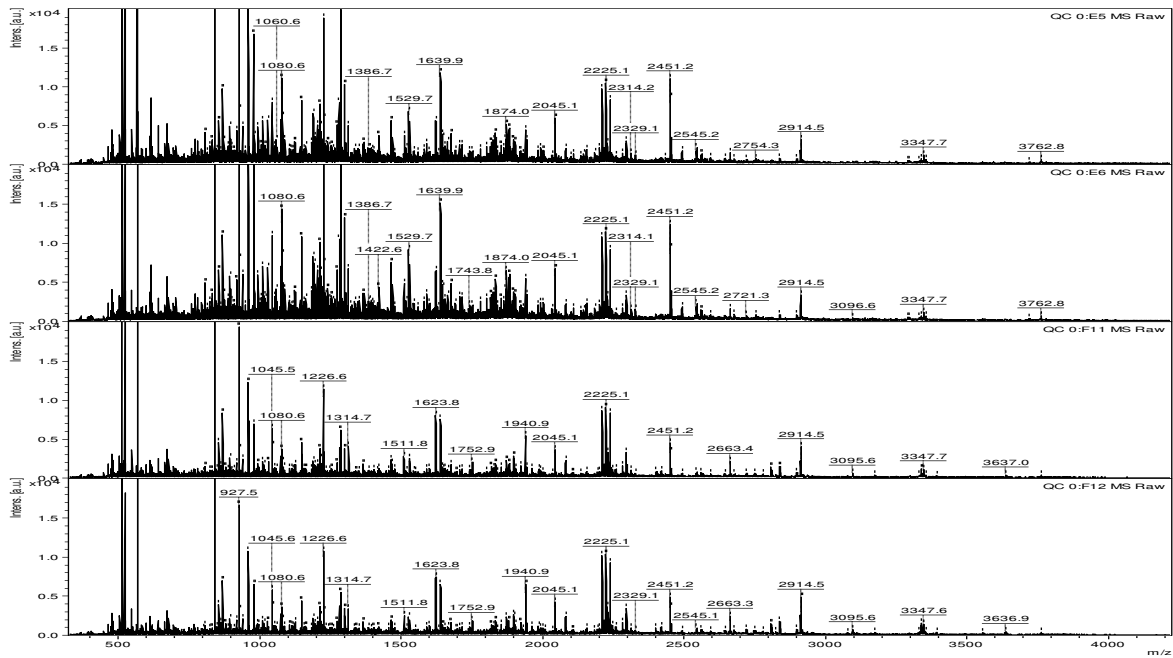


Figure3.2 Shows 4 different QC samples were added to our PCa serum samples randomly, and they show almost identical spectra.

3.1.3 MALDI -TOF – MS analysis of Prostate cancer serum samples

The assessment of the analysis for all the prostate cancer samples (<7, and >7 in Gleason sum) were applied by checking the spectra derived from MALDI-MS visually (figure 3.3)

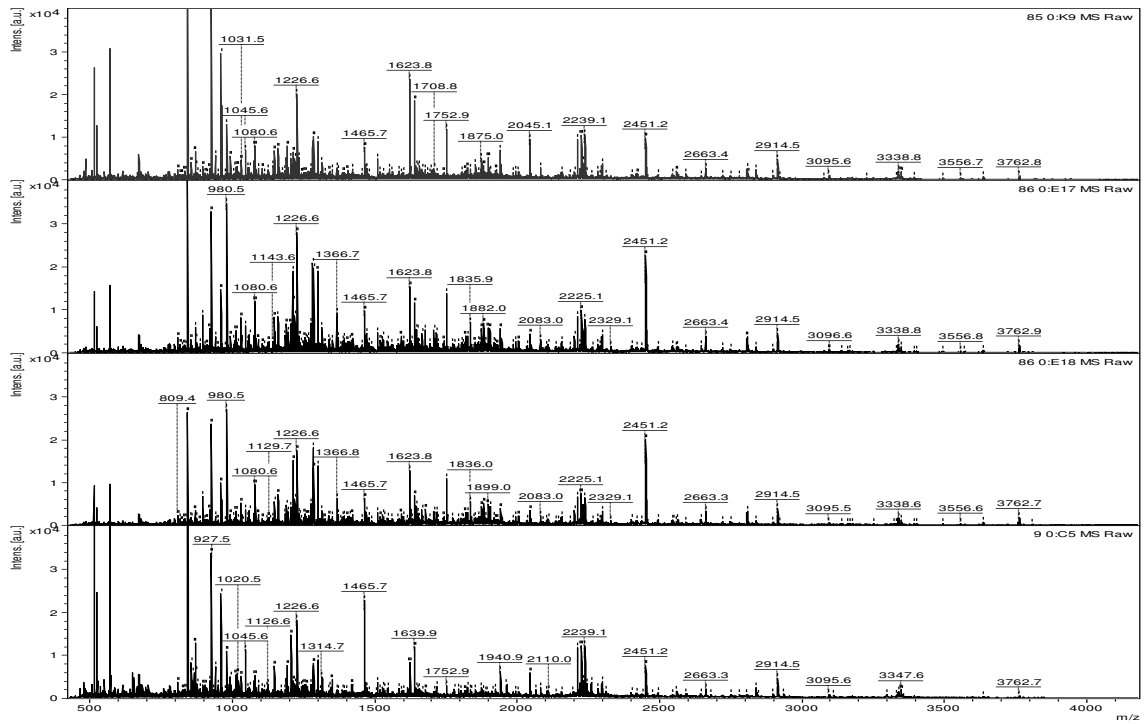


Figure 3.3 Four different prostate cancer serum samples spectra and shows good spectra with trypsin peaks

MALDI-MS is not inherently quantitative, in which the identical peaks from duplicate samples will not give exactly the same intensity value for specific peak. However all the PCa samples were Zip Tipped and digested enzymatically by adding trypsin (0.7 μ L) as we mentioned in the method section (2.7.1). Thus the trypsin analysis peaks should be included in all the spectra to show that reaction has taken place and a MASCOT search for BSA should be successful. Furthermore some PCa serum samples with bad spectra were excluded.

3.2 PCa samples and ANNs results

3.2.1 PCa patients' data and predictive ions by stepwise (ANNs)

The bad spectra for few prostate cancer serum samples were excluded and the rest of the data submitted to stepwise (ANNs) and predictive peptide ions were generated in table 3.1.

Table 3.1 The top 10 predictive ions obtained from stepwise (ANNs) for two different groups Of our study (nonaggressive prostate cancer patients < 7 Vs aggressive prostate cancer <7)

	Input ID(m/z)	Average Train Perf	Average Test Perf	Average Valid. Perf	Average Train Error	Average Test Error	Average Valid. Error
1	1268.6	0.653846	0.666667	0.555556	0.21965	0.218295	0.268825
2	998.6	0.730769	0.777778	0.666667	0.19550	0.187192	0.226763
3	910.4	0.807692	0.777778	0.777778	0.15308	0.152831	0.185394
4	1452.8	0.807692	0.888889	0.777778	0.13991	0.128612	0.182888
5	1439.6	0.846154	0.888889	0.777778	0.11535	0.124906	0.175517
6	2083.2	0.846154	0.888889	0.777778	0.12507	0.111161	0.159141
7	1717	0.884615	0.888889	0.777778	0.10276	0.108143	0.166488
8	3495	0.884615	0.888889	0.888889	0.08860	0.095931	0.144493
9	1423.8	0.884615	0.888889	0.833333	0.09766	0.101593	0.13994
10	2618.2	0.923077	0.888889	0.777778	0.08187	0.100358	0.157662

The rank of predictive ions in table 3.1 relies on the intensity for these ions and their ability to discriminate between two groups (aggressive Vs nonaggressive) using the ANN algorithm. In other word the ion with highest ability to predict between two groups with lowest error value will be the top ion, while the last predictive ion with lower power to predict and a higher error value. However the top three ions in table 3.1 were used to

generate a population chart (for our 44 PCa samples, 22 with Gleason sum >7 and 22 <7) which can show the ability to discriminate between our two study groups.

3.2.2 Generation a model of prediction between (nonaggressive “<7 Gleason sum” Vs aggressive” >7 Gleason sum”)

The three ions 1268.6, 998.6, and 910.4 were processed in Statistica 7 software to generate a dataset that could distinguish one group from the other. The reason only these three panel ions were used is the fact that there was no improvement in the performance of predictions between the two groups after ion number 3 (m/z 910.4) refer to table 3.1. Each ion from this panel represents 77% of the total population (44 PCa samples), which ion 1 (m/z 1268.6) represents 55%, ion 2 (m/z 998.6) represents 10%, and ion 3 (m/z 910.4) represents 10 %. The efficiency of the ANN model was tested by using Response Operator curve (ROC), which allows testing of the specificity and sensitivity for all the predictive 50 models that were used for panel ions (m/z 1268.6,998.6, and 910.4) figure 3.4. The ideal ROC curve for all the 50 models is considered to be close to 1, which is true positive.

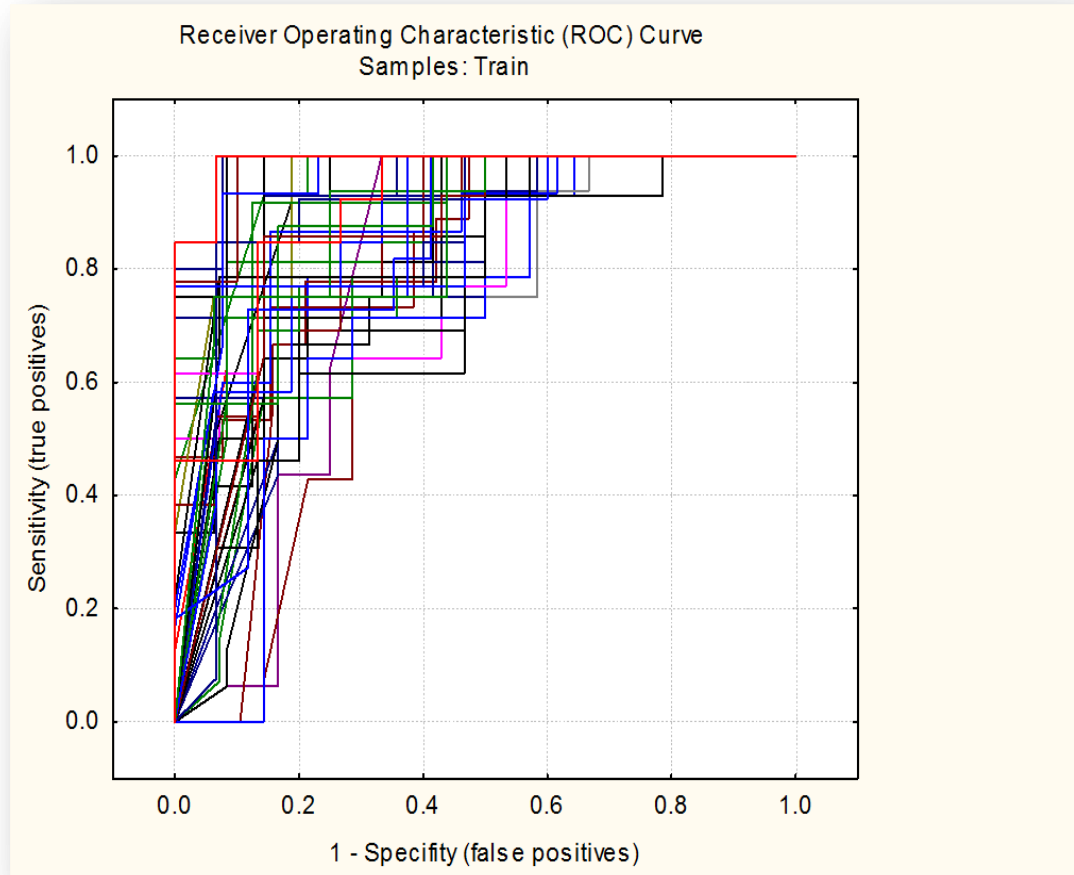
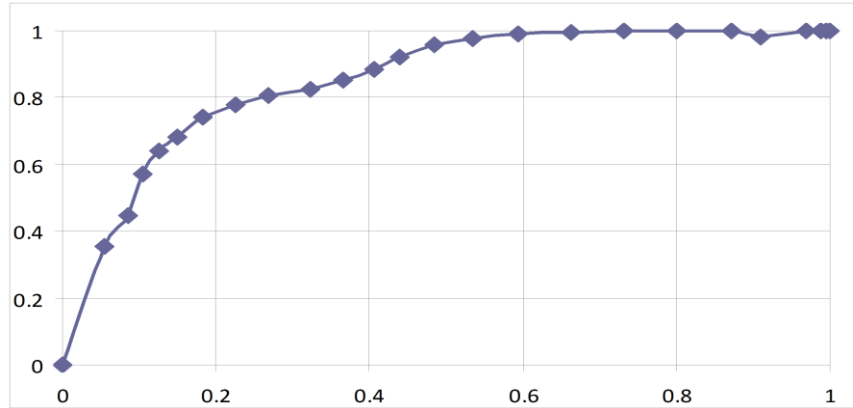


Figure 3.4 ROC curve generated by Statistica 7 software showing all 50 models that were used to train the top three ions (1268.6, 998.6, and 910.4) to discriminate between our two study groups (nonaggressive Vs aggressive). The performance of the ROC curve shows a good sensitivity (true positive) and specificity (false positive).

The mean of these 50 models were taken along with their area under curve (AUC), and the mean curve shows the specificity and sensitivity (figure 3.5). The AUC should be above 0.7 for a good prediction performance, which is in our study 0.879219, showing a positive result.



$$\text{AUC} = 0.879219$$

Figure 3.5 A Roc curve showing the mean of the 50 models that were used to train the model using the panel of the three peptide ions (1268.6, 998.6, and 910.4) for prediction between aggressive and nonaggressive prostate cancer patients. The area under the curve is 0.879219 which shows a good performance for the generated model. The Y axis represents sensitivity (true positive), X axis represents specificity (false positive).

After the assessment of the ANN model performance, a population chart was generated using from the mean of the 50 models used to train the system in order to discriminate between two our groups (figure 3.6).

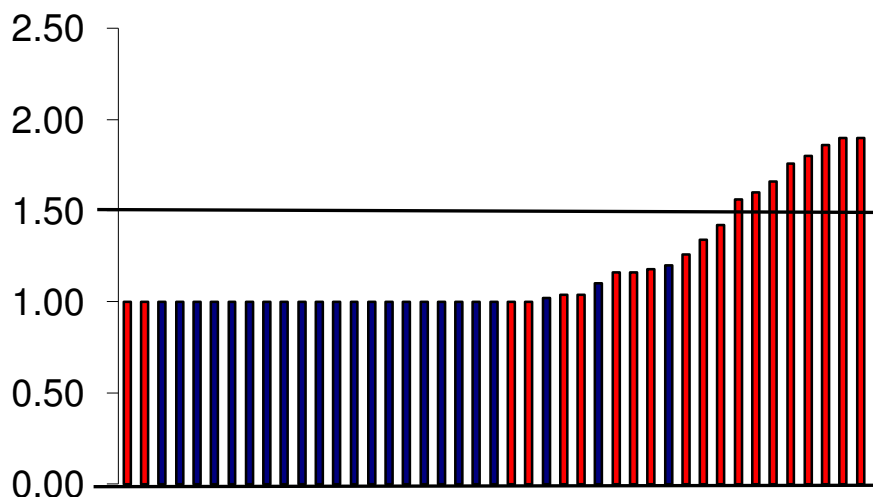


Figure 3.6. Population chart for 44 PCa patients' serum samples, red colour represents (>7 group in Gleason sum) and blue represents (<7 group in Gleason sum) for three ions m/z 1268.6, 998.6, and 910.4. A value of <1.5 = predicts nonaggressive prostate cancer patients, value of >1.5 = predicts aggressive prostate cancer patients, x-axis values indicate PCa patients samples.

Clearly, the discrimination between the two groups using these ions (panel ions) is obvious, with some samples misclassified. For the PCa samples with <7 Gleason sum group, three samples out of 22 are misclassified with prediction reaching to 86 % of the population. While the panel ions can predict 81% of the population for the PCa samples with Gleason sum >7 . As a total, the panel ions (1268.6, 998.6, and 910.4) have the power to discriminate between our two study groups for 85% of total prostate cancer patients.

However each predictive ion from our panel ions has the ability to discriminate between aggressive and nonaggressive PCa patients with a specific percentage. The top predictive ion(1268.8) can predict with 55 % of the population, while the next two ions (998.6, and 910.4) can predict with 10 % of the population for each one of them (figure 3.7).

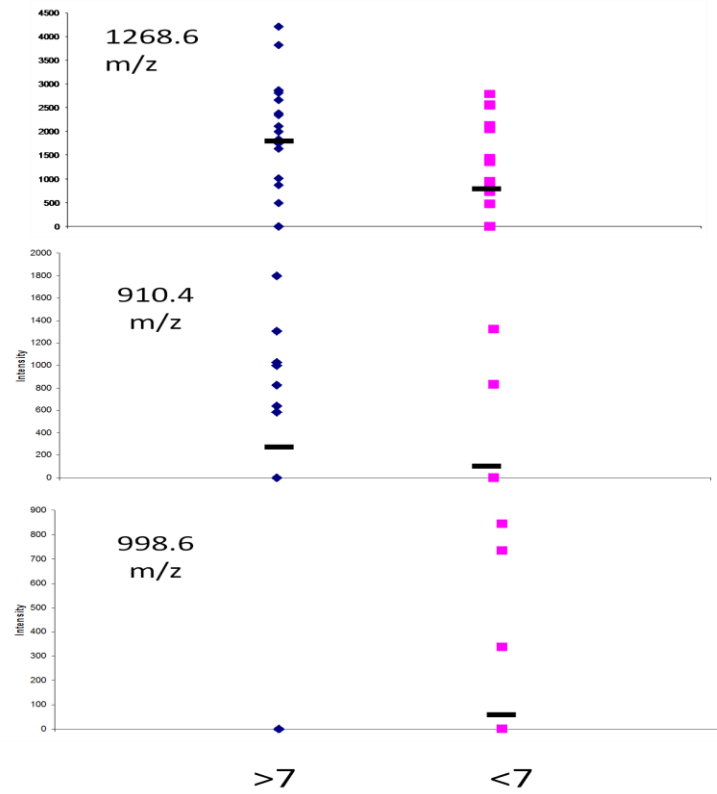


Figure 3.7 Shows the mean intensity for the three predictive ions, y axis represent the intensity, x axis represent aggressive and nonaggressive PCa patients (blue plot “aggressive” pink plot “nonaggressive”)

3.3 Assessment of MARS-HU 14 immuno-depleted column

The efficiency and specificity of MARS-HU 14 immunodepleted column was assessed visually using 1-D SDS PAGE. 24 prostate cancer serum samples were depleted (12 aggressive, and 12 nonaggressive) and after each 10 the MARS column was assessed by running a QC serum sample, then comparing it with an un-depleted QC visually in 1D SDS PAGE (figure 3.8).

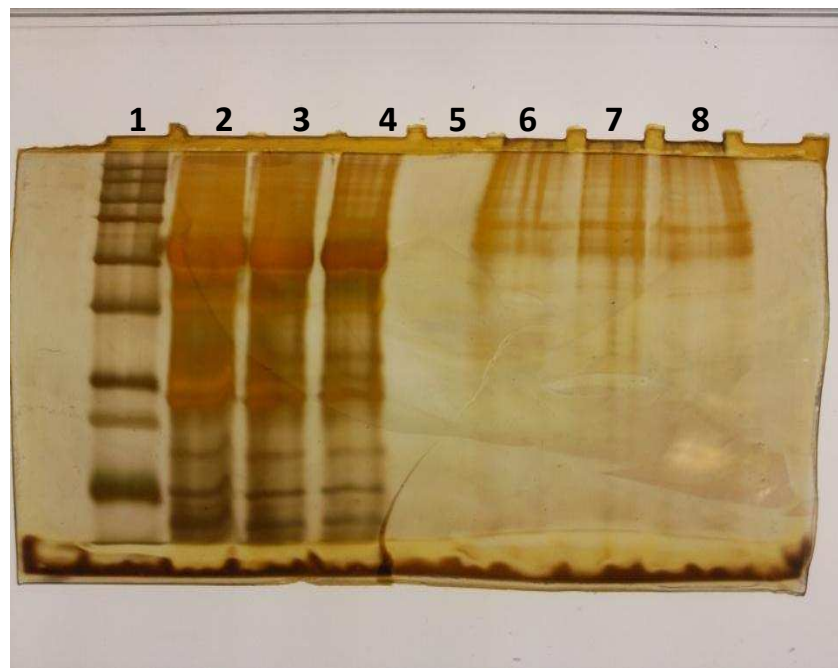


Figure 3.8. Digital image for 1-D SDS PAGE for undepleted QC serum Vs depleted QC serum by MARS-Hu 14 spin column. The first lane from the left standard ladder (Bio-Rad™ WesternC precision plus protein standards), the next three lanes undepletd QC (QC serum diluted in Buffer A MARS column), the last three lanes depleted QC serum

The efficiency of the MARS column was shown by observing a decreased complexity of high abundance proteins (ALB, immunoglobulin's). Furthermore the proteins identifications from LC MALDI-MS/MS shows fewer number of high abundance (the high 14 proteins abundance in serum) see the proteins list in appendix section.

3.4 LC-MALDI-MS/MS and protein identifications

All the 24 depleted PCa serum samples (12 samples with <7 in Gleason sum “nonaggressive”, and 12 samples with >7 in Gleason sum “aggressive”) were tryptically digested followed by ZipTipped. The tryptic peptides for each sample were fractionated using nanoLC then analysed by MALDI-MS/MS. Proteins mapping for each sample obtained with a positive MOWSE scores and high peptide sequence coverage for many proteins in the list. Some unique proteins were identified for each group (table 3.2) and the proteins have been identified for the panel of three predictive ions from the ANN (1268.6, 998.8, and 910.4) listed in table 3.3. (for more details for all the proteins identified refer to appendix sections).

Table 3.2 Unique identified proteins for all the 24 depleted (12 nonaggressive, 12 aggressive PCa patients) samples analysed by LC-MALDI

Nonaggressive prostate cancer	Aggressive prostate cancer
Ankyrin repeat and SOCS box protein 18	Ankyrin repeat domain-containing protein 17
ATP-binding cassette sub-family A member 13	ATP-binding cassette sub-family A member 12
Corticosteroid-binding globulin	Calcium-transporting ATPase type 2C member 2
Epsin-2	Calpain-15
Glial fibrillary acidic protein	Focal adhesion kinase 1
Glutamate receptor-interacting protein 1	FYVE, RhoGEF and PH domain-containing protein
Hemoglobin subunit beta	Golgi-specific brefeldin A-resistance guanine
Leucine-rich repeat serine/threonine-protein	Myosin-13
Low affinity immunoglobulin gamma Fc region	Platelet factor 4 variant
Pregnancy zone protein	Plexin-A4
Prostaglandin-H2 D-isomerase	Serine/threonine-protein kinase SRPK2
Protein Z-dependent protease inhibitor	Stabilin-1
Putative hydroxypyruvate isomerase	Steroid hormone receptor ERR1
RelA-associated inhibitor	TRIO and F-actin-binding protein
Retinol-binding protein 4	
Rho GTPase-activating protein 7	
Serotransferrin	
TNF receptor-associated factor 3	

Table 3.3 predictive peptide ions for PCa samples (12 with <7 in Gleason sum, 12 with >7 in Gleason sum) analysed by LC-MALDI-MS/MS and proteins identification generated using MASCOT software search (0.05 is the Significant value for MASCOT proteins identification)

<7	patient id	Gleason sum	PSA (ng/ml)	Proteins ID (910.4 m/z)	Protein ID(998.6 m/z)	Protein ID (1268.6 m/z)
	L1	3+3	7.1		Gelsolin(pep#23) Apolipoprotein B-100(pep#103)	Hemopexin(pep#27)
	L2	3+3	7.6			Hemopexin(pep#21)
	L2	3+3	3.14		Gelsolin (pep#19) ApolipoproteinB-100(pep#117)	ApolipoproteinB-100(pep#117) Hemopexin(pep#23)
	L3	3+3	4.7		Gelsolin(pep#19) ApolipoproteinB-100(pep#137)	Hemopexin(pep#30)
	L4	3+3	5.9		Gelsolin(pep#19)	Hemopexin(pep#23)
	L5	3+3	12		Gelsolin(pep#20)	ApolipoproteinB-100(pep#96) Hemopexin(pep#21)
	L6	3+3	3.8		Gelsolin(pep#14)	Hemopexin(pep#16)
	L7	3+3	11			
	L8	3+3	9		ApolipoproteinB-100(pep#56) Gelsolin(pep#12) Gelsolin(pep#25)	Hemopexin(pep#21)
	L9	3+3	7.4		ApolipoproteinB-100(pep#128)	Hemopexin(pep#52)
	L10	3+3	8		Gelsolin(pep#19)	Hemopexin(pep#33)
	L11	3+3	10		Gelsolin(pep#9)	Hemopexin(pep#20) Apolipoprotein B-100(pep#60)
	L12	3+3	6.8		Gelsolin(pep#16) ApolipoproteinB-100(pep#65)	Hemopexin(pep#31) Angiotensinogen(pep#12)
>7	H1	7	6.8		Gelsolin(pep#18)	Hemopexin(pep# 34)
	H2	3+4	5.7		Gelsolin(pep#16)	Hemopexin(pep# 19)
	H3	3+4	8.8		Gelsolin(pep#18)	Hemopexin(pep# 24)
	H4	3+4	6.8			Hemopexin(pep# 21)
	H5	3+4	14			
	H6	4+3	12			
	H7	3+4	36		Gelsolin(pep#25)	Hemopexin(pep# 32)
	H8	4+3	22.8		Gelsolin(pep#9)	ApolipoproteinB-100(pep#79) Hemopexin(pep#21)
	H9	3+4,4+5	5.9		Gelsolin(pep#13)	Hemopexin(pep#22)
	H10	3+4	5.2			Hemopexin(pep#22)
	H11	3+4	4.6		Gelsolin(pep#22)	Hemopexin(pep#25)
	H12	4+4	11	Complement C5(pep#18)	Gelsolin(pep#16)	Hemopexin(pep#27) Angiotensinogen(pep#9)

3.5 Proteins expression differences between nonaggressive and aggressive prostate cancer patients

The analysis for all the 24 PCa samples (12 nonaggressive, and 12 aggressive) by LC-MALDI-MS/MS, allow to us to generated a model using ProfileAnalysis™ 1.1 software (Bruker, Daltonics, UK). This model can show the difference in proteins expression for our two group study when we submit it to WARP-LC software prior to MS-MS analysis. The 24 samples (12 pooled sample < 7 in Gleason sum, 12 pooled samples >7 in Gleason sum) were digested and Zip Tipped followed by nanoLC analysis. The model generated from the previous LC-MS run for all the 24 depleted samples individually were applied to WARP-LC software and proteins mapping obtained using MASCOT Search software (table 3.4, 3.5).

Table 3.4 Proteins list for all the 12 pooled samples (<7 in Gleason sum) shows the MOWSE score, MW (kDa), and number of peptide coverage

Protein	Score	MW [kDa]	# Pept.
Unknown	56.83	0	7
Unknown	48.31690887	0	3
Unknown	44.72690887	0	3
Unknown	43.21	0	3
Unknown	38.72	0	2
Unknown	37.55	0	2
Unknown	36.84	0	2
Unknown	35.1	0	1
Unknown	34.62	0	1
Unknown	31.39	0	1
Unknown	30.46	0	1
Actin, cytoplasmic I	53.63	41.70973	3
Afamin	140.8969089	69.02401	6
Alpha-1-antichymotrypsin	1096.42	47.62054	18
Alpha-1B-glycoprotein	563.01	54.23858	11
Alpha-2-HS-glycoprotein	353.87	39.29971	7
Alpha-2-macroglobulin	199.1669089	163.18888	12
Angiotensinogen	238.95	53.12051	6
Ankyrin repeat and SOCS box protein 18	47.03	50.77091	2
Antithrombin-III	804.7669089	52.56886	22
Apolipoprotein A-I	221.43	30.75893	11
Apolipoprotein A-IV	976.3	45.37147	22
Apolipoprotein B-100	4503.159124	515.24085	122
Apolipoprotein C-I	36.95	9.32609	1
Apolipoprotein C-III	189.58	10.8455	2
Apolipoprotein E	179.5469089	36.13175	9
ATP-binding cassette sub-family A member 12	57.67	293.04884	5
Beta-2-glycoprotein 1	248.38	38.27266	7
Carboxypeptidase B2	109.25	48.38141	5
Carboxypeptidase N subunit 2	119.01	60.57615	3
Ceruloplasmin	2080.857635	122.12759	38
Clusterin	129.37	52.46101	6
Coagulation factor X	72.32	54.69651	3
Complement C1q subcomponent subunit B	236.59	26.44241	6
Complement C1q subcomponent subunit C	267.8769089	25.75714	4
Complement C1s subcomponent	258.8169089	76.6348	10
Complement C2	133.82	83.21431	7
Complement C3	527.6907266	187.02987	19
Complement C4-B	2474.37218	192.67254	59
Complement C5	625.3869089	188.18613	22
Complement component C6	99.45	104.718	5
Complement component C8 alpha chain	70.99	65.12104	4
Complement component C8 beta chain	267.67	67.00347	9
Complement component C8 gamma chain	89.11	22.26354	4
Complement component C9	240.1569089	63.1327	7
Complement factor B	710.9269089	85.47852	18
Complement factor H	729.3638177	139.0047	26
Complement factor H-related protein 1	165.99	37.62596	4
Cyclin N-terminal domain-containing protein 1	32.15	36.89737	1
Fibrinogen alpha chain	120.58	94.91441	4
Fibronectin	957.5448129	262.44208	27
Gelsolin	444.9	85.64419	14
Haptoglobin	100.97	45.17656	5

Hemopexin	1122.476909	51.64327	27
Heparin cofactor 2	607.0638177	57.0342	16
Heterogeneous nuclear ribonucleoprotein A1-like 2	31.52	34.20427	2
Heterogeneous nuclear ribonucleoproteins A2/B1	31.52	37.40673	2
Histidine-rich glycoprotein	330.59	59.54087	7
Insulin-like growth factor-binding protein complex acid labile	220.3838177	65.9938	12
Inter-alpha-trypsin inhibitor heavy chain H1	1086.040727	101.32561	20
Inter-alpha-trypsin inhibitor heavy chain H2	1085.876909	106.39661	28
Inter-alpha-trypsin inhibitor heavy chain H3	284.7169089	99.78653	10
Inter-alpha-trypsin inhibitor heavy chain H4	1052.876909	103.29298	30
Interleukin-25	31.89	20.31696	1
Kallistatin	202.93	48.51116	9
Keratin, type II cytoskeletal 1	254.6476355	65.999	11
Keratin, type II cytoskeletal 2 epidermal	45.21	65.39322	2
Kininogen-1	827.51	71.91215	12
Leucine-rich alpha-2-glycoprotein	123.07	38.15411	3
Lumican	109.0269089	38.40479	5
Pigment epithelium-derived factor	140.89	46.3133	6
Plasma protease C1 inhibitor	402.46	55.11939	15
Plasminogen	357.7069089	90.51016	11
Platelet basic protein	113.62	13.88542	3
POTE ankyrin domain family member F	49.74690887	121.36669	3
Protein AMBP	126.75	38.97398	4
Prothrombin	135.0969089	69.99212	9
Putative hydroxypyruvate isomerase	39.87	30.38656	2
Putative zinc-alpha-2-glycoprotein-like	71.69	22.96546	3
Pyruvate kinase isozymes M1/M2	32.14	57.90002	1
Retinol-binding protein 4	59.68	22.99526	2
Serum amyloid P-component	409.81	25.37113	10
Stromal interaction molecule 1	41.04	77.37529	2
Sushi, nidogen and EGF-like domain-containing protein 1	44.68	152.10421	3
Thyroxine-binding globulin	88.08381774	46.29461	4
Trypsin-1	72.06	26.54109	1
Vitamin D-binding protein	493.31	52.92903	11
Vitronectin	277.12	54.27117	8
Zinc-alpha-2-glycoprotein	320.94	34.2371	10

Table 3.5. Proteins list for all the 12 pooled samples (>7 in Gleason sum) shows the MOWSE score, MW (kDa), and number of peptide coverage

Protein	Score	MW [kDa]	# Pept.
Unknown	69.05	0	1
Unknown	62.44	0	2
60S ribosomal protein L6	95.9	33.54094	2
Afamin	104.8	69.02401	4
Alpha-1-antichymotrypsin	672.85	47.62054	14
Alpha-1B-glycoprotein	433.05	54.23858	11
Alpha-2-HS-glycoprotein	314.46	39.29971	7
Alpha-2-HS-glycoprotein	174.01	39.41876	5
Alpha-2-macroglobulin	444.39691	163.18888	15
Alpha-enolase	76.14	47.11121	2
Alpha-S1-casein	126.08	24.51343	3
Angiotensinogen	73.58	53.34043	2
Angiotensinogen	196.45	53.12051	5
Apolipoprotein A-I	235.49	30.75893	10
Apolipoprotein A-IV	696.99	45.37147	21
Apolipoprotein B-100	3635.2103	515.24085	114
Apolipoprotein C-III	182.96	10.8455	2
Beta-2-glycoprotein 1	103.62	38.27266	2
Ceruloplasmin	1558.1907	122.12759	31
Complement C1q subcomponent subunit B	149.08	26.44241	3
Complement C1q subcomponent subunit C	166.51691	25.75714	3
Complement C1s subcomponent	189.02691	76.6348	7
Complement C3	484.45382	187.02987	19
Complement C4-B	1506.8045	192.67254	47
Complement C5	396.71382	188.18613	15
Complement component C6	60.33	104.718	2
Complement component C7	181.09	93.45729	6
Complement component C9	111.03	63.1327	3
Complement factor B	250.29691	85.47852	12
Complement factor H	507.62764	139.0047	21
Fibronectin	701.56172	262.44208	23
Ficolin-3	109.16	32.88199	4
Gelsolin	269.88	85.64419	10
Hemopexin	959.77382	51.64327	25
Heparin cofactor 2	194.10691	57.0342	8
Histidine-rich glycoprotein	162.03	59.54087	6
Insulin-like growth factor-binding protein complex acid labile subunit	53.96	66.91791	3
Inter-alpha-trypsin inhibitor heavy chain H1	714.21382	101.32561	14
Inter-alpha-trypsin inhibitor heavy chain H2	636.72	106.39661	20
Inter-alpha-trypsin inhibitor heavy chain H4	710.34	103.29298	22
Kallistatin	105.12	48.51116	5
Keratin, type I cytoskeletal 10	239.7	58.79169	9
Keratin, type I cytoskeletal 9	232.34	62.02681	4
Keratin, type II cytoskeletal 1	481.88454	65.999	15
Keratin, type II cytoskeletal 4	86.07	57.24983	4
Kininogen-1	340.23	71.91215	7
Leucine-rich alpha-2-glycoprotein	132.85	38.15411	5
Lumican	70.796909	38.40479	3
Pigment epithelium-derived factor	94.71	46.3133	5
Plasma protease C1 inhibitor	104.16	55.57615	3
Plasminogen	321.12691	90.51016	11
Platelet basic protein	95.73	13.88542	2
Prothrombin	111.74691	69.99212	6

Retinol-binding protein 4	97.406909	22.99526	4
Serum amyloid P-component	254.26	25.37113	8
Trypsin	167.57	24.39381	2
Trypsin-1	53.52	26.54109	1
Vitamin D-binding protein	133.27	53.30706	5
Vitronectin	290.49	54.27117	6
Zinc-alpha-2-glycoprotein	187.92	34.2371	10

Protein (peptide ion intensity) expression differences between nonaggressive Vs aggressive were assessed using ProfileAnalysis, which allow examination of the fold change between our two categories (figure 3.9).

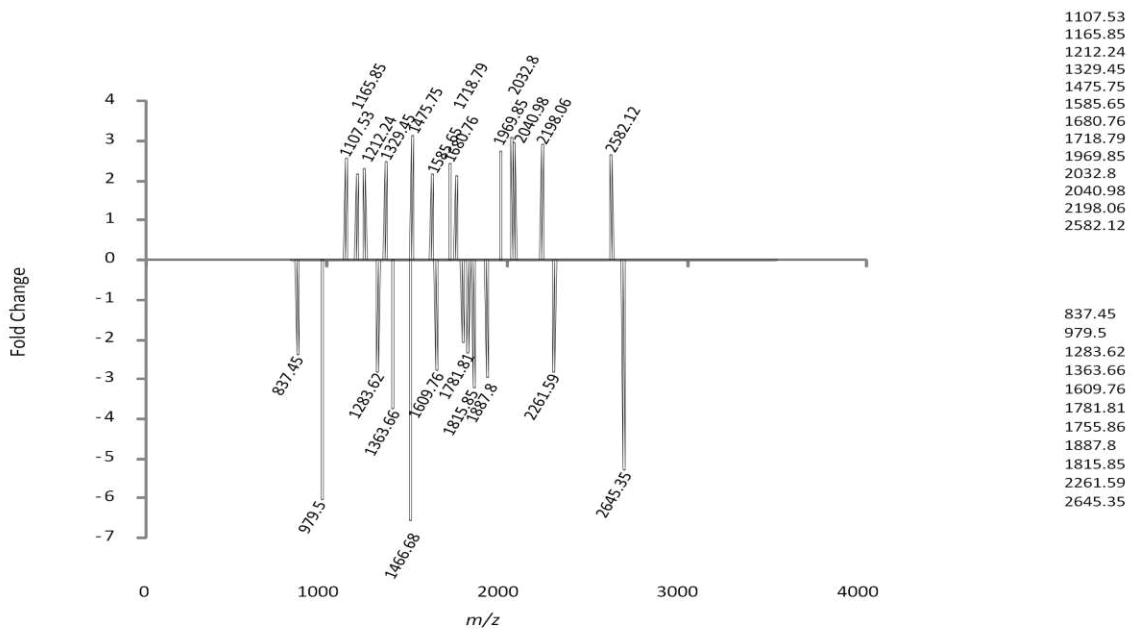


Figure 3.9 shows the difference in peptide expression between nonaggressive Vs aggressive PCa samples generated by ProfileAnalysis software

The number of proteins identified for our two pooled samples, one for aggressive and the other for nonaggressive, shows several unique and overlapping proteins for each group (figure 3.10)

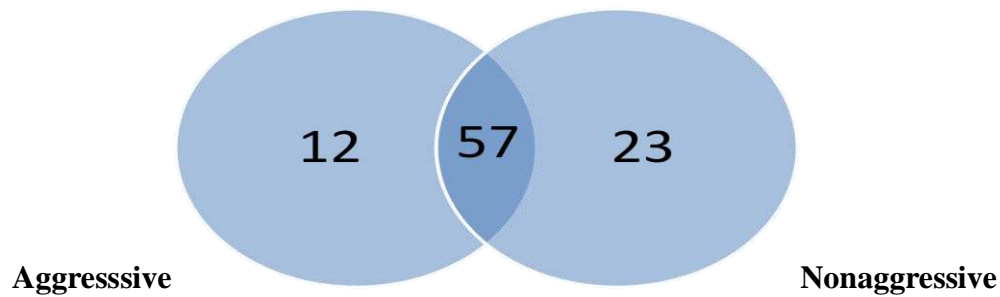


Figure 3.10. Venn diagram showing the number of proteins identified for two pooled samples each category, 12 unique proteins for aggressive prostate cancer patients, 23 nonaggressive prostate cancer patients, and there are shared 57 proteins.

However there are only five ions that were able to be identified to proteins (Table 3.6). Four of them have positive fold change, while only one had negative fold change for the nonaggressive PCa group compared with aggressive.

Table 3.6. Peptide associated with identified proteins by tandem MS/MS shows the difference in their regulation for nonaggressive comparing with aggressive PCa samples.

ions (<i>m/z</i>)	protein identified	peptide sequence	Regulation
1212.24	Apolipoprotein B-100	NSEEFAAAMSR	↑
1329.45	Apolipoprotein B-100	KLTISEQNIQR	↑
1585.65	Apolipoprotein A-IV	LKEEIGKELEELR	↑
2032.8	Apolipoprotein B-100	HSGSFQSQVELSNDQEK	↑
1887.8	Afamin	SDVGFLPPFPTLDPEEK	↓

4- Discussion

4.1 The Use of automated sample preparation in proteomic studies

One of the big challenges for researchers in the proteomic field, over the years, is to carry out experiments manually with sufficient reproducibility. Manual handling of many of the experiments ultimately end up with high incidents of sample to sample variation due to several factors which occur during the experimental preparation. This is one of the greatest limitations in serum biomarker studies. Fortunately the advancements in instrumentation allowed the processing and preparation of serum samples automatically prior to MS analysis with automated robotic liquid handling platforms. Recently the fractionation and cleaning up of the serum samples by C₁₈ Zip Tipping, as well as fractionation (LC-MALDI) can be done automatically. The Xcise robotic system (Shimadzu, Manchester UK) has the ability to prepare the sample for proteomics analysis automatically, which can reduce any bias due to manual handling. This instrument shows a good performance in the discovery process of new biomarkers (Vafadar-Isfahni et al., 2010). In this study the Xcise robotic system was used in order to prepare the PCa samples (nonaggressive Vs aggressive) prior to MS analysis. The system shows good performance in which only 3 out of 118 PCa samples were excluded, which showed bad spectra. Another advantage of this system is the speed to process this number of samples in short time (approximately five hours) without considerable sample to sample variation.

4.2 The use of Bioinformatics and ANNs in proteomic approaches

The data generated from mass spectrometry (MS) remains a big challenge for researchers because of its complexity and multidimensionality. Fortunately, the advancement in the bioinformatics field allows us to significantly reduce the complexity and make the data manageable. Utilisation of any bioinformatics method in proteomics should be carefully considered before any study commences. The use of ANNs in proteomics biomarker discovery and its ability to discriminate between difference groups study shows promising results, such as the study carried out by Vafadar-Isfahni et al., in 2010 . The ANN was employed in this study to generate a model capable of discriminating between nonaggressive and aggressive serum peptide profiles, using a stepwise approach. The use of a stepwise approach reduces any overlapping that might occur during the generation of each new model by the ANN. The benefit of the ANN is to pull out the most discriminatory ions as a panel which classifies the two groups with high sensitivity and specificity. In this study, the panel of three peptide biomarker ions (m/z 1268.6, 998.6, and 910.4) was able to discriminate patients with aggressive PCa from nonaggressive patients with accuracy of 77%.

The population chart (figure 3.6) shows five misclassified patients in both groups (nonaggressive Vs aggressive). This could be for several reasons; firstly it should be considered that both groups are very similar to each other. In other words, one group with (3+3=6) as the Gleason sum and the other one with (3+4, 4+3 =7) in Gleason sum, the difference is only one. A second reason could be that the initial diagnostic by the pathologist was not accurate. In this case the urologist or the nurse may have missed some area of the prostate gland when they took the biopsy sample, which would make the

diagnosis by the pathologist not comprehensive. A third reason is the possibility that the prostate cancer could have developed from the time of the biopsy examination to the time the blood was collected for the clinical trial especially for three of the misclassified samples in the nonaggressive group with aggressive patients. The fourth reason could be what is termed “prostate cancer volume”, studies show the patients with Gleason sum 6 are more likely to increase their prostate cancer volume after radical prostatectomy (Dong et al., 2008). Thus the 3 nonaggressive misclassified samples in the aggressive group could have increased their prostate cancer volume if they had this kind of treatment.

Although the model generated by the ANN to discriminate between our groups had a good performance, the ANN still suffered from some overfitting even with the use of the stepwise method. Another disadvantage of the ANN is the inability to have the same predictive panel of ions every time you submit the same data to the ANN, due to the random nature of the algorithms. Another limitation for the ANN when we use it as a bioinformatic tool to generate a model for MS data is the difficulty in matching the output from the ANN to MS spectra, visually (i.e. matching an ANN classifier ion to a real peak in the spectrum) For example if we take one of our predictive ions that can discriminate between nonaggressive and aggressive prostate cancer patients and if we checked its spectra for different patients from different groups we could not visualise any significant difference, because MALDI-MS is not inherently quantitative. So the future direction should concentrate much more on effective and reliable correlation between the predicted and experimental data.

4.3 The appraisal of the MARS-Hu 14 spin column for our proteomics study

The high complexity of serum and the high dynamic range of protein concentrations make the discovery of new biomarkers obtained from serum a complicated process. As the low abundance proteins promise new biomarkers (such as tumor markers), the depletion of high abundance proteins has become more important and critical. The MARS-Hu 14 spin column used in our study was assessed using 1D-SDS-PAGE separation and the results shows its ability to enrich the low abundance proteins and deplete most of the 14 proteins targetted. The remaining proteins were identified as moderate to low abundance proteins such as; Ankyrin repeat domain-containing protein 17, Focal adhesion kinase 1, Spermatogenesis-associated protein 7, Apolipoprotein C-I, Apolipoprotein C-III, Complement C1q subcomponent subunit C, and there are more (see the appendix section for more details) (Ahemd 2009) . The main limitation of the column is the unanticipated removal of some of the low abundance proteins which a specifically or nonspecifically binds or attach to the high abundant proteins such as albumin.

4.4 Identification of the predictive ions from the MS profiling of PCa serum samples using ANN

Protein identities of our three predictive ions (1268.6, 998.6, and 910.4 m/z) that were obtained from the ANN were found following MSM on the fractionated samples. The depletion of high abundance proteins in our PCa samples followed by LC-MALDI allowed the identification of several proteins. The top predictive ion - 1268.6 (m/z) was

identified as Haemopexin. Haemopexin is a glycoprotein, and it plays an important role as a carrier for plasma haeme (Haeme Scavenger). Haemoglobin is a carrier protein that transports oxygen from the lung to all the body tissues through the blood, and this haemoglobin consists of four globin subunits each one of them contains a haeme group. Each haeme molecule consisting of a porphyrin ring and iron atom has the ability to bind with the oxygen atom, which can be transported. The free haeme in plasma indicates some pathologic conditions such as haemolysis. Haeme is auto-oxidise molecule which can intercalate with the lipid membrane of the cell, so free haeme in the plasma is considered a risk for health. Thus Haemopexin has an important role in binding plasma haeme, and therefore its expression indicates some pathologic conditions such as inflammation and cancer (Piccard et al., 2006). Another role of Haemopexin is activating Matrix metalloproteinases (MMPs). MMPs are that have the ability to breakdown the extracellular matrix, which facilitate localised cancer to invade to another organ (Pia et al., 2005).

The second predictive ion 998.6 (m/z) was identified as a peptide of the protein Gelsolin. Gelsolin is an intracellular protein and a member of actin-binding proteins which are found in mitochondria and cytosol with molecular weight 82 (kDa) and found in blood in concentration 100 to 250 $\mu\text{L/ml}$ (Goetzel et al., 2000). Gelsolin can also be found in the blood stream (extracellular) and plays important role in motility and differentiation of cells and it is stimulated by calcium (Ca^{++}). Gelsolin is found to be a substrate for caspase-3, and has the ability to promote apoptosis and protect cells from apoptosis as well. The expression of these proteins in prostate cancer has been shown in many studies (Nishimura et al., 2003).

For the same predictive ion 998.6 (m/z) another possible protein identity was found (there are several possible identities as the ANN bins the peptide ion m/z value to the nearest 0.1 Da, leaving some slight ambiguity as to the exact mass to use in the MSMS/database search), which is Apolipoprotein B-100 (apo B100). This protein has a role as primary form of very low density lipoproteins (VLDL) and low density lipoprotein (LDL) which is responsible of removing insoluble water lipids such as cholesterol from the body. The process of transferring the cholesterol to cell membrane occurs when Apo B100 works as a receptor to facilitate this process. In the present day some laboratories assess Apo B100 as a marker of cardiac disease patients. Studies show a relationship between prostate cancer therapy such as androgen deprivation and heart disease (Keatings et al 2006). Other studies show that Apo B100 will be increased when the patients have Estrogen treatment (Usui et al., 2002). Table 3.2 shows that predictive ion 998.6 (m/z) was identified as a peptide of Apo B100 only in nonaggressive patients (<7 in Gleason sum).

4.5 Identification of unique proteins in nonaggressive Vs aggressive PCa samples

The main aim of this section of the study was to find the protein expression differences between nonaggressive and aggressive PCa patients and define prognostic biomarkers. 24 depleted PCa serum samples (12 aggressive, 12 nonaggressive) were analysed by LC-MALDI MS/MS, and the identification of candidate proteins were obtained by tandem MS/MS mass spectrometry using MASCOT search dataset. The number of proteins identified in each sample was in range of 80 to 140 proteins. The majority of identified proteins are the same in aggressive and nonaggressive PCa patients (tables of the entire proteins list were identified for all the 24 PCa patients in the appendix section). Some proteins were identified as unique proteins for a specific

group. 18 candidate proteins for nonaggressive PCa identified were different from the aggressive group, and 14 proteins were identified and expressed only in aggressive PCa patients.

However, it has been noticed that some of our unique proteins identified have been previously associated with prostate cancer. For example (Pregnancy zone protein) which has expressed in nonaggressive PCa patients. (Pregnancy zone protein) has been identified to increase when the patient's have treated hormonally. Oestrogen is a female hormone that has been used in the last few decades as hormone treatment for prostate cancer. Although the advantage of the treatment with this hormone, there is high risk to have blood clot. Studies show that (Pregnancy zone protein) will be increased if the patient has oestrogen treatment (Beckman et al 1973). Another example for a unique identified protein for nonaggressive PCa patients is (Rho GTPase-activating protein 7). Studies show that (Rho GTPase-activating protein 7) has the ability to downregulate in cancers and can inhibit the growth of prostate cancer (Durkin et al., 2007).

On the other hand the uniquely identified proteins in aggressive PCa patients have been found to be associated with prostate cancer, and some candidate proteins are related to different types of cancers. One of these proteins is (Focal adhesion kinase 1), which has a very important role in the regulation of the migration of cells. Thus (Focal adhesion kinase 1) play a critical role in metastatic cancers, which will facilitate cancerous cells invading different organs such as bone marrow. This protein has the ability to cross the extracellular matrix (ECM) from one primary organ to another without the help of matrix metalloproteinases (MMPs), which are used to breakdown the ECM. Furthermore, studies prove that (Focal adhesion kinase 1) can control the advancement phenotype of androgen-independent prostate cancer (Johnson et al; 2008). However we should

consider that some of these proteins were expressed in only one patient, these types of proteins may be identified with many other physiological conditions of the patients including age and infections. These patient proteins shouldn't be treated as a good candidate biomarkers since its occurrences were limited only single or few patients. A wider population study is necessary to ascertain these types of proteins to a biomarker candidate status.

4.6 Differences in peptide expression in nonaggressive (<7 in Gleason sum) Vs aggressive (≥ 7 in Gleason sum) patients for all the 24 pooled depleted samples

Two pooled samples were used to differentiate between the aggressive and nonaggressive PCa samples based on their "expression" (comparison of peptide ion intensity). We had 24 PCa samples and pooled 12 nonaggressive PCa samples in one sample, and pooled 12 aggressive PCa samples in a second sample. These two pooled samples were depleted and analysed by LC-MALDI. Prior to analysis of these samples, a model was generated by the software (ProfileAnalysis, Bruker Daltonics). The model contains ions (m/z) and their intensities and was obtained based upon data from all 24 samples that were run previously by individual LC-MALDI. This model shows the fold change in peptide expression for aggressive and nonaggressive PCa pooled samples (figure 3.9). The figure shows the difference in peptide expression based on their fold change as interpreted as up (positive) or down (Negative) regulation. 13 Ions (m/z) had a positive fold change in the nonaggressive PCa patients compared with aggressive group. In contrast 11 ions (m/z)

had a negative fold change in the nonaggressive samples compared with aggressive sample.

These significant ions were subjected to WARP-LC software (Bruker Daltonics) prior to tandem MS/MS and list of proteins identified for the two pooled samples. 81 proteins were identified for the pooled nonaggressive patients, while 60 proteins were identified in the pooled aggressive sample (table 3.3, 3.4). 57 proteins were expressed in both groups, while 23 were expressed only on nonaggressive pooled sample and 12 expressed only in aggressive pooled sample (figure 3.10). However five of the ions were associated with three identified proteins (table 3.5). 1212.24, 1329.45, and 2032.8 (m/z) ions were identified as peptide of protein Apo B100, which has been already discussed above. These ions had a positive fold change in the nonaggressive sample compared with the aggressive. The ion 1585.65 (m/z) was identified as Apolipoprotein A-IV. This ion showed increased fold change in the nonaggressive group compared with the aggressive group. Apolipoprotein A-IV is a glycoprotein expressed in intestine and secreted into bloodstream, which has very important role in lipid metabolism, especially for triglycerides (Tso et al., 2001)) The concentration of Apolipoprotein A-IV in blood is dramatically changed due to the dietary. This protein considered as the main component of high density lipoprotein HDL (the good cholesterol in the body), and associated with cardiac disease. One study shows that Apolipoprotein A-IV is increased in BPH patients (Srivastava 2008). The last ion (m/z 1887.8) was identified as protein “Afamin”. Afamin is one of a glycoprotein family which is synthesised in liver and secreted into the circulation. Afamin plays role as a vitamin-E carrier, which is protect the body from

oxidative stress (Jerkovic et al., 2005). There are no studies reported which depict any relationship between Afamin and prostate cancer.

As shown above, Apo B 100 protein was identified by two methodologies - ANNs and ProfileAnalysis, indicating its significance as a possible prognostic biomarker to differentiate between aggressive and nonaggressive prostate cancer types.

Future work

Further analysis/validation Apo B 100 protein along with other identified proteins such as Enzyme-linked immunosorbent assay (ELISA) or Western blot need to be carried out to confirm the MALDI mass spectrometric data. Another analysis can be carried out is the quantification of candidate peptides using isobaric tags for relative and absolute quantification (iTRAQ) technology (Tonack et al., 2009). Once it is confirmed as a candidate marker, this has to be studied in a separate cohort of patients drawn from different geographical distribution or ethnicity to address its validity to use as a universal biomarker for prostate cancer staging.

Conclusion

This study was designed with the objective of the identification of prognostic biomarkers in a prostate cancer cohort with two forms of prostate cancer, aggressive and nonaggressive. The pathological classification of these two forms based on the popular Gleason score identified aggressive types as above seven and the nonaggressive types as

below seven. Reproducibility of the MS spectra were successfully assessed by running BSA standards and the serum QC samples along with the test samples in a randomised manner using robotic systems. ANN analysis of the MS data shortlisted three ions (m/z 1268.8, 998.6, 910.4) which have a combined predictive capability of 77% of the total population studied. These ions successfully stratified the patients into two groups. The sensitivity and the specificity of the model was then assessed by response operator curve which gave a value close to one indicating the robustness of the ions as classifiers. In a second section of the study, immunodepletion columns were successfully used for the selective removal of the major high abundant proteins. Further fractionation by nano-LC and MSMS generated 80-120 protein identities with multiple peptides identified for many proteins. The protein and the peptide list generated after the LC MALDI was manually searched for the presence of 3 ions shortlisted with ANN and they were identified as Haemppexin, Gelsolin and Apolipoprotein 100. Detailed comparison of the protein identities classified in to three group 18 proteins were uniquely present in the non aggressive group and 14 proteins were unique for aggressive group. Literature survey suggested that the majority of these proteins are functionally associated with prostate or other cancer development and progression. The studies with the pooled samples of aggressive and nonaggressive PCa samples with a different model generation approach (ProfileAnalysis) also came up with Apolipoprotein B100 suggesting the potential of this protein as a potential biomarker candidate for stratifying aggressive and nonaggressive PCa. However, further experiments need to be carried out with a separate PCa population prior to envisaging the wider use of this marker in prostate cancer patients.

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6. Appendix

List for the identified proteins for all 24 PCa samples (12 nonaggressive and 12 aggressive)

Patient one (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	78.3009952	0	13
unknown	76.92	0	7
unknown	60.0369089	0	5
unknown	58.69	0	4
unknown	57.8469089	0	5
unknown	56.48	0	4
unknown	55.9469089	0	5
unknown	54.7	0	4
unknown	53.6969089	0	6
unknown	48.77	0	3
unknown	47	0	3
unknown	46.42	0	1
unknown	45.19	0	2
unknown	41.67	0	1
unknown	41.23	0	2
unknown	41.21	0	3
unknown	37.1369089	0	2
unknown	36.96	0	2
unknown	35.67	0	2
unknown	34.4	0	1
unknown	34.3569089	0	2
unknown	32.23	0	1
unknown	31.55	0	1
unknown	31.15	0	1
Afamin	330.446909	69.02401	9
Alpha-1-antichymotrypsin	1325.92	47.62054	29
Alpha-1-antitrypsin	459.546909	46.70702	9
Alpha-1B-glycoprotein	1207.00691	54.23858	31
Alpha-2-antiplasmin	464.623818	54.53107	15
Alpha-2-HS-glycoprotein	641.34	39.29971	21
Alpha-2-macroglobulin	585.886909	163.18888	20
Angiotensinogen	516.86	53.12051	10
Ankyrin repeat and SOCS box protein 18	49.07	50.77091	8
Antithrombin-III	1171.27691	52.56886	24
Apolipoprotein A-I	1185.47	30.75893	25
Apolipoprotein A-II	36.41	11.1679	1
Apolipoprotein A-IV	1489.59	45.37147	32

Apolipoprotein B-100	6762.25948	515.24085	128
Apolipoprotein C-I	100.93	9.32609	2
Apolipoprotein C-II	217.29	11.27675	4
Apolipoprotein C-III	323.09	10.8455	3
Apolipoprotein E	523.706909	36.13175	18
ATP-binding cassette sub-family A member 13	98.4638177	575.87114	13
Attractin	92.78	158.43246	5
Beta-2-glycoprotein 1	259.92	38.27266	7
Beta-Ala-His dipeptidase	180.48	56.65611	6
Biotinidase	156.99	61.09326	5
Carboxypeptidase B2	220.796909	48.38141	7
Carboxypeptidase N subunit 2	206.84	60.57615	5
Ceruloplasmin	3730.31073	122.12759	63
Cholinesterase	87.2169089	68.37427	6
Clusterin	476.673818	52.46101	8
Coagulation factor V	218.01	251.51354	9
Coagulation factor X	118.76	54.69651	4
Coagulation factor XII	134.48	67.77391	6
Complement C1q subcomponent subunit A	366.23	26.00019	2
Complement C1q subcomponent subunit B	243.64	26.44241	8
Complement C1q subcomponent subunit C	344.856909	25.75714	6
Complement C1r subcomponent	422.516909	80.06681	12
Complement C1r subcomponent-like protein	122.78	53.46434	6
Complement C1s subcomponent	492.576909	76.6348	12
Complement C2	666.406909	83.21431	15
Complement C3	1390.49073	187.02987	33
Complement C4-A	4099.84218	192.65045	85
Complement C4-B	4179.65218	192.67254	86
Complement C5	866.563818	188.18613	23
Complement component C6	468.81	104.718	9
Complement component C7	384.33	93.45729	5
Complement component C8 alpha chain	56.52	65.12104	4
Complement component C8 beta chain	578	67.00347	13
Complement component C8 gamma chain	202.38	22.26354	5
Complement component C9	539.476909	63.1327	14
Complement factor B	1365.20382	85.47852	27
Complement factor H	930.586909	139.0047	28
Complement factor H-related protein 1	166.49	37.62596	3
Complement factor H-related protein 3	69.47	37.29875	2
Complement factor I	462.416909	65.6766	12
Corticosteroid-binding globulin	178.47	45.11191	4
Epsin-2	59.14	68.43928	3
Fibrinogen alpha chain	135.82	94.91441	6
Fibronectin	2177.94554	262.44208	51
Gelsolin	1172.37	85.64419	24
Glial fibrillary acidic protein	47.06	49.84965	3
Glutamate receptor-interacting protein 1	67.4469089	122.34728	5
Glutathione peroxidase 3	74.67	25.5369	3
Haptoglobin	330.006909	45.17656	9
Hemoglobin subunit alpha	37.85	15.24793	1

Hemoglobin subunit beta	48.95	15.98829	2
Hemopexin	1540.44382	51.64327	52
Heparin cofactor 2	1003.68382	57.0342	21
Hepatocyte growth factor activator	72.8369089	70.63609	4
Histidine-rich glycoprotein	877.166909	59.54087	19
Insulin-like growth factor-binding protein complex acid labile subunit	694.514813	65.9938	17
Inter-alpha-trypsin inhibitor heavy chain H1	1698.40691	101.32561	41
Inter-alpha-trypsin inhibitor heavy chain H2	2045.54691	106.39661	40
Inter-alpha-trypsin inhibitor heavy chain H3	740.026909	99.78653	16
Inter-alpha-trypsin inhibitor heavy chain H4	1959.48691	103.29298	50
Kallistatin	319.806909	48.51116	15
Keratin, type II cytoskeletal 1	83.4307266	65.999	7
Keratin, type II cytoskeletal 4	53.08	57.24983	3
Keratin, type II cytoskeletal 80	48.8669089	50.49378	4
Kininogen-1	840.68	71.91215	13
Leucine-rich alpha-2-glycoprotein	393.04	38.15411	9
Leucine-rich repeat serine/threonine-protein kinase 1	91.3969089	227.69779	12
Low affinity immunoglobulin gamma Fc region receptor III-A	40.59	29.07069	1
Lumican	269.103818	38.40479	7
Lymphoid-restricted membrane protein	58.87	62.06908	3
N-acetylmuramoyl-L-alanine amidase	355.61	62.17788	10
Nebulin	131.763527	772.4543	31
Pigment epithelium-derived factor	605.51	46.3133	11
Plasma kallikrein	118.2	71.32284	7
Plasma protease C1 inhibitor	702.38	55.11939	16
Plasma serine protease inhibitor	165.29	45.6727	6
Plasminogen	490.406909	90.51016	14
Platelet basic protein	157.53	13.88542	4
Pregnancy zone protein	42.23	163.75958	2
Prostaglandin-H2 D-isomerase	62.15	21.01534	1
Protein AMBP	378.89	38.97398	10
Protein Z-dependent protease inhibitor	86.1869089	50.67422	9
Prothrombin	416.776909	69.99212	19
Putative hydroxypyruvate isomerase	57.15	30.38656	4
Putative trypsin-6	90.62	26.52219	7
RelA-associated inhibitor	60.78	89.03574	5
Retinol-binding protein 4	105.62	22.99526	2
Rho GTPase-activating protein 7	78.27	170.486	6
Serotransferrin	39.59	76.99961	1
Serum albumin	229.410727	69.32149	8
Serum amyloid P-component	567.02	25.37113	11
Serum paraoxonase/arylesterase 1	120.46	39.72418	2
Sex hormone-binding globulin	140.64	43.75182	5
Tetranectin	270.77	22.55228	6
Thrombospondin-1	380.813818	129.29956	16
Thyroxine-binding globulin	138.306909	46.29461	5
TNF receptor-associated factor 3	54.73	64.44808	7
Transthyretin	91.98	15.87705	3
Uncharacterised protein C10orf67	53.3369089	21.44912	4
Uncharacterised protein C10orf90	41.21	77.86188	2

Vitamin D-binding protein	809.04	52.92903	16
Vitronectin	546.536909	54.27117	11
Zinc-alpha-2-glycoprotein	425.3	34.2371	13

Patient 2 (nonaggressive)

Afamin	248.98691	70.96274	5
Alpha-1-antichymotrypsin	822.77	47.7916	11
Alpha-1B-glycoprotein	666.74	54.8088	10
Alpha-2-antiplasmin	187.40691	54.87319	6
Alpha-2-HS-glycoprotein	313.71	40.09801	4
Alpha-2-macroglobulin	55.51	164.61441	3
Angiotensinogen	330.24	53.40562	5
Apolipoprotein A-I	481.94	30.75893	13
Apolipoprotein A-IV	1429.79	45.37147	26
Apolipoprotein B-100	2683.1896	516.66639	60
Apolipoprotein C-III	252.56	10.8455	2
Apolipoprotein E	189.15691	36.2458	6
Beta-2-glycoprotein 1	93.35	39.58415	2
Beta-Ala-His dipeptidase	98.8	56.77015	4
Biotinidase	36.67	62.0056	1
Carboxypeptidase B2	132.97	48.95162	3
Carboxypeptidase N subunit 2	121.09	61.43147	4
Ceruloplasmin	2158.4969	122.98291	27
Clusterin	210.49691	53.03122	5
Coagulation factor X	101.75	56.06503	3
Complement C1q subcomponent subunit B	237.03	26.67049	6
Complement C1q subcomponent subunit C	307.59691	25.98522	4
Complement C1s subcomponent	404.59691	78.17438	12
Complement C2	210.14	84.58283	9
Complement C3	405.76	188.56945	6
Complement C4-A	2566.7384	194.24706	40
Complement C5	365.01	189.89678	12
Complement component C6	161.34	108.36738	4
Complement component C7	122.62691	96.65049	3
Complement component C8 alpha chain	68.79	66.83168	2
Complement component C8 beta chain	343.43	68.71412	7
Complement component C8 gamma chain	62.74	22.43461	2
Complement component C9	384.57691	64.61526	7
Complement factor B	1292.0338	86.84704	21
Complement factor H	802.46691	143.68046	18
Complement factor H-related protein 1	171.9	38.76639	4
Complement factor H-related protein 3	49.98	38.4962	2
Complement factor I	188.31691	68.0715	4
Corticosteroid-binding globulin	38.36	45.28297	1
Fibrinogen alpha chain	166.11	95.65569	4
Fibronectin	1182.4638	266.03443	26
Ficolin-3	158.88	33.39518	4
FYVE, RhoGEF and PH domain-containing protein 4	33.03	87.59782	1
Gelsolin	495.46	86.04334	9
Glial fibrillary acidic protein	54.84	49.90667	2
Glutathione peroxidase 3	41.42	25.76499	2
Hemopexin	969.64	52.38455	20
Heparin cofactor 2	498.56382	57.20527	12
Histidine-rich glycoprotein	337.92	60.51023	7

Insulin-like growth factor-binding protein complex acid labile subunit	272.26382	66.73507	7
Inter-alpha-trypsin inhibitor heavy chain H1	1566.7169	101.78179	22
Inter-alpha-trypsin inhibitor heavy chain H2	1594.1069	106.85278	24
Inter-alpha-trypsin inhibitor heavy chain H3	377.49691	100.07164	6
Inter-alpha-trypsin inhibitor heavy chain H4	1279.4769	103.52107	21
Kallistatin	87.98	48.68222	3
Keratin, type I cytoskeletal 10	396.54	59.01978	12
Keratin, type I cytoskeletal 9	232.47	62.2549	8
Keratin, type II cytoskeletal 1	908.81454	66.17007	15
Keratin, type II cytoskeletal 2 epidermal	428.16691	65.67832	10
Keratin, type II cytoskeletal 2 oral	47.49	66.40031	2
Keratin, type II cytoskeletal 3	56.92	64.63566	2
Keratin, type II cytoskeletal 4	109.89	57.64898	3
Keratin, type II cytoskeletal 5	90.556909	62.56806	3
Keratin, type II cytoskeletal 6B	173.48691	60.3154	4
Keratin, type II cytoskeletal 7	102.69	51.43037	4
Keratin, type II cytoskeletal 71	54.49	57.72714	3
Keratin, type II cytoskeletal 72	50.786909	56.46967	3
Keratin, type II cytoskeletal 73	71.3	59.45695	4
Keratin, type II cytoskeletal 74	52.64	58.22879	3
Keratin, type II cytoskeletal 75	44.6	59.80914	2
Keratin, type II cytoskeletal 79	47.86	58.05923	2
Keratin, type II cytoskeletal 8	149.49	53.67113	5
Kininogen-1	875.49	72.99556	12
Lumican	111.62	38.74692	2
Lymphoid-restricted membrane protein	37.55	62.75334	1
N-acetylmuramoyl-L-alanine amidase	116.66	62.7481	4
Nebulin	74.65554	775.41942	9
Pigment epithelium-derived factor	228.29	46.48436	7
Plasma kallikrein	47.96	73.43264	1
Plasma protease C1 inhibitor	86.69	55.34748	4
Plasminogen	497.90691	93.24719	10
Platelet basic protein	238.31	14.17052	4
Platelet factor 4 variant	37.82	11.77337	1
Protein AMBP	170.55	39.88632	4
Prothrombin	222.80691	71.47468	6
Putative trypsin-6	80.79	27.0924	2
Serum amyloid P-component	387.07	25.48517	8
Spermatogenesis-associated protein 7	58.14	68.18992	2
Stabilin-1	47.406909	286.92647	2
Tetranectin	119.34	22.95143	1
Thrombospondin-1	263.95	133.29106	6
Titin	113.44338	3843.1187	33
Vitamin D-binding protein	586.8	54.52563	10
Vitamin K-dependent protein S	76.54	77.12671	4
Vitronectin	433.2	55.06947	7
Zinc-alpha-2-glycoprotein	116.41	34.46519	4

Patient 3 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
Unknown	85.42382	0	13
Unknown	63.79691	0	8
Unknown	63.35	0	4

Unknown	55.07691	0	8
Unknown	52.72	0	4
Unknown	51.16	0	3
Unknown	49.28	0	3
Unknown	48.43	0	3
Unknown	47.36382	0	5
Unknown	46.38	0	3
Unknown	46.3	0	2
Unknown	45.18	0	3
Unknown	45.16382	0	4
Unknown	41.38	0	3
Unknown	38.86	0	1
Unknown	36	0	1
Unknown	31.45	0	1
Afamin	449.3169	69.02401	13
Alpha-1-antichymotrypsin	1079.18	47.62054	21
Alpha-1B-glycoprotein	927.44	54.23858	19
Alpha-2-antiplasmin	308.8538	54.53107	10
Alpha-2-HS-glycoprotein	445.09	39.29971	10
Alpha-2-macroglobulin	309.8069	163.18888	10
Angiotensinogen	387.89	53.12051	11
Ankyrin repeat and SOCS box protein 18	46.09	50.77091	3
Antithrombin-III	627.1869	52.56886	19
Apolipoprotein A-I	696.1269	30.75893	20
Apolipoprotein A-IV	1646.23	45.37147	32
Apolipoprotein B-100	4745.71	515.24085	96
Apolipoprotein C-III	236.74	10.8455	2
Apolipoprotein E	369.3469	36.13175	14
Beta-2-glycoprotein 1	264.36	38.27266	7
Beta-Ala-His dipeptidase	313.74	56.65611	8
Biotinidase	58.98	61.09326	2
C5a anaphylatoxin chemotactic receptor	39.86	39.29501	2
Carboxypeptidase B2	202.0169	48.38141	7
Carboxypeptidase N subunit 2	141.87	60.57615	5
Cartilage acidic protein 1	62.13	71.37581	2
CD44 antigen	60.05	81.5034	3
Ceruloplasmin	3324.038	122.12759	49
Clusterin	246.7369	52.46101	7
Coagulation factor IX	77.57	51.745	1
Coagulation factor X	110.33	54.69651	2
Coagulation factor XII	88.08	67.77391	4
Complement C1q subcomponent subunit A	163.35	26.00019	3
Complement C1q subcomponent subunit B	250.12	26.44241	7
Complement C1q subcomponent subunit C	339.3569	25.75714	6
Complement C1r subcomponent	208.04	80.06681	10
Complement C1s subcomponent	571.0369	76.6348	14
Complement C2	519.5869	83.21431	16
Complement C3	1024.751	187.02987	26
Complement C4-A	2830.965	192.65045	54
Complement C5	672.8869	188.18613	23
Complement component C6	371.13	104.718	12
Complement component C7	401.3	93.45729	8
Complement component C8 alpha chain	110.55	65.12104	4
Complement component C8 beta chain	549.71	67.00347	15
Complement component C8 gamma chain	250.66	22.26354	5
Complement component C9	441.4969	63.1327	14
Complement factor B	1100.004	85.47852	23

Complement factor H	1296.754	139.0047	34
Complement factor H-related protein 1	137.15	37.62596	4
Complement factor H-related protein 3	82.2	37.29875	3
Complement factor I	239.5138	65.6766	9
Corticosteroid-binding globulin	123.26	45.11191	5
FERM domain-containing protein 4A	45.58	115.3873	3
Fibrinogen alpha chain	349.48	94.91441	8
Fibronectin	1488.839	262.44208	36
Ficolin-3	190.72	32.88199	6
Gelsolin	1078.03	85.64419	19
Glutathione peroxidase 3	56.43	25.5369	3
Haptoglobin	230.32	45.17656	6
Hemoglobin subunit alpha	73.67	15.24793	1
Hemopexin	1300.587	51.64327	33
Heparin cofactor 2	783.6338	57.0342	15
Hepatocyte growth factor activator	90.16691	70.63609	2
Histidine-rich glycoprotein	737.0969	59.54087	18
Hyaluronan-binding protein 2	55	62.63044	1
Insulin-like growth factor-binding protein complex acid labile subunit	336.6138	65.9938	11
Inter-alpha-trypsin inhibitor heavy chain H1	1750.164	101.32561	28
Inter-alpha-trypsin inhibitor heavy chain H2	1472.167	106.39661	32
Inter-alpha-trypsin inhibitor heavy chain H3	535.3569	99.78653	16
Inter-alpha-trypsin inhibitor heavy chain H4	1298.327	103.29298	31
Kallistatin	179.37	48.51116	9
Kininogen-1	1028.74	71.91215	14
Leucine-rich alpha-2-glycoprotein	201.46	38.15411	4
Lumican	225.1738	38.40479	7
Mannan-binding lectin serine protease 2	100.93	75.68464	5
Mannose-binding protein C	64.25	26.127	2
Myosin-13	46.55	223.54012	3
N-acetylmuramoyl-L-alanine amidase	192.86	62.17788	8
Neuron navigator 3	72.86	255.46086	6
Neuronal acetylcholine receptor subunit beta-2	42.7	56.98207	4
Pigment epithelium-derived factor	309.95	46.3133	9
Plasma kallikrein	55.46	71.32284	3
Plasma protease C1 inhibitor	363.03	55.11939	12
Plasma serine protease inhibitor	98.51	45.6727	4
Plasminogen	595.8869	90.51016	15
Platelet basic protein	228.16	13.88542	6
Platelet glycoprotein V	35.98	60.92068	2
Protein AMBP	212.3	38.97398	6
Protein FAM184A	63.55691	132.88315	6
Protein Z-dependent protease inhibitor	41.83	50.67422	1
Prothrombin	230.4569	69.99212	11
Protocadherin-12	33.06691	128.91494	2
Retinol-binding protein 4	34.57	22.99526	1
Serum amyloid P-component	406.92	25.37113	7
Serum paraoxonase/arylesterase 1	36.52	39.72418	1
Sex hormone-binding globulin	114.4	43.75182	4
StAR-related lipid transfer protein 9	88.19145	506.43489	10
Tetranectin	183.05	22.55228	4
Thrombospondin-1	549.5007	129.29956	17
Thrombospondin-2	83.34	129.90777	3
Thyroxine-binding globulin	108.9538	46.29461	4
Trypsin-1	72.97	26.54109	3
Vitamin D-binding protein	716.8	52.92903	14

Vitamin K-dependent protein S	121.19	75.07393	7
Vitronectin	515.0838	54.27117	10
Zinc finger protein 791	43.25	66.82764	2
Zinc-alpha-2-glycoprotein	396.52	34.2371	14

Patient 4 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
Afamin	108.40691	69.02401	5
Alpha-1-antichymotrypsin	599.56	47.62054	13
Alpha-1B-glycoprotein	521.11	54.23858	13
Alpha-2-HS-glycoprotein	368.65	39.29971	7
Amyloid-like protein 1	63.53	72.13118	2
Angiotensinogen	280.97	53.12051	6
Apolipoprotein A-I	207.42	30.75893	10
Apolipoprotein A-IV	1306.68	45.37147	30
Apolipoprotein B-100	1843.2019	515.24085	60
Apolipoprotein C-III	131.41	10.8455	2
Apolipoprotein E	100.58691	36.13175	8
Beta-2-glycoprotein 1	157.84	38.27266	5
Biotinidase	33.76	61.09326	1
Carboxypeptidase B2	83.396909	48.38141	3
Ceruloplasmin	1549.6238	122.12759	31
Clusterin	228.91382	52.46101	9
Complement C1q subcomponent subunit B	79.43	26.44241	5
Complement C1q subcomponent subunit C	196.32691	25.75714	4
Complement C1s subcomponent	237.07691	76.6348	11
Complement C2	77.54	83.21431	3
Complement C3	302.67382	187.02987	8
Complement C4-B	1663.6915	192.67254	45
Complement C5	226.82	188.18613	11
Complement componen	80.33	93.45729	3
Complement component C8 beta chain	130.12	67.00347	3
Complement component C8 gamma chain	44.28	22.26354	3
Complement component C9	230.91691	63.1327	10
Complement factor B	743.87691	85.47852	23
Complement factor H	502.50691	139.0047	23
Complement factor H-related protein 1	137.47	37.62596	4
Cystatin-C	71.86	15.78908	3
FERM domain-containing protein 4A	39.47	115.3873	2
Fibrinogen alpha chain	111.87	94.91441	5
Fibronectin	507.89481	262.44208	16
Ficolin-3	61.69	32.88199	3
Gelsolin	442.19	85.64419	11
Hemopexin	711.85	51.64327	29
Heparin cofactor 2	289.97	57.0342	7
Hepatocyte growth factor activator	36.653818	70.63609	2
Histidine-rich glycoprotein	264.27	59.54087	8
Insulin-like growth factor-binding protein complex acid labile subunit	157.07382	65.9938	9
Inter-alpha-trypsin inhibitor heavy chain H1	992.78073	101.32561	21
Inter-alpha-trypsin inhibitor heavy chain H2	823.54	106.39661	19
Inter-alpha-trypsin inhibitor heavy chain H3	301.13691	99.78653	10
Inter-alpha-trypsin inhibitor heavy chain H4	699.29	103.29298	22
Kininogen-1	683.2	71.91215	13
Lumican	98.17	38.40479	3

Osteopontin	98.88	35.40124	1
Pigment epithelium-derived factor	158.57	46.3133	9
Plasma protease C1 inhibitor	42.9	55.11939	2
Plasminogen	352.29691	90.51016	12
Platelet basic protein	88.3	13.88542	4
Protein AMBP	97.86	38.97398	6
Prothrombin	85.816909	69.99212	4
Serum albumin	106.44382	69.32149	5
Serum amyloid P-component	230.98	25.37113	5
Spermatogenesis-associated protein 7	34.86	67.67673	1
Tetranectin	62.3	22.55228	3
Thrombospondin-1	94.426909	129.29956	8
Trypsin-1	46.74	26.54109	1
Trypsin-1	46.24	54.69651	3
Vitamin D-binding protein	310.15	52.92903	10
Vitamin K-dependent protein S	79.28	75.07393	5
Vitronectin	178.78	54.27117	5
Zinc-alpha-2-glycoprotein	63.15	34.2371	3
unknown	58.09	0	5
unknown	49.713818	0	5
unknown	45.44	0	2
unknown	33.83	0	2

Patient 5 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	52.24	0	3
unknown	45.17	0	2
unknown	42.99	0	2
unknown	41.6	0	1
unknown	40.3	0	1
unknown	36.53	0	1
Afamin	225.66691	70.96274	6
Alpha-1-antichymotrypsin	934.21	47.7916	14
Alpha-1B-glycoprotein	1026.27	54.8088	17
Alpha-2-antiplasmin	330.69691	54.87319	9
Alpha-2-HS-glycoprotein	325.24	40.09801	8
Antithrombin-III	500.08691	53.02504	12
Apolipoprotein A-I	577.14691	30.75893	15
Apolipoprotein A-IV	1777.2	45.37147	31
Apolipoprotein B-100	5071.2079	516.66639	103
Apolipoprotein C-III	253.95	10.8455	2
Apolipoprotein E	672.21691	36.2458	15
Beta-2-glycoprotein 1	170.34	39.58415	5
Beta-actin-like protein 3	36.75	42.33097	3
Beta-Ala-His dipeptidase	123.47	56.77015	4
Biotinidase	58.69	62.0056	2
Carboxypeptidase B2	258.66	48.95162	6
Carboxypeptidase N catalytic chain	140.08	52.53842	4
Carboxypeptidase N subunit 2	208.82	61.43147	5
Cartilage acidic protein 1	48.49	72.17411	2
Ceruloplasmin	2299.8569	122.98291	36

Clusterin	360.18691	53.03122	6
Coagulation factor IX	83.1	53.11351	1
Coagulation factor V	103.42691	252.65397	9
Coagulation factor X	142.86	56.06503	4
Coagulation factor XIII A chain	121.31	83.7278	1
Complement C1q subcomponent subunit B	199.6	26.67049	5
Complement C1q subcomponent subunit C	299.17691	25.98522	5
Complement C1r subcomponent	57.56	81.60639	3
Complement C1s subcomponent	413.10691	78.17438	13
Complement C2	395.04	84.58283	11
Complement C3	634.46691	188.56945	14
Complement C4-B	2791.1884	194.21212	50
Complement C5	544.04	189.89678	19
Complement component C6	414.95	108.36738	11
Complement component C7	465.26691	96.65049	12
Complement component C8 alpha chain	95.53	66.83168	2
Complement component C8 beta chain	527.25	68.71412	12
Complement component C8 gamma chain	157.36	22.43461	4
Complement component C9	513.92691	64.61526	11
Complement factor B	1115.5438	86.84704	20
Complement factor H	608.89691	143.68046	20
Complement factor H-related protein 1	184.29	38.76639	4
Complement factor H-related protein 3	62.3	38.4962	2
Complement factor I	298.75691	68.0715	8
Corticosteroid-binding globulin	124.26	45.28297	4
Fibrinogen alpha chain	259.72	95.65569	6
Fibronectin	1166.4338	266.03443	27
Ficolin-3	226.39	33.39518	7
Gelsolin	1494.33	86.04334	23
Hemopexin	1016.85	52.38455	27
Heparin cofactor 2	731.44382	57.20527	13
Hepatocyte growth factor activator	43.54	72.85992	2
Histidine-rich glycoprotein	745.72691	60.51023	14
Hyaluronan-binding protein 2	57.91	64.74024	1
Insulin-like growth factor-binding protein complex acid labile subunit	298.90382	66.73507	10
Inter-alpha-trypsin inhibitor heavy chain H1	1922.7769	101.78179	27
Inter-alpha-trypsin inhibitor heavy chain H2	1552.4869	106.85278	28
Inter-alpha-trypsin inhibitor heavy chain H3	744.61691	100.07164	14
Inter-alpha-trypsin inhibitor heavy chain H4	1390.27	103.52107	28
Kallistatin	107.67	48.68222	3
Keratin, type I cytoskeletal 16	90.906909	51.57833	4
Keratin, type II cytoskeletal 1	182.93382	66.17007	7
Keratin, type II cytoskeletal 6B	146.02691	60.3154	6
Kininogen-1	1075.19	72.99556	12
Lumican	221.86073	38.74692	7
Mannan-binding lectin serine protease 2	54.55	77.22422	2
N-acetylmuramoyl-L-alanine amidase	210.17691	62.7481	7
Pigment epithelium-derived factor	399.53	46.48436	9
Plasma kallikrein	83	73.43264	3
Plasma protease C1 inhibitor	120.03	55.34748	8
Plasminogen	469.96382	93.24719	13
Platelet basic protein	204.68	14.17052	3
Platelet factor 4 variant	57.64	11.77337	1
Platelet glycoprotein V	36.65	61.43388	1
POTE ankyrin domain family member E	48.32	122.88226	4
Protein AMBP	163.21	39.88632	5
Prothrombin	291.54691	71.47468	7

Putative trypsin-6	41.78	27.0924	3
Serum amyloid P-component	605.66	25.48517	8
Sex hormone-binding globulin	191.29	43.9799	7
Spermatogenesis-associated protein 7	51.6	68.18992	2
Tetranectin	97.54	22.95143	2
Thrombospondin-1	373.66691	133.29106	11
Thrombospondin-2	83.43	133.78523	3
Vitamin D-binding protein	521.13	54.52563	13
Vitamin K-dependent protein S	114.4	77.12671	5
Vitronectin	408.36	55.06947	9
Zinc-alpha-2-glycoprotein	381.42	34.46519	11

Patient 6 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	39.52	0	1
unknown	36.52	0	1
Afamin	100.3369	70.96274	5
Alpha-1-antichymotrypsin	750.95	47.7916	15
Alpha-1B-glycoprotein	618.31	54.8088	11
Alpha-2-antiplasmin	86.51691	54.87319	3
Alpha-2-HS-glycoprotein	288.74	40.09801	5
Apolipoprotein A-IV	1387.42	45.37147	25
Apolipoprotein B-100	2578.86	516.6664	64
Apolipoprotein C-III	233.83	10.8455	3
Apolipoprotein E	297.2769	36.2458	9
ATP-binding cassette sub-family A member 12	37.08	295.3867	1
Beta-2-glycoprotein 1	110.04	39.58415	2
Carboxypeptidase B2	111.71	48.95162	3
Carboxypeptidase N subunit 2	93.5	61.43147	4
Ceruloplasmin	1530.467	122.9829	25
Clusterin	252.1269	53.03122	6
Complement C1q subcomponent subunit B	102.22	26.67049	3
Complement C1q subcomponent subunit C	182.1569	25.98522	4
Complement C1r subcomponent	114.51	81.60639	6
Complement C1s subcomponent	138.8869	78.17438	7
Complement C3	345.16	188.5695	9
Complement C4-A	1272.485	194.2471	35
Complement C5	207.7	189.8968	9
Complement component C6	134.1	108.3674	5
Complement component C8 beta chain	144.48	68.71412	7
Complement component C8 gamma chain	100.06	22.43461	3
Complement component C9	392.1769	64.61526	9
Complement factor B	607.5269	86.84704	16
Complement factor H	614.7138	143.6805	16
Complement factor H-related protein 1	156.72	38.76639	2
Complement factor I	91.95691	68.0715	3
Fibronectin	867.1138	266.0344	21
Gelsolin	321.85	86.04334	7
Haptoglobin	279.31	45.86082	7
Hemopexin	899.02	52.38455	21
Heparin cofactor 2	544.4769	57.20527	11

Histidine-rich glycoprotein	362.4	60.51023	6
Insulin-like growth factor-binding protein complex acid labile subunit	224.6138	66.73507	9
Inter-alpha-trypsin inhibitor heavy chain H1	1131.147	101.7818	19
Inter-alpha-trypsin inhibitor heavy chain H2	911.11	106.8528	15
Inter-alpha-trypsin inhibitor heavy chain H3	302.3369	100.0716	8
Inter-alpha-trypsin inhibitor heavy chain H4	678.8969	103.5211	17
Keratin, type I cytoskeletal 10	94.24	59.01978	5
Keratin, type I cytoskeletal 9	122.0569	62.2549	6
Keratin, type II cytoskeletal 1	437.9345	66.17007	11
Keratin, type II cytoskeletal 2 epidermal	77.98691	65.67832	4
Keratin, type II cytoskeletal 7	61.16	51.43037	2
Keratin, type II cytoskeletal 8	70.09	53.67113	3
Kininogen-1	772.34	72.99556	12
Leucine-rich alpha-2-glycoprotein	145.05	38.3822	4
Lumican	151.75	38.74692	3
N-acetylmuramoyl-L-alanine amidase	41.22	62.7481	1
Pigment epithelium-derived factor	120.98	46.48436	3
Plasma protease C1 inhibitor	233.64	55.34748	6
Plasminogen	290.3769	93.24719	8
Platelet basic protein	157.78	14.17052	3
Protein AMBP	168.82	39.88632	6
Prothrombin	147.4569	71.47468	8
Putative trypsin-6	66.9	27.0924	2
Serum amyloid P-component	195.24	25.48517	5
Tetranectin	70.67	22.95143	2
Thrombospondin-1	227.3869	133.2911	11
Vitamin D-binding protein	479.79	54.52563	9
Vitronectin	343.4	55.06947	7
Zinc-alpha-2-glycoprotein	103.94	34.46519	4

Patient 7 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	52.24	0	3
unknown	45.17	0	2
unknown	42.99	0	2
unknown	41.6	0	1
unknown	40.3	0	1
unknown	36.53	0	1
Afamin	225.6669	70.96274	6
Alpha-1-antichymotrypsin	934.21	47.7916	14
Alpha-1B-glycoprotein	1026.27	54.8088	17
Alpha-2-antiplasmin	330.6969	54.87319	9
Antithrombin-III	500.0869	53.02504	12
Apolipoprotein A-I	577.1469	30.75893	15
Apolipoprotein A-IV	1777.2	45.37147	31
Apolipoprotein B-100	5071.208	516.6664	103
Apolipoprotein C-III	253.95	10.8455	2
Apolipoprotein E	672.2169	36.2458	15
Beta-2-glycoprotein 1	170.34	39.58415	5
Beta-actin-like protein 3	36.75	42.33097	3
Beta-Ala-His dipeptidase	123.47	56.77015	4

Biotinidase	58.69	62.0056	2
Carboxypeptidase B2	258.66	48.95162	6
Carboxypeptidase N catalytic chain	140.08	52.53842	4
Carboxypeptidase N subunit 2	208.82	61.43147	5
Cartilage acidic protein 1	48.49	72.17411	2
Ceruloplasmin	2299.857	122.9829	36
Clusterin	360.1869	53.03122	6
Coagulation factor IX	83.1	53.11351	1
Coagulation factor V	103.4269	252.654	9
Coagulation factor X	142.86	56.06503	4
Coagulation factor XIII A chain	121.31	83.7278	1
Complement C1q subcomponent subunit B	199.6	26.67049	5
Complement C1q subcomponent subunit C	299.1769	25.98522	5
Complement C1r subcomponent	57.56	81.60639	3
Complement C1s subcomponent	413.1069	78.17438	13
Complement C2	395.04	84.58283	11
Complement C3	634.4669	188.5695	14
Complement C4 (Fragments)	224.39	103.0177	8
Complement C4-B	2791.188	194.2121	50
Complement C5	544.04	189.8968	19
Complement component C6	414.95	108.3674	11
Complement component C7	465.2669	96.65049	12
Complement component C7	323.8869	96.65671	9
Complement component C8 alpha chain	95.53	66.83168	2
Complement component C8 beta chain	527.25	68.71412	12
Complement component C8 gamma chain	157.36	22.43461	4
Complement component C9	513.9269	64.61526	11
Complement factor B O	1115.544	86.84704	20
Complement factor H	608.8969	143.6805	20
Complement factor H-related protein 1	184.29	38.76639	4
Complement factor H-related protein 3	62.3	38.4962	2
Complement factor I	298.7569	68.0715	8
Corticosteroid-binding globulin	124.26	45.28297	4
Fibrinogen alpha chain	259.72	95.65569	6
Fibronectin	1166.434	266.0344	27
Ficolin-3	226.39	33.39518	7
Gelsolin	1494.33	86.04334	23
Hemopexin	1016.85	52.38455	27
Heparin cofactor 2	731.4438	57.20527	13
Hepatocyte growth factor activator	43.54	72.85992	2
Histidine-rich glycoprotein	745.7269	60.51023	14
Hyaluronan-binding protein 2	57.91	64.74024	1
Insulin-like growth factor-binding protein complex acid labile subunit	298.9038	66.73507	10
Inter-alpha-trypsin inhibitor heavy chain H1	1922.777	101.7818	27
Inter-alpha-trypsin inhibitor heavy chain H2	1552.487	106.8528	28
Inter-alpha-trypsin inhibitor heavy chain H3	744.6169	100.0716	14
Inter-alpha-trypsin inhibitor heavy chain H4	1390.27	103.5211	28
Kallistatin	107.67	48.68222	3
Keratin, type I cytoskeletal 16	90.90691	51.57833	4
Keratin, type II cytoskeletal 1	182.9338	66.17007	7
Keratin, type II cytoskeletal 6B	146.0269	60.3154	6
Kininogen-1	1075.19	72.99556	12
Lumican	221.8607	38.74692	7
Mannan-binding lectin serine protease 2	54.55	77.22422	2
N-acetylmuramoyl-L-alanine amidase	210.1769	62.7481	7
Pigment epithelium-derived factor	399.53	46.48436	9
Plasma kallikrein	83	73.43264	3

Plasma protease C1 inhibitor	120.03	55.34748	8
Plasminogen	469.9638	93.24719	13
Platelet basic protein	204.68	14.17052	3
Platelet factor 4 variant	57.64	11.77337	1
Platelet glycoprotein V	36.65	61.43388	1
POTE ankyrin domain family member E	48.32	122.8823	4
Protein AMBP	163.21	39.88632	5
Prothrombin	291.5469	71.47468	7
Putative trypsin-6	41.78	27.0924	3
Serum amyloid P-component	605.66	25.48517	8
Sex hormone-binding globulin	191.29	43.9799	7
Tetranectin	97.54	22.95143	2
Thrombospondin-1	373.6669	133.2911	11
Thrombospondin-2	83.43	133.7852	3
Vitamin D-binding protein	521.13	54.52563	13
Vitamin K-dependent protein S	114.4	77.12671	5
Vitronectin	408.36	55.06947	9
Zinc-alpha-2-glycoprotein	381.42	34.46519	11

Patient 8 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	60.87	0	4
unknown	56.11	0	3
unknown	52.81	0	3
unknown	52.75	0	2
unknown	52.52691	0	3
unknown	50.70691	0	5
unknown	49.95	0	3
unknown	48.28	0	3
unknown	47.78691	0	2
unknown	47.68	0	2
unknown	37.6	0	3
unknown	36.49	0	1
unknown	33.15	0	2
unknown	32.19	0	1
unknown	31.75	0	2
Afamin	311.1669	69.02401	9
Alpha-1-antichymotrypsin	865.68	47.62054	18
Alpha-1B-glycoprotein	795.38	54.23858	14
Alpha-2-antiplasmin	143.3469	54.53107	4
Alpha-2-HS-glycoprotein	334.76	39.29971	7
Alpha-2-macroglobulin	76.3	163.1889	5
Angiotensinogen	381.02	53.12051	7
Ankyrin repeat and SOCS box protein 18	51.09	50.77091	1
Antithrombin-III	187.5969	52.56886	8
Apolipoprotein A-I	277.3669	30.75893	10
Apolipoprotein A-IV	1387.23	45.37147	25
Apolipoprotein B-100	2226.595	515.2409	63
Apolipoprotein C-I	63.14	9.32609	2
Apolipoprotein C-III	103.6	10.8455	2
Apolipoprotein E	196.26	36.13175	10
ATP-binding cassette sub-family A member 12	45.14	293.0488	4
Beta-2-glycoprotein 1	188.79	38.27266	6
Calcium-transporting ATPase type 2C member 2	34.8	103.1209	1

Carboxypeptidase N catalytic chain	106.17	52.25331	4
Carboxypeptidase N subunit 2	145.93	60.57615	3
Ceruloplasmin O	2693.634	122.1276	45
Clusterin	217.1369	52.46101	7
Coagulation factor X	145.05	54.69651	4
Coiled-coil and C2 domain-containing protein 1A	40.18	103.9983	3
Complement C1q subcomponent subunit B	202.27	26.44241	5
Complement C1q subcomponent subunit C	163.9069	25.75714	5
Complement C1r subcomponent	135.91	80.06681	6
Complement C1r subcomponent-like protein	44.76	53.46434	1
Complement C1s subcomponent	298.6969	76.6348	8
Complement C2	507.5769	83.21431	13
Complement C3	361.82	187.0299	8
Complement C4-B	2506.131	192.6725	46
Complement C5	295.43	188.1861	11
Complement component C6	213.84	104.718	6
Complement component C7	325.85	93.45729	7
Complement component C8 alpha chain	89.32	65.12104	6
Complement component C8 beta chain	354.62	67.00347	17
Complement component C8 gamma chain	209.97	22.26354	4
Complement component C9	503.04	63.1327	10
Complement factor B	719.5207	85.47852	18
Complement factor D	58.37	27.01586	2
Complement factor H	685.0569	139.0047	20
Complement factor H-related protein 1	184.71	37.62596	4
Complement factor H-related protein 2	92.61	30.63063	2
Complement factor I	123.4969	65.6766	4
Corticosteroid-binding globulin	144.37	45.11191	4
Dynein heavy chain 10, axonemal	56.88481	514.484	7
FERM domain-containing protein 4A	77.05	115.3873	8
Fibrinogen alpha chain	150.7	94.91441	6
Fibronectin OS=Homo sapiens	771.2248	262.4421	21
Ficolin-3	109.78	32.88199	3
Gelsolin	885.97	85.64419	12
Glutathione peroxidase 3	45.94	25.5369	4
Hemopexin	1112.78	51.64327	26
Heparin cofactor 2	737.7338	57.0342	16
Hepatocyte growth factor activator	54.14691	70.63609	3
Histidine-rich glycoprotein	245.44	59.54087	5
Insulin-like growth factor-binding protein complex acid labile subunit	394.8838	65.9938	11
Inter-alpha-trypsin inhibitor heavy chain H1	1421.844	101.3256	21
Inter-alpha-trypsin inhibitor heavy chain H2	1078.75	106.3966	20
Inter-alpha-trypsin inhibitor heavy chain H3	379.1769	99.78653	11
Inter-alpha-trypsin inhibitor heavy chain H4	911.2469	103.293	19
Intercellular adhesion molecule 3	31.22	59.50283	2
Kallistatin	152.47	48.51116	5
Keratin, type I cytoskeletal 10	277.64	58.79169	10
Keratin, type I cytoskeletal 28	67.88	50.53592	3
Keratin, type I cytoskeletal 9	310.3469	62.02681	10
Keratin, type II cuticular Hb4	59.76	64.85455	3
Keratin, type II cytoskeletal 1	686.8576	65.999	13
Keratin, type II cytoskeletal 2 epidermal	497.9938	65.39322	11
Keratin, type II cytoskeletal 2 oral	62.68	65.8301	4
Keratin, type II cytoskeletal 3	94.39	64.46459	5
Keratin, type II cytoskeletal 6B	163.9069	60.03029	6
Keratin, type II cytoskeletal 7	123.26	51.37335	4
Keratin, type II cytoskeletal 72	62.64691	55.84244	4

Keratin, type II cytoskeletal 73	50.04	58.88674	3
Keratin, type II cytoskeletal 74	64.68	57.82964	5
Keratin, type II cytoskeletal 75	106.06	59.52403	5
Keratin, type II cytoskeletal 79	94.88	57.77412	4
Keratin, type II cytoskeletal 8	119.89	53.67113	4
Kininogen-1	812.34	71.91215	11
Leucine-rich alpha-2-glycoprotein	209.28	38.15411	4
Lumican	227.8869	38.40479	5
Lymphoid-restricted membrane protein	36.09	62.06908	1
Mannan-binding lectin serine protease 2	40.1	75.68464	1
N-acetylmuramoyl-L-alanine amidase	166.55	62.17788	6
Pigment epithelium-derived factor	320.29	46.3133	7
Plasma kallikrein	48.63691	71.32284	2
Plasma protease C1 inhibitor	257.89	55.11939	7
Plasminogen	534.0269	90.51016	16
Platelet basic protein	213.65	13.88542	5
Protein AMBP	167.6	38.97398	7
Prothrombin	213.8969	69.99212	8
Putative trypsin-6	78.68	26.52219	2
Serum amyloid P-component	398.1	25.37113	7
Sex hormone-binding globulin	92.43	43.75182	4
Spermatogenesis-associated protein 7	63.26	67.67673	2
Thrombospondin-1	167.81	129.2996	6
Thyroxine-binding globulin	48.06	46.29461	1
Uncharacterised protein C10orf67	31.13691	21.44912	4
Uncharacterised protein C10orf92	32.52	95.43611	1
Vitamin D-binding protein	633.2	52.92903	13
Vitamin K-dependent protein S	77.84	75.07393	4
Vitronectin	330.36	54.27117	5
Zinc finger protein 791	45.77691	66.82764	3
Zinc-alpha-2-glycoprotein	235.64	34.2371	8

Patient 9 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	79.684439	0	13
unknown	54.75	0	4
unknown	52.88	0	4
unknown	49.38	0	5
unknown	46.29	0	3
unknown	41.45	0	3
unknown	39.2	0	1
unknown	38.23	0	1
unknown	38.036909	0	3
unknown	36.48	0	2
unknown	35.696909	0	4
unknown	35.04	0	2
unknown	34.64	0	1
unknown	34.25	0	1
unknown	33.88	0	1
unknown	30.51	0	1
Afamin	230.03691	70.96274	8
Alpha-1-antichymotrypsin	1206.89	47.7916	24
Alpha-1B-glycoprotein	936.92	54.8088	15
Alpha-2-antiplasmin	306.44691	54.87319	8

Alpha-2-HS-glycoprotein	330.72	40.09801	7
Alpha-2-macroglobulin	376.89	164.61441	17
Angiotensinogen	230.61	53.40562	10
Antithrombin-III	900.39691	53.02504	18
Apolipoprotein A-I	593.99691	30.75893	19
Apolipoprotein A-IV	1320.77	45.37147	30
Apolipoprotein B-100	6769.0779	516.66639	133
Apolipoprotein C-III	210.47	10.8455	2
Apolipoprotein E	469.24691	36.2458	12
ATP synthase subunit alpha, mitochondrial	65.56	59.82764	5
ATP-binding cassette sub-family A member 12	80.716909	295.38672	10
Beta-2-glycoprotein 1	151.54	39.58415	5
Beta-Ala-His dipeptidase	95.97	56.77015	5
Carboxypeptidase B2	262.25	48.95162	8
Carboxypeptidase N subunit 2	256.24	61.43147	7
Cartilage acidic protein 1	87.17	72.17411	5
Ceruloplasmin	2846.6548	122.98291	57
Chordin	46.58	104.70335	4
Clusterin	245.37691	53.03122	6
Coagulation factor X	139.06	56.06503	6
Coagulation factor XII	53.85	70.05477	3
Complement C1q subcomponent subunit A	163.98	26.28529	3
Complement C1q subcomponent subunit B	232.86	26.67049	6
Complement C1q subcomponent subunit C	223.89691	25.98522	3
Complement C1r subcomponent	195.98	81.60639	7
Complement C1r subcomponent-like protein	39.43	54.20562	2
Complement C1s subcomponent	389.44691	78.17438	12
Complement C2	492.07691	84.58283	14
Complement C3	1719.1284	188.56945	44
Complement C4-B	3350.0753	194.21212	73
Complement C5	1115.4369	189.89678	32
Complement component C6	322.17	108.36738	10
Complement component C7	410.93691	96.65049	11
Complement component C8 beta chain	484.76	68.71412	16
Complement component C8 gamma chain	235.13	22.43461	7
Complement component C9	491.16691	64.61526	14
Complement factor B	826.85691	86.84704	20
Complement factor H	747.27691	143.68046	24
Complement factor H-related protein 1	170.26	38.76639	4
Complement factor H-related protein 2	102.63	31.54298	5
Complement factor H-related protein 3	57.52	38.4962	2
Complement factor I	255.94382	68.0715	9
Condensin-2 complex subunit D3	41.166909	170.95017	4
Cyclin N-terminal domain-containing protein 1	32.71	37.2395	1
Fibrinogen alpha chain	229.21691	95.65569	9
Fibronectin	1099.8945	266.03443	30
Ficolin-3	149.67	33.39518	6
Gelsolin	899.29691	86.04334	19
Glial fibrillary acidic protein	66.42	49.90667	5
Glutathione peroxidase 3	67.4	25.76499	3
Haptoglobin	124.01	45.86082	4
Hemopexin	925.54	52.38455	30
Heparin cofactor 2	779.38382	57.20527	16
Hepatocyte growth factor activator	40.67	72.85992	1
Histidine-rich glycoprotein OS=Homo sapiens GN=HRG PE=1 SV=1	453.02	60.51023	14
Insulin-like growth factor-binding protein complex acid labile subunit	496.75481	66.73507	18
Inter-alpha-trypsin inhibitor heavy chain H1	1586.2607	101.78179	30

Inter-alpha-trypsin inhibitor heavy chain H2	1576.55	106.85278	38
Inter-alpha-trypsin inhibitor heavy chain H3	266.41691	100.07164	11
Inter-alpha-trypsin inhibitor heavy chain H4	1599.8969	103.52107	41
Kallistatin	324.52	48.68222	12
Keratin, type I cytoskeletal 10	144.78	59.01978	7
Keratin, type I cytoskeletal 9	67.45	62.2549	7
Keratin, type II cytoskeletal 1	336.90073	66.17007	9
Keratin, type II cytoskeletal 2 epidermal	65.046909	65.67832	3
Keratin, type II cytoskeletal 4	55.47	57.64898	4
Keratin, type II cytoskeletal 7	63.926909	51.43037	4
Keratin, type II cytoskeletal 8	85.91	53.67113	7
Keratin, type II cytoskeletal 80	58.69	51.00697	2
Kininogen-1	964.62	72.99556	10
Leucine-rich alpha-2-glycoprotein	371	38.3822	10
Lumican	231.42691	38.74692	5
Lymphoid-restricted membrane protein	41.51	62.75334	4
Myosin-XV	145.21764	397.43458	18
N-acetylmuramoyl-L-alanine amidase	153.41691	62.7481	7
Neuropilin-1	39.22	104.32307	4
Pigment epithelium-derived factor	222.48	46.48436	5
Plasma kallikrein	53.72	73.43264	2
Plasma protease C1 inhibitor	418.96	55.34748	15
Plasminogen	385.16382	93.24719	11
Platelet basic protein	199.56	14.17052	5
Protein AMBP	166.82	39.88632	5
Protein FAM102A	62.26	42.2151	3
Prothrombin	257.02	71.47468	7
Putative trypsin-6	70.16	27.0924	3
Retinol-binding protein 4	97.48	23.33739	2
Serum amyloid P-component	558.05	25.48517	11
Sex hormone-binding globulin	101.83	43.9799	5
Spectrin beta chain, brain 3	86.700995	290.00505	10
Tetranectin	207.9	22.95143	5
Threonyl-tRNA synthetase, mitochondrial	73.79	81.84076	5
Thrombospondin-1	476.24691	133.29106	15
Thrombospondin-2	77.55	133.78523	5
Thyroxine-binding globulin	147.06382	46.63674	7
Trifunctional enzyme subunit alpha, mitochondrial	64.05	83.68815	7
Vitamin D-binding protein	458.86	54.52563	10
Vitamin K-dependent protein S	91.91	77.12671	6
Vitronectin	508.88	55.06947	14
Zinc-alpha-2-glycoprotein	295.84	34.46519	12

Patient 10 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	91.25691	0	13
unknown	61.15	0	6
unknown	53.27	0	3
unknown	52.56382	0	4
unknown	46.98691	0	5
unknown	46.7479	0	4
unknown	46.45	0	2
unknown	45.62691	0	4
unknown	43.43691	0	4

unknown	42.84	0	2
unknown	42.7	0	1
unknown	42.53691	0	3
unknown	38.27	0	1
unknown	36.33	0	2
unknown	36.17	0	2
unknown	33.06	0	1
unknown	32.62	0	1
unknown	31.92691	0	2
Afamin	157.8569	70.96274	7
Alpha-1-antichymotrypsin	817.4	47.7916	14
Alpha-1-antitrypsin	98.46	46.87808	3
Alpha-1B-glycoprotein	522.68	54.8088	9
Alpha-2-antiplasmin	211.9569	54.87319	8
Alpha-2-HS-glycoprotein	286.81	40.09801	6
Alpha-2-macroglobulin	527.61	164.6144	16
Amyloid-like protein 1	31.72	72.81544	1
Angiotensinogen	287.36	53.40562	6
Antithrombin-III	443.6469	53.02504	13
Apolipoprotein A-I	303.4069	30.75893	12
Apolipoprotein A-IV	1554.28	45.37147	30
Apolipoprotein B-100	2539.289	516.6664	70
Apolipoprotein C-III	214.34	10.8455	2
Apolipoprotein E	195.6669	36.2458	10
Beta-2-glycoprotein 1	101.5	39.58415	2
Biotinidase	34.02	62.0056	1
Carboxypeptidase B2	154.16	48.95162	3
Carboxypeptidase N subunit 2	136.28	61.43147	4
CDC45-related protein	40.51	66.21084	2
Ceruloplasmin	2487.188	122.9829	43
Clusterin	185.6669	53.03122	6
Coagulation factor X	117.6	56.06503	4
Complement C1q subcomponent subunit B	188.63	26.67049	4
Complement C1q subcomponent subunit C	275.0869	25.98522	5
Complement C1r subcomponent	148.13	81.60639	7
Complement C1s subcomponent	378.8969	78.17438	11
Complement C2	342.2338	84.58283	15
Complement C3	532.1607	188.5695	14
Complement C4-B	2314.348	194.2121	47
Complement C5 OS=Homo sapiens GN=C5 PE=1 SV=4	418.5669	189.8968	15
Complement component C6	202.14	108.3674	8
Complement component C7	510.1838	96.65049	10
Complement component C8 alpha chain	65.09	66.83168	1
Complement component C8 beta chain	280.01	68.71412	8
Complement component C8 gamma chain	148.76	22.43461	3
Complement component C9	287.9469	64.61526	8
Complement factor B	1177.214	86.84704	24
Complement factor H	889.2369	143.6805	24
Complement factor H-related protein 1	192.16	38.76639	5
Complement factor I	204.6269	68.0715	6
Corticosteroid-binding globulin	66.75	45.28297	4
Dedicator of cytokinesis protein 11	63.99073	240.1422	8
Fibrinogen alpha chain	211.18	95.65569	8
Fibronectin	739.0338	266.0344	22
Ficolin-3	112.22	33.39518	3
Gelsolin	821.47	86.04334	18
Glutathione peroxidase 3	32.48	25.76499	2

Haptoglobin	149.0869	45.86082	8
Hemoglobin subunit alpha	45.13	15.30495	1
Hemopexin	1036.95	52.38455	23
Heparin cofactor 2	564.5738	57.20527	14
Hepatocyte growth factor activator	64.33	72.85992	4
Histidine-rich glycoprotein	560.57	60.51023	10
Insulin-like growth factor-binding protein complex acid labile subunit	165.2069	66.73507	6
Inter-alpha-trypsin inhibitor heavy chain H1	1457.164	101.7818	24
Inter-alpha-trypsin inhibitor heavy chain H2	1282.237	106.8528	23
Inter-alpha-trypsin inhibitor heavy chain H3	632.8869	100.0716	12
Inter-alpha-trypsin inhibitor heavy chain H4	1025.667	103.5211	23
Kallistatin	85.75	48.68222	4
Keratin, type I cytoskeletal 10	79.28	59.01978	5
Keratin, type II cytoskeletal 1	175.1807	66.17007	8
Kininogen-1	824.47	72.99556	10
Leucine-rich alpha-2-glycoprotein	63.85	38.3822	3
Lumican	178.3238	38.74692	5
Mannan-binding lectin serine protease 2	42.25	77.22422	1
N-acetylmuramoyl-L-alanine amidase	71.64	62.7481	4
Neuronal acetylcholine receptor subunit beta-2	31.69	57.78037	1
Osteopontin	96.42	35.5723	1
Phosphatidylinositol-glycan-specific phospholipase D	46.91	92.9052	4
Pigment epithelium-derived factor	238.3169	46.48436	10
Plasma kallikrein	44.99691	73.43264	3
Plasma protease C1 inhibitor	193.8	55.34748	7
Plasminogen	407.3269	93.24719	11
Platelet basic protein	224.34	14.17052	4
Protein AMBP	203.82	39.88632	4
Prothrombin	134.2269	71.47468	5
Protocadherin-12	36.13	129.5992	3
Serine/threonine-protein kinase haspin	39.39	89.6592	4
Serine/threonine-protein kinase LMTK2	42.03	165.9964	3
Serum albumin	72.56691	71.31725	3
Serum amyloid P-component	376.1	25.48517	7
Sialic acid-binding Ig-like lectin 10	47.28	77.45596	4
Spermatogenesis-associated protein 73	38.06	68.18992	2
Stabilin-1	62.91691	286.9265	4
Talin-1	53.77	271.7659	4
Talin-2	89.28	273.7813	8
Tetranectin	134.14	22.95143	2
Thrombospondin-1	160.8138	133.2911	9
TRIO and F-actin-binding protein	67.15382	264.125	10
Vitamin D-binding protein	502.66	54.52563	12
Vitamin K-dependent protein S	102.91	77.12671	7
Vitronectin	530.97	55.06947	10
Zinc-alpha-2-glycoprotein	140.78	34.46519	6

Patient 11 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
unkown	51.01	0	4
unkown	50.12	0	4
unkown	46.74	0	3
unkown	38.97	0	2
unkown	35.8	0	1

unkown	35.75	0	1
unkown	32.65	0	1
Antithrombin-III	241.94	53.02504	5
Apolipoprotein A-I	207.937	30.75893	8
Apolipoprotein A-IV	1323.27	45.37147	29
Apolipoprotein B-100	2575.83	516.66639	65
Apolipoprotein C-I	73.83	9.32609	3
Apolipoprotein C-III	162.87	10.8455	2
Apolipoprotein E	418.807	36.2458	15
Beta-2-glycoprotein 1	123.84	39.58415	4
Beta-Ala-His dipeptidase	202	56.77015	7
Biotinidase	102.987	62.0056	4
Carboxypeptidase B2	142.9	48.95162	5
Carboxypeptidase N catalytic chain	137.23	52.53842	4
Carboxypeptidase N subunit 2	155.81	61.43147	4
Ceruloplasmin	2150.24	122.98291	44
Clusterin	149.047	53.03122	6
Coagulation factor IX	43.8	53.11351	3
Coagulation factor V	49.1169	252.65397	3
Coagulation factor X	125.28	56.06503	2
Complement C1q subcomponent subunit A	174.96	26.28529	2
Complement C1q subcomponent subunit B	217.12	26.67049	6
Complement C1q subcomponent subunit C	328.247	25.98522	7
Complement C1r subcomponent	125.37	81.60639	7
Complement C1r subcomponent-like protein	44.03	54.20562	2
Complement C1s subcomponent	357.227	78.17438	11
Complement C2	286.657	84.58283	10
Complement C3	373.891	188.56945	12
Complement C4-B	2351.45	194.21212	50
Complement C5	346.627	189.89678	15
Complement component C6	174.32	108.36738	8
Complement component C7	264.537	96.65049	5
Complement component C8 beta chain	386.04	68.71412	13
Complement component C8 gamma chain	132.32	22.43461	3
Complement component C9	379.84	64.61526	10
Complement factor B	910.924	86.84704	17
Complement factor H	790.727	143.68046	22
Complement factor H-related protein 1	180.43	38.76639	5
Complement factor H-related protein 3	60.72	38.4962	2
Complement factor I	173.497	68.0715	8
Corticosteroid-binding globulin	134.95	45.28297	4
Fibrinogen alpha chain	166.25	95.65569	4
Fibronectin	1140.39	266.03443	30
Ficolin-3	268.97	33.39518	8
Gelsolin	838.44	86.04334	16
Glutathione peroxidase 3	60.88	25.76499	5
Hemopexin	998.69	52.38455	31
Heparin cofactor 2	419.957	57.20527	12
Hepatocyte growth factor activator	49.83	72.85992	4
Histidine-rich glycoprotein	514.48	60.51023	13
Insulin-like growth factor-binding protein complex acid labile subunit	501.274	66.73507	12
Inter-alpha-trypsin inhibitor heavy chain H1	1766.39	101.78179	29
Inter-alpha-trypsin inhibitor heavy chain H2	1457.41	106.85278	33
Inter-alpha-trypsin inhibitor heavy chain H3	572.034	100.07164	11
Inter-alpha-trypsin inhibitor heavy chain H4	1109.83	103.52107	25
Kallistatin	158.43	48.68222	3
Kininogen-1	565.73	72.99556	13

Leucine-rich alpha-2-glycoprotein	146.81	38.3822	4
Lumican	147.044	38.74692	4
Mannan-binding lectin serine protease 2	39.71	77.22422	2
Mitogen-activated protein kinase 3	35.31	43.45024	1
Myelin transcription factor 1	45.7169	123.90716	3
N-acetylmuramoyl-L-alanine amidase	93.9	62.7481	4
Nebulin	108.501	775.41942	20
Neuropilin-1	52.7	104.32307	3
Pigment epithelium-derived factor	440.49	46.48436	11
Plasma kallikrein	41.97	73.43264	2
Plasminogen	459.194	93.24719	12
Platelet basic protein	150.81	14.17052	3
Platelet glycoprotein V	47.81	61.43388	2
Protein AMBP	176.31	39.88632	5
Protein deltex-3	39.29	38.64782	2
Prothrombin	129.847	71.47468	5
Putative trypsin-6	78.9	27.0924	3
Serum amyloid P-component	496.12	25.48517	8
Tetranectin	45.75	22.95143	2
Thrombospondin-1	340.834	133.29106	15
Titin	151.285	3843.1187	48
Uncharacterised protein C10orf92	43.5669	96.51952	3
Vitamin D-binding protein	413.34	54.52563	10
Vitamin K-dependent protein S	64.24	77.12671	3
Vitronectin	384.32	55.06947	8
Zinc-alpha-2-glycoprotein	213.15	34.46519	9

Patient 12 (nonaggressive)

Protein	Score	MW [kDa]	# Pept.
Alpha-1-antichymotrypsin	710.29	47.7916	12
Alpha-1B-glycoprotein	532.21	54.8088	11
Alpha-2-antiplasmin	95.6969	54.8732	3
Alpha-2-HS-glycoprotein	254.09	40.098	6
Angiotensinogen	136.63	53.4056	4
Apolipoprotein A-IV	810.87	45.3715	22
Apolipoprotein B-100	1653.14	516.666	46
Apolipoprotein C-III	193.03	10.8455	2
Apolipoprotein E	231.837	36.2458	10
Beta-2-glycoprotein 1	35.84	39.5842	1
Beta-Ala-His dipeptidase	79.42	56.7702	4
Carboxypeptidase B2	202.56	48.9516	4
Carboxypeptidase N subunit 2	108.53	61.4315	2
Cartilage acidic protein 1	46.92	72.1741	3
Centrosomal protein of 290 kDa	121.049	290.892	12
Ceruloplasmin	1765.08	122.983	27
Clusterin	223.877	53.0312	6
Coagulation factor X	108.98	56.065	3
Coagulation factor XIII A chain	68.73	83.7278	2
Complement C1q subcomponent subunit B	134.05	26.6705	4
Complement C1q subcomponent subunit C]	198.547	25.9852	3
Complement C1s subcomponent	132.687	78.1744	5
Complement C2	115.71	84.5828	6
Complement C3	246.314	188.569	9
Complement C4-A	1238.15	194.247	31

Complement C5	134.02	189.897	6
Complement component C6	90.13	108.367	4
Complement component C7	122.784	96.6505	5
Complement component C8	343.95	68.7141	8
Complement component C9	242.027	64.6153	7
Complement factor B	578.677	86.847	17
Complement factor H	374.957	143.68	12
Complement factor H-related protein 1	142.9	38.7664	3
Complement factor H-related protein 3	35.02	38.4962	2
Complement factor I	107.527	68.0715	4
FERM domain-containing protein 4A	37.18	115.958	1
Fibrinogen alpha chain	108.03	95.6557	4
Fibronectin	874.884	266.034	22
Ficolin-3	115.4	33.3952	4
Gelsolin	650.43	86.0433	14
Haptoglobin	72.15	45.8608	2
Hemopexin	650.66	52.3846	16
Heparin cofactor 2	375.987	57.2053	11
Histidine-rich glycoprotein	198.12	60.5102	5
Insulin-like growth factor-binding protein complex acid labile subunit	196.014	66.7351	9
Inter-alpha-trypsin inhibitor heavy chain H1	1066.11	101.782	18
Inter-alpha-trypsin inhibitor heavy chain H2	868.87	106.853	18
Inter-alpha-trypsin inhibitor heavy chain H3	391.547	100.072	8
Inter-alpha-trypsin inhibitor heavy chain H4	730.52	103.521	16
Kallistatin	46.41	48.6822	3
Kininogen-1	671.28	72.9956	12
Lumican	54.58	38.7469	1
Lymphoid-restricted membrane protein	30.78	62.7533	1
Mannan-binding lectin serine protease 2	44.05	77.2242	1
N-acetylmuramoyl-L-alanine amidase	53.9	62.7481	3
Pigment epithelium-derived factor	170.91	46.4844	6
Plasma kallikrein	42.93	73.4326	2
Plasminogen	328.077	93.2472	10
Platelet basic protein	159.71	14.1705	3
Protein AMBP	68.6	39.8863	4
Prothrombin	152.15	71.4747	5
Serine/threonine-protein kinase LMTK2	38.45	165.996	2
Serum amyloid P-component	353.23	25.4852	7
Spermatogenesis-associated protein 7	61.38	68.1899	2
Structural maintenance of chromosomes protein 3	53.3158	141.853	6
Tetranectin	122.29	22.9514	2
Thrombospondin-1	179.31	133.291	8
Trypsin-1	41.76	27.1113	2
Vitamin D-binding protein	280.83	54.5256	8
Vitronectin	245.94	55.0695	6
Zinc-alpha-2-glycoprotein	85.11	34.4652	3

Patient 13 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	45.0379	0	3
unknown	41.38	0	1
Afamin	465.7569	70.9627	12
Alpha-1-acid glycoprotein 1	52.67	23.7249	2
Alpha-1-antichymotrypsin	877.55	47.7916	15

Alpha-1B-glycoprotein	915.39	54.8088	14
Alpha-2-antiplasmin	208.3969	54.8732	7
Alpha-2-HS-glycoprotein	308.55	40.098	6
Angiotensinogen	367.22	53.4056	8
Apolipoprotein A-I	431.2569	30.7589	14
Apolipoprotein A-IV	1593.1	45.3715	30
Apolipoprotein B-100	4825.461	516.666	104
Apolipoprotein C-III	243.77	10.8455	2
Apolipoprotein E	447.7369	36.2458	14
Beta-2-glycoprotein 1	91.96	39.5842	2
Beta-Ala-His dipeptidase	160.24	56.7702	6
Carboxypeptidase B2	209.69	48.9516	5
Carboxypeptidase N subunit 2	203.56	61.4315	5
Ceruloplasmin	2281.268	122.983	39
Clusterin	461.1969	53.0312	8
Coagulation factor IX	59.2	53.1135	2
Coagulation factor V	117.31	252.654	6
Coagulation factor X	106.04	56.065	3
Coagulation factor XII	75.43	70.0548	2
Complement C1q subcomponent subunit A	143.7	26.2853	2
Complement C1q subcomponent subunit B	152.83	26.6705	3
Complement C1q subcomponent subunit C	271.5569	25.9852	5
Complement C1r subcomponent	233.56	81.6064	7
Complement C1s subcomponent	414.6469	78.1744	10
Complement C2	421.3969	84.5828	13
Complement C3	749.0438	188.569	23
Complement C4-B	2854.281	194.212	53
Complement C5	610.42	189.897	21
Complement component C6	324.77	108.367	8
Complement component C7	232.3669	96.6505	5
Complement component C8 alpha chain	68.37691	66.8317	3
Complement component C8 beta chain	378.31	68.7141	10
Complement component C8 gamma chain	154.19	22.4346	3
Complement component C9	332.4869	64.6153	9
Complement factor B	996.7569	86.847	21
Complement factor H	984.4569	143.68	24
Complement factor H-related protein 1	178.74	38.7664	5
Complement factor H-related protein 3	73.8	38.4962	2
Complement factor I	218.0769	68.0715	6
Fibrinogen alpha chain	211.39	95.6557	6
Fibronectin	1916.985	266.034	40
Ficolin-3	137.81	33.3952	5
Gelsolin	922.2469	86.0433	18
Haptoglobin	64.84	39.2407	3
Hemopexin	999.61	52.3846	24
Heparin cofactor 2	661.0338	57.2053	18
Hepatocyte growth factor activator	95.2	72.8599	3
Histidine-rich glycoprotein	445.95	60.5102	7
Hyaluronan-binding protein 2	62.44	64.7402	2
Insulin-like growth factor-binding protein complex acid labile subunit	653.3248	66.7351	16
Inter-alpha-trypsin inhibitor heavy chain H1	1759.954	101.782	26
Inter-alpha-trypsin inhibitor heavy chain H2	1537.587	106.853	31
Inter-alpha-trypsin inhibitor heavy chain H3	386.9369	100.072	7
Inter-alpha-trypsin inhibitor heavy chain H4	1234.207	103.521	28
Kallistatin	139.42	48.6822	5
Keratin, type II cytoskeletal 1	278.3876	66.1701	7
Keratin, type II cytoskeletal 2 epidermal	46.80691	65.6783	2

Kininogen-1	975.18	72.9956	13
Lumican	233.4469	38.7469	5
N-acetylmuramoyl-L-alanine amidase	152.1069	62.7481	5
Pigment epithelium-derived factor	368.5	46.4844	10
Plasma kallikrein	70.54691	73.4326	5
Plasma protease C1 inhibitor	171	55.3475	5
Plasminogen	435.1538	93.2472	11
Platelet basic protein	147.69	14.1705	3
Protein AMBP	189.36	39.8863	5
Prothrombin	275.0569	71.4747	9
Serine/threonine-protein kinase MARK1	42.07	89.4604	2
Serum amyloid P-component	497.6	25.4852	7
Tetranectin	123.45	22.9514	2
Thrombospondin-1	466.9369	133.291	12
Thrombospondin-2	68.57	133.785	2
Thyroxine-binding globulin	57.62691	46.6367	2
Trypsin-1	61.76	27.1113	1
Vitamin D-binding protein	608.3	54.5256	11
Vitamin K-dependent protein S	74.34	77.1267	5
Vitronectin	531.09	55.0695	11
Zinc-alpha-2-glycoprotein	304.95	34.4652	11

Patient 14(aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	56.923818	0	7
unknown	38.17	0	3
unknown	33.73	0	1
unknown	32.33	0	2
unknown	31.35	0	1
Afamin	134.17691	70.96274	5
Alpha-1-antichymotrypsin	613.83	47.7916	15
Alpha-1B-glycoprotein	676.09	54.8088	14
Alpha-2-antiplasmin	67.826909	54.87319	4
Alpha-2-HS-glycoprotein	285.77	40.09801	7
Alpha-2-macroglobulin	327.46	164.6144	12
Angiotensinogen	143.5	53.40562	3
Apolipoprotein A-I	710.24	30.75893	19
Apolipoprotein A-IV	1168.06	45.37147	28
Apolipoprotein B-100	3974.8199	516.6664	100
Apolipoprotein C-III	220.57	10.8455	2
Apolipoprotein E	222.33691	36.2458	10
Beta-2-glycoprotein 1	93	39.58415	2
Biotinidase	55.23	62.0056	3
Carboxypeptidase B2	84.6	48.95162	3
Carboxypeptidase N subunit 2	134.18	61.43147	3
Ceruloplasmin	1788.8238	122.9829	31
Clusterin	133.52	53.03122	4
Coagulation factor X	72.4	56.06503	2
Complement C1q subcomponent subunit A	64.78	26.28529	4
Complement C1q subcomponent subunit B	124.31	26.67049	6
Complement C1q subcomponent subunit C	188.56691	25.98522	3
Complement C1r subcomponent	124.56	81.60639	4
Complement C1s subcomponent	244.67691	78.17438	11
Complement C2	173.98	84.58283	5

Complement C3	521.72382	188.5695	19
Complement C4-B	1602.1176	194.2121	45
Complement C5	301.37	189.8968	15
Complement component C6	174.08	108.3674	7
Complement component C7	219.13382	96.65049	7
Complement component C8 alpha chain	29.35	66.83168	1
Complement component C8 beta chain	192.74	68.71412	6
Complement component C9	164.36	64.61526	7
Complement factor B	426.75382	86.84704	12
Complement factor H	445.08691	143.6805	18
Complement factor H-related protein 1	55.67	38.76639	5
Complement factor H-related protein 3	44.35	38.4962	2
Corticosteroid-binding globulin	82.13	45.28297	5
Fibrinogen alpha chain	78.43	95.65569	6
Fibronectin	493.35382	266.0344	15
Ficolin-3	98.95	33.39518	4
Gelsolin	753.15691	86.04334	16
Haptoglobin	53.3	45.86082	3
Hemoglobin subunit alpha	46.24	15.30495	1
Hemopexin	725.27	52.38455	19
Heparin cofactor 2	271.97	57.20527	9
Hepatocyte growth factor activator	53.45	72.85992	2
Histidine-rich glycoprotein	283.57	60.51023	7
Insulin-like growth factor-binding protein complex acid labile subunit	291.83382	66.73507	11
Inter-alpha-trypsin inhibitor heavy chain H1	1427.1138	101.7818	24
Inter-alpha-trypsin inhibitor heavy chain H2	1009.31	106.8528	21
Inter-alpha-trypsin inhibitor heavy chain H3	211.20691	100.0716	7
Inter-alpha-trypsin inhibitor heavy chain H4	754.59	103.5211	22
Keratin, type I cytoskeletal 10	92.66	59.01978	5
Keratin, type I cytoskeletal 28	39.73	51.16315	2
Kininogen-1	625.73	72.99556	11
Leucine-rich alpha-2-glycoprotein	100.65	38.3822	3
Lumican	86.22	38.74692	2
Myb-binding protein 1A	40.34	149.731	2
N-acetylmuramoyl-L-alanine amidase	76.356909	62.7481	4
Pigment epithelium-derived factor	215.59	46.48436	8
Plasma kallikrein	43.31	73.43264	1
Plasma protease C1 inhibitor	146.88	55.34748	8
Plasminogen	231.88	93.24719	8
Probable tRNA (uracil-O(2)-)-methyltransferase	36.62	85.9441	2
Protein AMBP	105.72	39.88632	3
Prothrombin	92.036909	71.47468	6
Serum amyloid P-component	212.29	25.48517	6
Serum paraoxonase/arylesterase 1	55.83	39.89525	2
Sex hormone-binding globulin	46.47	43.9799	3
Tetranectin	66.23	22.95143	2
Thrombospondin-1	46.58	133.2911	6
Vitamin D-binding protein	382.25	54.52563	10
Vitamin K-dependent protein S	47.75	77.12671	5
Vitronectin	235.57	55.06947	8
Zinc-alpha-2-glycoprotein	73.05	34.46519	4

Patient 15 (aggressive)

Protein	Score	MW [kDa]	# Pept.
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Afamin	242.0669	70.96274	8
Alpha-1-antichymotrypsin	906.58	47.7916	16
Alpha-1B-glycoprotein	924.86	54.8088	13
Alpha-2-antiplasmin	165.3669	54.87319	5
Alpha-2-HS-glycoprotein	372.44	40.09801	7
Alpha-2-macroglobulin	316.13	164.6144	12
Angiotensinogen	293.42	53.40562	6
Apolipoprotein A-I	836.6069	30.75893	20
Apolipoprotein A-IV	1374.83	45.37147	26
Apolipoprotein B-100	6430.523	516.6664	124
Apolipoprotein C-III	249.5	10.8455	2
Apolipoprotein E	491.3769	36.2458	14
Beta-2-glycoprotein 1	135.54	39.58415	3
Beta-Ala-His dipeptidase	177.75	56.77015	7
Biotinidase	80.43	62.0056	2
Carboxypeptidase B2	211.37	48.95162	7
Carboxypeptidase N subunit 2	204.18	61.43147	6
Ceruloplasmin	2723.865	122.9829	39
Clusterin	276.5769	53.03122	6
Coagulation factor V	96.04	252.654	6
Coagulation factor X	101.61	56.06503	3
Complement C1q subcomponent subunit A	146.22	26.28529	1
Complement C1q subcomponent subunit B	182.28	26.67049	4
Complement C1q subcomponent subunit C	272.0669	25.98522	5
Complement C1r subcomponent	321.46	81.60639	7
Complement C1s subcomponent	314.7769	78.17438	11
Complement C2	403.1969	84.58283	9
Complement C3	640.0838	188.5695	18
Complement C4-B	3464.688	194.2121	55
Complement C5	742.2569	189.8968	26
Complement component C6	253.08	108.3674	7
Complement component C7	294.4969	96.65049	6
Complement component C8 alpha chain	74.44	66.83168	2
Complement component C8 beta chain	398.19	68.71412	11
Complement component C8 gamma chain	148.76	22.43461	2
Complement component C9	309.28	64.61526	9
Complement factor B	691.5238	86.84704	18
Complement factor D	58.77	27.52906	3
Complement factor H	892.5569	143.6805	23
Complement factor H-related protein 1	153.77	38.76639	5
Complement factor I	152.8969	68.0715	5
Corticosteroid-binding globulin	117.71	45.28297	3
Fibrinogen alpha chain	143.49	95.65569	6
Fibronectin	1390.188	266.0344	32
Ficolin-3	175.11	33.39518	5
Gelsolin	1118.337	86.04334	19
Glutathione peroxidase 3	41.15	25.76499	2
Hemopexin	1047.73	52.38455	24
Heparin cofactor 2	603.0669	57.20527	12
Hepatocyte growth factor activator	73.23	72.85992	2
Histidine-rich glycoprotein	684.53	60.51023	12
Insulin-like growth factor-binding protein complex acid labile subunit	496.8548	66.73507	15
Inter-alpha-trypsin inhibitor heavy chain H1	1613.397	101.7818	23
Inter-alpha-trypsin inhibitor heavy chain H2	1553.21	106.8528	28
Inter-alpha-trypsin inhibitor heavy chain H3	505.2969	100.0716	12
Inter-alpha-trypsin inhibitor heavy chain H4	1277.79	103.5211	25
Kallistatin	149.33	48.68222	6

Kininogen-1	863.37	72.99556	11
Leucine-rich alpha-2-glycoprotein	266.13	38.3822	4
Lumican	167.6069	38.74692	5
N-acetylmuramoyl-L-alanine amidase	166.7969	62.7481	6
Pigment epithelium-derived factor	283.94	46.48436	7
Plasma kallikrein	54.47	73.43264	1
Plasma protease C1 inhibitor	357.02	55.34748	12
Plasma serine protease inhibitor	104	45.78674	6
Plasminogen	403.5438	93.24719	12
Platelet basic protein	118.86	14.17052	3
Protein AMBP	155.22	39.88632	3
Prothrombin	252.3169	71.47468	8
RNA-binding motif protein, Y chromosome, family 1 member A1/C	39.94	56.09176	2
Serum amyloid P-component	534.55	25.48517	9
Serum paraoxonase/arylesterase 1	34.86	39.89525	1
Sex hormone-binding globulin	93.77	43.9799	5
Tetranectin	226.36	22.95143	4
THO complex subunit 2	69.70382	184.5411	5
Thrombospondin-1	259.2569	133.2911	11
Thrombospondin-2	77.33	133.7852	4
Trypsin-1	78.22	27.1113	1
Vitamin D-binding protein	464.41	54.52563	10
Vitamin K-dependent protein S	88.48	77.12671	6
Vitronectin	311.55	55.06947	6
Zinc-alpha-2-glycoprotein	196.99	34.46519	10

Patient 16 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	65.537904	0	4
unknown	59.5	0	5
unknown	57.436909	0	4
unknown	57.13	0	4
unknown	55.2	0	4
unknown	52.58	0	3
unknown	51.28	0	3
unknown	48.7	0	3
unknown	46.61	0	2
unknown	45.510995	0	6
unknown	44.62	0	2
unknown	41.91	0	2
unknown	38.95	0	2
unknown	38.27	0	2
unknown	37.056909	0	4
unknown	36.1	0	2
unknown	35.11	0	2
unknown	35.05	0	2
unknown	32.29	0	1
unknown	32.08	0	1
unknown	31.79	0	1
unknown	31.21	0	1
unknown	30.26	0	1
Afamin	376.17691	69.02401	10
Alpha-1-antichymotrypsin	1108.71	47.62054	21
Alpha-1B-glycoprotein	827.46	54.23858	18

Alpha-2-antiplasmin	232.81382	54.53107	8
Alpha-2-HS-glycoprotein	490.12	39.29971	8
Alpha-2-macroglobulin	431.99691	163.18888	14
Angiotensinogen	491.94	53.12051	10
Antithrombin-III	786.29691	52.56886	17
Apolipoprotein A-I	725.62	30.75893	17
Apolipoprotein A-IV	1664.49	45.37147	29
Apolipoprotein B-100	5972.4455	515.24085	119
Apolipoprotein C-I	55.88	9.32609	2
Apolipoprotein C-III	265.93	10.8455	3
Apolipoprotein E	532.06691	36.13175	16
Apolipoprotein M	39.58	21.23944	2
ATP-binding cassette sub-family F member 1	55.750995	95.86647	5
ATP-binding cassette sub-family G member 5	36.62	72.45688	2
Attractin	67.96	158.43246	4
Beta-2-glycoprotein 1	167.56	38.27266	5
Beta-Ala-His dipeptidase	138.64	56.65611	5
Biotinidase	50.86	61.09326	3
Calcium-transporting ATPase type 2C member 2	57.22	103.12085	4
Calpain-15	46.51	117.23917	3
Carboxypeptidase B2	212.90691	48.38141	5
Carboxypeptidase N catalytic chain	161.82	52.25331	8
Carboxypeptidase N subunit 2	218.65	60.57615	5
Ceruloplasmin	3250.0476	122.12759	48
Clusterin	451.52382	52.46101	9
Coagulation factor IX	73.99	51.745	3
Coagulation factor V	155.3	251.51354	9
Coagulation factor X	109.35	54.69651	4
Coagulation factor XII	63.74	67.77391	5
Complement C1q subcomponent subunit A	211.17	26.00019	2
Complement C1q subcomponent subunit B	228.63	26.44241	4
Complement C1q subcomponent subunit C	274.99691	25.75714	6
Complement C1r subcomponent	338.71	80.06681	11
Complement C1r subcomponent-like protein	54	53.46434	3
Complement C1s subcomponent	388.70691	76.6348	13
Complement C2	535.69382	83.21431	10
Complement C3	942.57073	187.02987	24
Complement C4-B	2896.2922	192.67254	53
Complement C5	668.73382	188.18613	20
Complement component C6	299.03	104.718	8
Complement component C7	347.52	93.45729	8
Complement component C8 alpha chain	126.61	65.12104	6
Complement component C8 beta chain	445.68	67.00347	12
Complement component C8 gamma chain	124.06	22.26354	3
Complement component C9	384.06691	63.1327	10
Complement factor B	912.93382	85.47852	23
Complement factor H	885.79382	139.0047	22
Complement factor H-related protein 1	164.82	37.62596	2
Complement factor H-related protein 3	80.49	37.29875	2
Complement factor I	162.97691	65.6766	9
Cytoplasmic dynein 1 heavy chain 1	100.66764	532.07184	12
Fibrinogen alpha chain	190.84691	94.91441	7
Fibronectin	928.03172	262.44208	25
Ficolin-3	95.42	32.88199	5
Gelsolin	1349.6669	85.64419	25
Glutathione peroxidase 3	73.99	25.5369	1
Hemopexin	1306.3438	51.64327	32

Heparin cofactor 2	709.37382	57.0342	16
Hepatocyte growth factor activator	56.126909	70.63609	3
Hepatocyte growth factor-like protein	85.45	80.26753	7
Histidine-rich glycoprotein	500.39	59.54087	8
Hyaluronan-binding protein 2	49.29	62.63044	3
Insulin-like growth factor-binding protein complex acid labile subunit	467.07481	65.9938	13
Inter-alpha-trypsin inhibitor heavy chain H1	1574.5407	101.32561	22
Inter-alpha-trypsin inhibitor heavy chain H2	1495.8669	106.39661	31
Inter-alpha-trypsin inhibitor heavy chain H3	300.20691	99.78653	8
Inter-alpha-trypsin inhibitor heavy chain H4	1183.69	103.29298	25
Kallistatin	196.5	48.51116	9
Kininogen-1	1070.18	71.91215	16
Leucine-rich alpha-2-glycoprotein	295.98	38.15411	4
Lumican	160.18691	38.40479	3
Lymphoid-restricted membrane protein	48.82	62.06908	4
Myosin-13	71.113818	223.54012	8
N-acetylmuramoyl-L-alanine amidase	232.6	62.17788	6
Nebulin	105.42504	772.4543	19
Pigment epithelium-derived factor	341.35	46.3133	6
Plasma kallikrein	75.72	71.32284	3
Plasma protease C1 inhibitor	336.44	55.11939	11
Plasminogen	453.50691	90.51016	14
Platelet basic protein	142.98	13.88542	2
Protein AMBP	172.17	38.97398	4
Prothrombin	351.56691	69.99212	12
Serine/threonine-protein kinase SRPK2	35.35	77.49418	1
Serum amyloid P-component	482.63	25.37113	8
Sex hormone-binding globulin	194.49	43.75182	8
Sterol regulatory element-binding protein 1	40.44	121.59908	2
Sulfhydryl oxidase 1	62.13	82.52551	6
T-cell surface glycoprotein CD3 epsilon chain	31.22	23.13247	1
Tetranectin	64.12	22.55228	2
Thrombospondin-1	499.43382	129.29956	14
Thyroxine-binding globulin	181.40382	46.29461	7
Transthyretin	115.85	15.87705	3
Tripeptidyl-peptidase 2	64.466909	138.26262	6
Tubby-related protein 2	37.83	58.62737	3
Tyrosine-protein phosphatase non-receptor type 11	69.626909	68.39332	7
Uncharacterised protein C10orf67	40.516909	21.44912	3
Vitamin D-binding protein	515.42	52.92903	11
Vitamin K-dependent protein S	112.52	75.07393	6
Vitronectin	410.18382	54.27117	9
Zinc-alpha-2-glycoprotein	299.68	34.2371	11

Patient 17 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	139.72454	0	15
unknown	81.843818	0	9
unknown	53.106909	0	3
unknown	51.32	0	3
unknown	45.05	0	3
unknown	44.873818	0	4
unknown	44.57	0	1
unknown	43.08	0	2

unknown	39.2	0	2
unknown	37.03	0	2
unknown	35.97	0	1
unknown	35.69	0	2
unknown	34.07	0	1
unknown	34.07	0	1
unknown	33.57	0	1
unknown	33.31	0	1
unknown	31.996909	0	1
unknown	30.09	0	1
Afamin	305.69691	70.96274	6
Alpha-1-antichymotrypsin	984.82	47.7916	16
Alpha-1B-glycoprotein	714.79	54.8088	11
Alpha-2-antiplasmin	245.23691	54.87319	7
Alpha-2-HS-glycoprotein	294.54	40.09801	5
Alpha-2-macroglobulin	199.9	164.61441	5
Angiotensinogen	351.61	53.40562	6
Antithrombin-III	394.95691	53.02504	9
Apolipoprotein A-I	1194.9969	30.75893	26
Apolipoprotein A-II	42.36	11.28194	1
Apolipoprotein A-IV	1617.5	45.37147	30
Apolipoprotein B-100	5149.441	516.66639	99
Apolipoprotein C-I	146.21	9.32609	3
Apolipoprotein C-III	298.1	10.8455	3
Apolipoprotein E	745.21691	36.2458	16
Beta-2-glycoprotein 1	113.44	39.58415	3
Beta-Ala-His dipeptidase	217.42	56.77015	6
Biotinidase	39.31	62.0056	1
Carboxypeptidase B2	203.92	48.95162	4
Carboxypeptidase N catalytic chain	91.25	52.53842	3
Carboxypeptidase N subunit 2	187.06	61.43147	4
Ceruloplasmin	2386.4869	122.98291	30
Clusterin	280.96691	53.03122	8
Coagulation factor V	128.40691	252.65397	7
Coagulation factor X	114.89	56.06503	3
Coiled-coil domain-containing protein 148	57.04	71.62924	5
Complement C1q subcomponent subunit B	184.04	26.67049	4
Complement C1q subcomponent subunit C	329.64691	25.98522	4
Complement C1r subcomponent	163.05	81.60639	6
Complement C1r subcomponent-like protein	45.57	54.20562	2
Complement C1s subcomponent	491.08691	78.17438	12
Complement C2	340.53	84.58283	11
Complement C3	1002.7538	188.56945	21
Complement C4-B	2738.0515	194.21212	49
Complement C5	483.26	189.89678	15
Complement component C6	283.46	108.36738	7
Complement component C7	403.43382	96.65049	7
Complement component C8 alpha chain	51.06	66.83168	3
Complement component C8 beta chain	399.29	68.71412	11
Complement component C8 gamma chain	159.32	22.43461	4
Complement component C9	460.13691	64.61526	9
Complement factor B	1119.9169	86.84704	22
Complement factor H	828.53691	143.68046	21
Complement factor H-related protein 1	202.58	38.76639	5
Complement factor H-related protein 3	57.83	38.4962	2
Complement factor I	255.32691	68.0715	6
Corticosteroid-binding globulin	139.61	45.28297	6

Dixin	43.69	77.88639	2
Fibrinogen alpha chain	130.09	95.65569	3
Fibronectin	1495.8138	266.03443	34
Ficolin-3	110.62	33.39518	4
Gelsolin	1059.86	86.04334	18
Glutathione peroxidase 3	53.88	25.76499	2
Haptoglobin	97.93	45.86082	4
Hemoglobin subunit alpha	88.39	15.30495	1
Hemopexin	1078.46	52.38455	22
Heparin cofactor 2	589.23382	57.20527	13
Hepatocyte growth factor activator	59.33	72.85992	2
Histidine-rich glycoprotein	383.95	60.51023	6
Insulin-like growth factor-binding protein complex acid labile subunit	487.51481	66.73507	12
Inter-alpha-trypsin inhibitor heavy chain H1	1913.6276	101.78179	29
Inter-alpha-trypsin inhibitor heavy chain H2	1535.73	106.85278	27
Inter-alpha-trypsin inhibitor heavy chain H3	596.96691	100.07164	13
Inter-alpha-trypsin inhibitor heavy chain H4	1277.53	103.52107	23
Interferon omega-1	30.07	22.53259	1
Kallistatin	145.49	48.68222	4
Keratin, type I cytoskeletal 10	93.04	59.01978	5
Keratin, type II cytoskeletal 1	180.71073	66.17007	4
Keratin, type II cytoskeletal 2 epidermal	88.636909	65.67832	5
Kininogen-1	829.83	72.99556	11
Leucine-rich alpha-2-glycoprotein	101.13	38.3822	2
Leucine-rich repeat-containing protein 40	51.82	68.72032	3
Lumican	159.28691	38.74692	3
Mannan-binding lectin serine protease 2	37.67	77.22422	2
Max-like protein X	46.74	33.56483	3
N-acetylmuramoyl-L-alanine amidase	75.67	62.7481	3
Necdin	34.38	36.11966	1
Pigment epithelium-derived factor	438.54	46.48436	10
Plasma kallikrein	90.486909	73.43264	3
Plasma protease C1 inhibitor	325.15	55.34748	9
Plasminogen	450.77691	93.24719	10
Platelet basic protein	180.51	14.17052	3
Probable cation-transporting ATPase 13A1	35.62	134.52325	1
Protein AMBP	150.43	39.88632	4
Prothrombin	212.45691	71.47468	5
Putative trypsin-6	38.04	27.0924	2
Serum amyloid P-component	503.78	25.48517	10
Serum paraoxonase/arylesterase 1	61.58	39.89525	2
Sex hormone-binding globulin	80.5	43.9799	4
Smoothelin	39.79	100.0298	2
Spermatogenesis-associated protein 7	57.66	68.18992	3
Structural maintenance of chromosomes protein 3	60.234813	141.85298	4
Tetranectin	218.26	22.95143	3
Thrombospondin-1	300.74691	133.29106	9
Thrombospondin-2	62.88	133.78523	3
Thyroxine-binding globulin	74.286909	46.63674	2
Titin	102.8173	3843.1187	46
Trypsin-1	48.71	27.1113	2
Uncharacterised protein C10orf92	30.51	96.51952	1
Vitamin D-binding protein	569.63	54.52563	11
Vitamin K-dependent protein S	35.3	77.12671	2
Vitronectin	339.5	55.06947	6
Zinc-alpha-2-glycoprotein	171.86	34.46519	6

Patient 18 (aggressive)

Protein	Score	MW [kDa]	# Pept.
Unknown	45.69	0	2
Unknown	41.19	0	1
Unknown	39.51	0	1
Unknown	37.75	0	2
Unknown	36.5	0	1
Unknown	35.8769089	0	1
Afamin	306.976909	70.96274	7
Alpha-1-antichymotrypsin	1026.32	47.7916	18
Alpha-1B-glycoprotein	673.07	54.8088	9
Alpha-2-antiplasmin	308.386909	54.87319	9
Alpha-2-HS-glycoprotein	321.48	40.09801	4
Angiotensinogen	431.74	53.40562	9
Apolipoprotein A-I	621.26	30.75893	16
Apolipoprotein A-IV	1782.22	45.37147	30
Apolipoprotein B-100	4473.27886	516.66639	96
Apolipoprotein C-III	295.47	10.8455	3
Apolipoprotein E	623.226909	36.2458	15
Beta-2-glycoprotein 1	131.86	39.58415	3
Beta-Ala-His dipeptidase	166.11	56.77015	5
Biotinidase	44.85	62.0056	1
Carboxypeptidase B2	214.43	48.95162	5
Carboxypeptidase N subunit 2	171.99	61.43147	5
Ceruloplasmin	1741.59691	122.98291	27
Clusterin	446.800727	53.03122	9
Coagulation factor X	117.88	56.06503	3
Complement C1q subcomponent subunit B	243.23	26.67049	6
Complement C1q subcomponent subunit C	303.326909	25.98522	5
Complement C1r subcomponent	153.97	81.60639	10
Complement C1s subcomponent	441.836909	78.17438	12
Complement C2	199.2	84.58283	10
Complement C3	911.936909	188.56945	21
Complement C4-B	2733.48836	194.21212	50
Complement C5	625.466909	189.89678	21
Complement component C6	156.46	108.36738	6
Complement component C7	324.016909	96.65049	9
Complement component C8 alpha chain	80.08	66.83168	5
Complement component C8 beta chain	408.86	68.71412	11
Complement component C8 gamma chain	109.65	22.43461	4
Complement component C9	388.886909	64.61526	10
Complement factor H	923.236909	143.68046	23
Complement factor H-related protein 1	131.51	38.76639	3
Complement factor H-related protein 3	58.26	38.4962	2
Complement factor I	214.356909	68.0715	7
Fibrinogen alpha chain	191.01	95.65569	5
Fibronectin	1675.41073	266.03443	36
Ficolin-3	120.42	33.39518	5
Gelsolin	1028.49	86.04334	22
Glutathione peroxidase 3	72.49	25.76499	5
Haptoglobin	107.9	45.86082	4

Hemopexin	1065.42	52.38455	25
Heparin cofactor 2	645.813818	57.20527	14
Hepatocyte growth factor activator	54.78	72.85992	2
Histidine-rich glycoprotein	415.23	60.51023	7
Hyaluronan-binding protein 2	58.65	64.74024	1
Insulin-like growth factor-binding protein complex acid labile subunit	484.573818	66.73507	13
Inter-alpha-trypsin inhibitor heavy chain H1	216.1	101.68631	6
Inter-alpha-trypsin inhibitor heavy chain H2	1478.98691	106.85278	25
Inter-alpha-trypsin inhibitor heavy chain H3	399.986909	100.07164	11
Inter-alpha-trypsin inhibitor heavy chain H4	1272.57	103.52107	26
Kallistatin	66.76	48.68222	2
Keratin, type I cytoskeletal 9	236.89	62.2549	7
Keratin, type II cytoskeletal 1	368.367635	66.17007	11
Keratin, type II cytoskeletal 2 epidermal	82.71	65.67832	3
Keratin, type II cytoskeletal 6A	61.72	60.29338	2
Kininogen-1	997.31	72.99556	11
Leucine-rich alpha-2-glycoprotein	134.03	38.3822	3
Lumican	199.52	38.74692	3
Pigment epithelium-derived factor	488.616909	46.48436	12
Plasma kallikrein	89.77	73.43264	5
Plasma protease C1 inhibitor	403.59	55.34748	10
Plasminogen	415.256909	93.24719	11
Platelet basic protein	187.75	14.17052	4
Protein AMBP	233.54	39.88632	6
Prothrombin	219.786909	71.47468	7
Putative trypsin-6	88.67	27.0924	2
Serum amyloid P-component	509.83	25.48517	9
Tetranectin	170.56	22.95143	3
Thrombospondin-1	480.946909	133.29106	14
Thyroxine-binding globulin	88.3338177	46.63674	4
Transthyretin	38.56	15.99109	2
Uncharacterised protein C10orf90	51.15	79.00231	2
Vitamin D-binding protein	522.25	54.52563	9
Vitamin K-dependent protein S	112.16	77.12671	7
Vitronectin	556.79	55.06947	9
Zinc-alpha-2-glycoprotein	302.18	34.46519	10

Patient 19 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	61.26	0	6
unknown	48.54073	0	4
unknown	48.22	0	2
unknown	46.84	0	4
unknown	46.7	0	2
unknown	42.94	0	4
unknown	37.12691	0	2
unknown	35.63	0	1
unknown	34.44	0	1
unknown	33.5	0	1
unknown	33.28	0	1
unknown	33.27	0	2
unknown	32.29	0	1
unknown	32.03691	0	1
Afamin	163.3769	70.96274	7

Alpha-1-antichymotrypsin	1065.48	47.7916	15
Alpha-1B-glycoprotein	673.23	54.8088	13
Alpha-2-antiplasmin	151.6169	54.87319	2
Alpha-2-HS-glycoprotein	207.62	40.09801	4
Alpha-2-macroglobulin	1344.84	164.6144	26
Angiotensinogen	361.84	53.40562	9
Antithrombin-III	704.0369	53.02504	12
Apolipoprotein A-I	627.6169	30.75893	14
Apolipoprotein A-IV	1558.25	45.37147	28
Apolipoprotein B-100	3342.462	516.6664	82
Apolipoprotein C-I	63.32	9.32609	1
Apolipoprotein C-II	32.09	11.27675	1
Apolipoprotein C-III	219.12	10.8455	2
Apolipoprotein E	206.01	36.2458	9
Beta-2-glycoprotein 1	40.37	39.58415	1
Beta-Ala-His dipeptidase	71.42	56.77015	2
Biotinidase	30.47	62.0056	1
Carboxypeptidase B2	172.81	48.95162	6
Carboxypeptidase N subunit 2	138.41	61.43147	3
Ceruloplasmin	1944.467	122.9829	35
Clusterin	149.7169	53.03122	6
Coagulation factor X	138.02	56.06503	3
Coiled-coil domain-containing protein 148	47.01691	71.62924	6
Complement C1q subcomponent subunit B	224.67	26.67049	4
Complement C1q subcomponent subunit C	296.3169	25.98522	7
Complement C1s subcomponent	246.8469	78.17438	9
Complement C2	207.66	84.58283	7
Complement C3	811.0707	188.5695	19
Complement C4-A	2679.448	194.2471	53
Complement C5	680.7269	189.8968	18
Complement component C6	178.25	108.3674	6
Complement component C7	290.8969	96.65049	4
Complement component C8 alpha chain	57.75	66.83168	4
Complement component C8 beta chain	366.27	68.71412	9
Complement component C8 gamma chain	201.36	22.43461	5
Complement component C9	352.55	64.61526	9
Complement factor B	773.3369	86.84704	17
Complement factor H	614.9838	143.6805	20
Complement factor H-related protein 1	161.68	38.76639	3
Complement factor I	75.46691	68.0715	3
Fanconi anemia group J protein	43.3	142.7846	2
Fibrinogen alpha chain	71.76	95.65569	3
Fibronectin	769.6807	266.0344	22
Gelsolin	644.04	86.04334	11
Glutamate [NMDA] receptor subunit 3A	35.1	126.5254	2
Glutathione peroxidase 3	60.23	25.76499	3
Haptoglobin	297.34	45.86082	8
Hemopexin	1008.54	52.38455	27
Heparin cofactor 2	530.9969	57.20527	13
Histidine-rich glycoprotein	424.66	60.51023	8
Inter-alpha-trypsin inhibitor heavy chain H1	1219.477	101.7818	22
Inter-alpha-trypsin inhibitor heavy chain H2	1018.52	106.8528	25
Inter-alpha-trypsin inhibitor heavy chain H3	340.8469	100.0716	7
Inter-alpha-trypsin inhibitor heavy chain H4	973.65	103.5211	19
Kallistatin	136.99	48.68222	7
Kininogen-1	682.03	72.99556	9
Leucine-rich alpha-2-glycoprotein	157.25	38.3822	4

Lumican	150.2638	38.74692	4
Pigment epithelium-derived factor	465.87	46.48436	9
Plasma kallikrein	68	73.43264	3
Plasma protease C1 inhibitor	234	55.34748	9
Plasminogen	344.3138	93.24719	9
Platelet basic protein	129.83	14.17052	3
Protein AMBP	138.02	39.88632	4
Prothrombin	79.07	71.47468	2
Putative hydroxypyruvate isomerase	36.63	30.50061	2
Putative trypsin-6	49.49	27.0924	3
Serum amyloid P-component	404.83	25.48517	8
Sex hormone-binding globulin	84.41	43.9799	4
Stabilin-1	46.39691	286.9265	2
Thyroxine-binding globulin	66.69	46.63674	2
Titin	156.9572	3843.119	46
TRIO and F-actin-binding protein	61.33382	264.125	7
Vitamin D-binding protein	560.69	54.52563	12
Vitronectin	346.42	55.06947	7
Zinc-alpha-2-glycoprotein	223.82	34.46519	7

Patient 20 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	72.577904	0	5
unknown	35.08	0	1
Afamin	188.64691	70.96274	6
Alpha-1-antichymotrypsin	868.48	47.7916	18
Alpha-1B-glycoprotein	671.31	54.8088	12
Alpha-2-antiplasmin	153.82691	54.87319	8
Alpha-2-HS-glycoprotein	277.06	40.09801	5
Alpha-2-macroglobulin	81.33	164.61441	4
Angiotensinogen	436.98	53.40562	8
Apolipoprotein A-I	744.20691	30.75893	19
Apolipoprotein A-IV	1442.91	45.37147	28
Apolipoprotein B-100	3953.9348	516.66639	98
Apolipoprotein C-III	236.49	10.8455	3
Apolipoprotein E	302.53691	36.2458	13
Beta-2-glycoprotein 1	95.18	39.58415	2
Beta-Ala-His dipeptidase	144.66	56.77015	6
Carboxypeptidase B2	67.94	48.95162	2
Carboxypeptidase N subunit 2	146.08	61.43147	3
Ceruloplasmin	2329.8879	122.98291	35
Clusterin	287.34691	53.03122	6
Coagulation factor X	105.8	56.06503	3
Coagulation factor XIII A chain	60.12	83.7278	1
Complement C1q subcomponent subunit A	170.49	26.28529	3
Complement C1q subcomponent subunit B	160.15	26.67049	5
Complement C1q subcomponent subunit C	245.05691	25.98522	4
Complement C1r subcomponent	107.16	81.60639	5
Complement C1s subcomponent	254.54691	78.17438	9

Complement C2	260.76691	84.58283	12
Complement C3	671.57	188.56945	19
Complement C4-B	1612.3715	194.21212	41
Complement C5	236.73	189.89678	9
Complement component C6	186.18	108.36738	7
Complement component C7	172.25382	96.65049	5
Complement component C8 alpha chain	52.82	66.83168	3
Complement component C8 beta chain	298.24	68.71412	9
Complement component C8 gamma chain	141.77	22.43461	2
Complement component C9	259.6	64.61526	6
Complement factor B	859.71382	86.84704	19
Complement factor H	585.78691	143.68046	18
Complement factor H-related protein 1	148.68	38.76639	3
Complement factor I	96.256909	68.0715	3
Corticosteroid-binding globulin	88.5	45.28297	4
Fibrinogen alpha chain	98.94	95.65569	4
Fibronectin	1258.4276	266.03443	31
Ficolin-3	96.55	33.39518	4
Gelsolin	883.57	86.04334	17
Hemopexin	955.6	52.38455	21
Heparin cofactor 2	555.47382	57.20527	13
Hepatocyte growth factor activator	60.5	72.85992	2
Histidine-rich glycoprotein	422.01	60.51023	6
Hyaluronan-binding protein 2	40.88	64.74024	1
Insulin-like growth factor-binding protein complex acid labile subunit	376.56382	66.73507	11
Inter-alpha-trypsin inhibitor heavy chain H1	1582.3507	101.78179	24
Inter-alpha-trypsin inhibitor heavy chain H2	1299.91	106.85278	25
Inter-alpha-trypsin inhibitor heavy chain H3	341.17691	100.07164	11
Inter-alpha-trypsin inhibitor heavy chain H4	1059.4269	103.52107	26
Kallistatin	134.03	48.68222	5
Kininogen-1	946.64	72.99556	11
Leucine-rich alpha-2-glycoprotein	82.46	38.3822	3
Lumican	113.44	38.74692	3
Monocyte differentiation antigen CD14	34.19	40.67798	1
N-acetylmuramoyl-L-alanine amidase	110.71	62.7481	6
Pigment epithelium-derived factor	202.58691	46.48436	8
Plasma protease C1 inhibitor	187.12	55.34748	7
Plasminogen	348.92691	93.24719	10
Platelet basic protein	192.03	14.17052	3
Protein AMBP	134.33	39.88632	5
Prothrombin	233.66691	71.47468	8
Putative trypsin-6	86.84	27.0924	2
Serum amyloid P-component	344.99	25.48517	7
Spermatogenesis-associated protein 7	55.37	68.18992	2
Tetranectin	159.36	22.95143	3
Thrombospondin-1	369.76691	133.29106	11
Thrombospondin-2	60.07	133.78523	3
Vitamin D-binding protein	546.66	54.52563	10
Vitamin K-dependent protein S	95.71	77.12671	6
Vitronectin	384.1	55.06947	8

Zinc-alpha-2-glycoprotein	214.91	34.46519	7
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Patient 21 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	54.03	0	1
unknown	52.67	0	2
unknown	50.02	0	2
unknown	43.4469	0	5
unknown	31.3	0	1
Afamin	118.48	70.9627	3
Alpha-1-antichymotrypsin	474.77	47.7916	9
Alpha-1B-glycoprotein	172.41	54.8088	4
Alpha-2-HS-glycoprotein	223.35	40.098	5
Angiotensinogen	83.19	53.4056	1
Ankyrin repeat domain-containing protein 17	33.07	275.97	1
Antithrombin-III	102.57	53.025	5
Apolipoprotein A-IV	424.12	45.3715	18
Apolipoprotein B-100	2481	516.666	55
Apolipoprotein C-III	101.24	10.8455	1
Apolipoprotein E	98.44	36.2458	4
Calpain-15	35.41	119.862	1
Ceruloplasmin	1315.69	122.983	21
Clusterin	236.927	53.0312	5
Coiled-coil domain-containing protein 148	40.95	71.6292	3
Complement C1q subcomponent subunit B	69.11	26.6705	2
Complement C1q subcomponent subunit C	54.92	25.9852	1
Complement C1s subcomponent	55.5	78.1744	2
Complement C3	297.957	188.569	7
Complement C4-B	1343.5	194.212	24
Complement C5	313.75	189.897	8
Complement component C8 beta chain	61.4	68.7141	1
Complement component C8 gamma chain	116.31	22.4346	4
Complement component C9	191.727	64.6153	3
Complement factor B	426.557	86.847	10
Complement factor H	289.68	143.68	7
Complement factor H-related protein 1	73.64	38.7664	1
Complement factor I	56.1269	68.0715	2
Fibrinogen alpha chain	309.18	95.6557	7
Fibronectin	209.637	266.034	6
Focal adhesion kinase 1	32.99	119.956	2
Gelsolin	307.9	86.0433	7
Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1	54.5969	208.367	4
Hemopexin	644.58	52.3846	14
Heparin cofactor 2	173.22	57.2053	3
Histidine-rich glycoprotein	194.06	60.5102	3
Insulin-like growth factor-binding protein complex acid labile subunit	49.0269	66.7351	2
Inter-alpha-trypsin inhibitor heavy chain H1	485.29	101.782	8
Inter-alpha-trypsin inhibitor heavy chain H2	461.64	106.853	10
Inter-alpha-trypsin inhibitor heavy chain H3	163.38	100.072	4
Inter-alpha-trypsin inhibitor heavy chain H4	345.37	103.521	9
Keratin, type II cytoskeletal 1	56.17	66.1701	2

Kininogen-1	436.15	72.9956	6
Leucine-rich alpha-2-glycoprotein	138.11	38.3822	3
Mannose-binding protein C	64.49	26.5262	3
Pigment epithelium-derived factor	54.46	46.4844	3
Plasminogen	99.1269	93.2472	4
Platelet factor 4 variant	60.58	11.7734	2
Plexin-A4	44.78	215.682	3
Protein AMBP	44.87	39.8863	2
Prothrombin	120.997	71.4747	5
Serum amyloid P-component	144.94	25.4852	3
Spermatogenesis-associated protein 7	32.49	68.1899	1
Stabilin-1	45.6	286.926	3
TRIO and F-actin-binding protein	62.3169	264.125	4
Vitamin D-binding protein	268.84	54.5256	8
Vitamin K-dependent protein Z	36.7	46.0262	1
Vitronectin	352.847	55.0695	6
Zinc-alpha-2-glycoprotein	97.19	34.4652	4

Patient 22 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unkown	42.76	0	2
unkown	42.4	0	4
unkown	39.89	0	1
unkown	38.126909	0	2
unkown	36.56	0	2
unkown	35.47	0	2
unkown	35.45	0	1
unkown	34.836909	0	1
unkown	33.89	0	1
unkown	33.7	0	1
unkown	33.64	0	1
unkown	32.35	0	1
unkown	32.35	0	1
unkown	31.74	0	1
Afamin	143.13	70.96274	5
Alpha-1-antichymotrypsin	922.48	47.7916	14
Alpha-1B-glycoprotein3	671.2	54.8088	11
Alpha-2-antiplasmin	217.70691	54.87319	6
Alpha-2-HS-glycoprotein	305.38	40.09801	4
Alpha-2-macroglobulin	57.4	164.61441	2
Angiotensinogen	307.07	53.40562	6
Antithrombin-III	112.47	53.02504	5
Apolipoprotein A-I	156.75	30.75893	6
Apolipoprotein A-IV	1012.12	45.37147	23
Apolipoprotein B-100	3442.8403	516.66639	79
Apolipoprotein C-I	61.35	9.32609	2
Apolipoprotein C-III	251.16	10.8455	3
Apolipoprotein E	372.41691	36.2458	10
ATP-binding cassette sub-family A member 12	51.576909	295.38672	4
Beta-2-glycoprotein 1	131.31	39.58415	3
Beta-Ala-His dipeptidase	86.91	56.77015	4
Calpain-15 1	30.36	119.86215	1
Carboxypeptidase B2	191.81	48.95162	4
Carboxypeptidase N subunit 2	77.77	61.43147	2

CDC45-related protein	34.35	66.21084	1
Ceruloplasmin	1952.3369	122.98291	29
Coagulation factor X	116.46	56.06503	4
Complement C1q subcomponent subunit B	158.94	26.67049	3
Complement C1q subcomponent subunit C	284.90691	25.98522	4
Complement C1s subcomponent	321.54691	78.17438	10
Complement C2	241.43	84.58283	7
Complement C3	434.05691	188.56945	11
Complement C4-A	2165.9053	194.24706	44
Complement C5	336.68	189.89678	9
Complement component C6	112	108.36738	4
Complement component C7	182.32691	96.65049	5
Complement component C8 beta chain	447.59	68.71412	11
Complement component C8 gamma chain	93.08	22.43461	2
Complement component C9	307.66691	64.61526	7
Complement factor B	1050.6369	86.84704	19
Complement factor H	731.44691	143.68046	17
Complement factor H-related protein 1	146.64	38.76639	3
Complement factor H-related protein 3	73.04	38.4962	2
Complement factor I	254.74691	68.0715	5
FERM domain-containing protein 4A	42	115.95752	2
Fibrinogen alpha chain	155.17	95.65569	4
Fibronectin	1115.0338	266.03443	22
Ficolin-3	207.9	33.39518	7
FYVE, RhoGEF and PH domain-containing protein 4	40.726909	87.59782	3
Gelsolin	483.7	86.04334	9
Haptoglobin	151.8	45.86082	4
Hemopexin	914.98	52.38455	20
Heparin cofactor 2	378.50382	57.20527	10
Histidine-rich glycoprotein	120.8	60.51023	4
Insulin-like growth factor-binding protein complex acid labile subunit	274.79382	66.73507	10
Inter-alpha-trypsin inhibitor heavy chain H1	1272.3869	101.78179	19
Inter-alpha-trypsin inhibitor heavy chain H2	1230.4769	106.85278	20
Inter-alpha-trypsin inhibitor heavy chain H3	358.06691	100.07164	7
Inter-alpha-trypsin inhibitor heavy chain H4	931.96691	103.52107	20
Kininogen-1	587.1	72.99556	9
Lumican	94.356909	38.74692	3
Lymphoid-restricted membrane protein	42.08	62.75334	3
Mannan-binding lectin serine protease 2	54.95	77.22422	1
N-acetylmuramoyl-L-alanine amidase	59.2	62.7481	3
Pigment epithelium-derived factor	263.59	46.48436	6
Plasma kallikrein	69.496909	73.43264	2
Plasminogen	427.84691	93.24719	10
Platelet basic protein	180.02	14.17052	3
Protein AMBP	100.37	39.88632	3
Prothrombin	185.47691	71.47468	6
Putative trypsin-6	75.14	27.0924	2
Serum amyloid P-component	408.94	25.48517	7
Spermatogenesis-associated protein 7	45.93	68.18992	2
Stabilin-1	50.946909	286.92647	3
Steroid hormone receptor ERR1	39.38	46.10786	3
Tetranectin	168.5	22.95143	3
Thrombospondin-1	91.9	133.29106	6
Vitamin D-binding protein	409.66	54.52563	9
Vitamin K-dependent protein S	43.21	77.12671	2
Vitronectin	332.82	55.06947	6
Zinc-alpha-2-glycoprotein	195.89	34.46519	6

Patient 24 (aggressive)

Protein	Score	MW [kDa]	# Pept.
unknown	54.03	0	1
unknown	52.67	0	2
unknown	50.02	0	2
unknown	43.44691	0	5
unknown	31.3	0	1
Afamin	118.48	70.96274	3
Alpha-1-antichymotrypsin	474.77	47.7916	9
Alpha-1B-glycoprotein	172.41	54.8088	4
Alpha-2-HS-glycoprotein	223.35	40.09801	5
Angiotensinogen	83.19	53.40562	1
Ankyrin repeat domain-containing protein 17	33.07	275.9698	1
Antithrombin-III	102.57	53.02504	5
Apolipoprotein A-IV	424.12	45.37147	18
Apolipoprotein B-100	2480.997	516.6664	55
Apolipoprotein C-III	101.24	10.8455	1
Apolipoprotein E	98.44	36.2458	4
Calpain-15	35.41	119.8622	1
Ceruloplasmin	1315.688	122.9829	21
Clusterin	236.9269	53.03122	5
Coiled-coil domain-containing protein 148	40.95	71.62924	3
Complement C1q subcomponent subunit B	69.11	26.67049	2
Complement C1q subcomponent subunit C	54.92	25.98522	1
Complement C1s subcomponent	55.5	78.17438	2
Complement C3	297.9569	188.5695	7
Complement C4-B	1343.501	194.2121	24
Complement C5	313.75	189.8968	8
Complement component C8 beta chain	61.4	68.71412	1
Complement component C8 gamma chain	116.31	22.43461	4
Complement component C9	191.7269	64.61526	3
Complement factor B	426.5569	86.84704	10
Complement factor H	289.68	143.6805	7
Complement factor H-related protein 1	73.64	38.76639	1
Complement factor I	56.12691	68.0715	2
Fibrinogen alpha chain	309.18	95.65569	7
Fibronectin	209.6369	266.0344	6
Focal adhesion kinase 1	32.99	119.9556	2
Gelsolin	307.9	86.04334	7
Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1	54.59691	208.3674	4
Hemopexin	644.58	52.38455	14
Heparin cofactor 2	173.22	57.20527	3
Histidine-rich glycoprotein	194.06	60.51023	3
Insulin-like growth factor-binding protein complex acid labile subunit	49.02691	66.73507	2
Inter-alpha-trypsin inhibitor heavy chain H1	485.29	101.7818	8
Inter-alpha-trypsin inhibitor heavy chain H2	461.64	106.8528	10
Inter-alpha-trypsin inhibitor heavy chain H3	163.38	100.0716	4
Inter-alpha-trypsin inhibitor heavy chain H4	345.37	103.5211	9
Keratin, type II cytoskeletal 1	56.17	66.17007	2
Kininogen-1	436.15	72.99556	6
Leucine-rich alpha-2-glycoprotein	138.11	38.3822	3
Mannose-binding protein C	64.49	26.52615	3
Pigment epithelium-derived factor	54.46	46.48436	3

Plasminogen	99.12691	93.24719	4
Platelet factor 4 variant	60.58	11.77337	2
Plexin-A4	44.78	215.6823	3
Protein AMBP	44.87	39.88632	2
Prothrombin	120.9969	71.47468	5
Serum amyloid P-component	144.94	25.48517	3
Spermatogenesis-associated protein 7	32.49	68.18992	1
Stabilin-1	45.6	286.9265	3
TRIO and F-actin-binding protein	62.31691	264.125	4
Vitamin D-binding protein	268.84	54.52563	8
Vitamin K-dependent protein Z	36.7	46.0262	1
Vitronectin	352.8469	55.06947	6
Zinc-alpha-2-glycoprotein	97.19	34.46519	4