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Application of SELDI-TOF-MS in protein profiling: state of the art

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Serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) appears to be an important diagnostic tool for a whole range of diseases. Sensitivities and specificities obtained with this new technology often seem superior to those obtained with current biomarkers. However, reproducibility and standardization are still problematic. This presentation explains the SELDI-TOF-MS technique and some important aspects for proteomics studies are discussed, like pre- and post-analytical aspects and quality control procedures.

The field of proteomics has developed rapidly in recent years. Until the mid-1990s mainly individual genes and proteins were studied. The point of proteomics is to characterize the behaviour of the system as a whole, rather than the behaviour of any single component. Proteomics is in fact the comprehensive study of the proteome: all the proteins in either a cell, tissue, organ, or organism. The proteome is dynamic and in constant flux due to a combination of factors. These factors include posttranslational modifications and functional regulation of gene expression (1). Moreover, in proteomics protein identification is not necessarily performed by complete sequence analysis, but can also be performed by partial sequence analysis with the aid of database matching tools.

To further improve and coordinate proteomics research, the international human proteome organization HUPO (www.hupo.org) has been founded in 2001, aiming to define and promote proteomics through international cooperation and collaborations by fostering the development of new technologies, techniques and training to better understand human disease.

Proteomic analysis requires the combination of various technologies, including biochemistry, mass spectrometry and bioinformatics. Important techniques for expression analysis of proteins are two-dimensional electrophoresis (2-DE) combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and/or liquid chromatography tandem mass spectrometry (LC/MS/MS). This paper is focused on a novel approach the SELDI-TOF-MS technique. Important issues in SELDI-TOF-MS analyses are protein profiling and detection of new biomarkers for different diseases. Although promising, the fact is that until now no widely applicable new approaches to patient diagnosis and therapy have become available. Research is still focused on improvement of reproducibility and sensitivity of the different techniques.

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SELDI-TOF-MS

There have been many reports on the application of SELDI-TOF-MS technology since its first introduction in 1993 by Hutchens and Yip (2). SELDI-TOF-MS is an approach that tries to overcome the requirements for purification and separation of proteins prior to mass spectrometry analysis (3). It is a novel approach to biomarker discovery that combines two powerful techniques: chromatography and mass spectrometry. One of the key features of SELDI-TOF-MS is its ability to provide a rapid protein expression profile from a variety of biological and clinical samples (4). It consists of selective protein extraction and retention on chromatographic chip surfaces and their subsequent analysis by a simple laser desorption ionization mass spectrometer (5). It differs in several aspects from conventional MALDI-TOF-MS. For MALDI-TOF-MS, analytes are directly spotted onto a plate. This is usually a metal plate. The applied samples are usually tryptic digests from proteins separated by 2-DE, although proteins purified by other separation methods are also compatible with the method. Before deposition of the analytes, the energy absorbing matrix (EAM) is placed on the plate or mixed in with the sample. The matrix will absorb energy from the laser causing the analytes to be ionized by MALDI-TOF-MS (6).

ProteinChip arrays

For the SELDI-TOF-MS technique different ProteinChip arrays (Ciphergen Biosystems Inc.) can be used. The chromatographic surfaces that make up the various ProteinChip arrays are uniquely designed to retain proteins from a complex sample mixture according to specific properties such as hydrophobicity, charge (4) (figure 1). The procedure for detecting protein biomarkers is very simple. A few microliters of the sample are dispensed onto the ProteinChip surface under specific binding conditions that determine which proteins will be retained by the surface. Protein specificity is achieved through the application of a series of washes with an appropriate solvent or buffer designed to elute unbound proteins and interfering substances, such as salts, detergents, lipids.

Only proteins actively interacting with the spot surfaces are analyzed in the Protein Biosystem series instrument (Ciphergen Biosystems Inc.), because all other components are washed off in advance (7).
By choosing different ProteinChip arrays with array-specific surface components, different proteins will be analyzed depending on the chip characteristics. In fact the interaction of the analyte and the chip introduces a purification step. After addition of sample and washing buffers, the EAM is applied to the ProteinChip array. The EAM will facilitate desorption and ionization in the PBS series instrument.

Desorption/ionization process
After introducing the ProteinChip array into the ProteinChip Reader, a laser beam is directed onto the sample on the spot. Upon laser activation, the sample becomes irradiated and the desorption and ionization proceeds to liberate gaseous ions from the ProteinChip arrays. These gaseous ions enter the TOF-MS region of the instrument, which measures the mass-to-charge ratio (*m/z*) of molecular ions of each protein, based on its velocity through a vacuum chamber (4). The time-of-flight corresponds inversely to the *m/z* value. As a first result, the molecules in the sample are represented in a graph with the *m/z* value on the x-axis and the corresponding signal intensity on the y-axis (7). (figure 2).

Advantages SELDI-TOF-MS
SELDI-TOF-MS has several advantages over other methods such as 2-DE combined with MALDI-TOF-MS and/or LC/MS/MS. SELDI-TOF-MS has a much higher throughput capability, requires significantly lower amounts of the sample, has small range sensitivity, offers higher resolution at low mass ranges, and is easy to use (8). SELDI-TOF-MS can effectively resolve polypeptides and peptides smaller than 20 kDa (9). The 2-DE approach, where proteins are at first separated by their isoelectric point and subsequently by their molecular weight, was developed to increase the resolving power for the analysis of complex protein mixtures. Whereas the enhanced resolution of 2-DE gels contributed greatly to our understanding of the wide variety of proteins in a given sample, it still includes the disadvantage of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method of giving preference to the most abundant proteins. In addition, proteins in the peptide range as well as those of high hydrophobicity or of extreme isoelectric points are typically neglected, resulting again in a loss of potentially interesting proteins (10). Moreover, 2-DE is labour intensive, time consuming, and difficult to standardize between laboratories (4).
The high throughput ability of the SELDI-TOF-MS system allows hundreds of samples to be screened for disease biomarker identification in a relatively short time period, providing investigators the opportunity to compare patient-to-patient variability (4). SELDI-TOF-MS is a recently established improvement on some of the concepts of MALDI-TOF-MS. ProteinChip arrays allow researchers to purify and detect a subset of proteins in the sample at the same time by using a variety of surface chemistries such as classic chromatographic surfaces (e.g., cation/anion exchanges) and biologically activated surfaces to capture specific molecular counterparts. This benefit is effective to especially the biological samples such as body fluids and conditioned medium containing a variety of proteins (11).

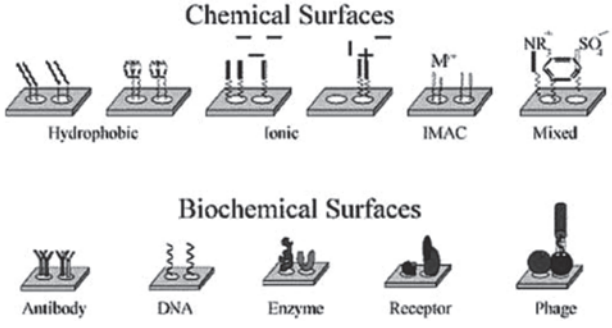

Figure 1. The different types of ProteinChip arrays. The chemical surfaces are chromatographic ProteinChip arrays with hydrophobic, cationic, anionic, metal ions for immobilized metal affinity binding (IMAC) or hydrophilic spots. The biochemical surfaces are designed for coupling of biomolecules in antibody-antigen assays, DNA-protein binding experiment, coupling of enzymes, receptor-ligand interaction and for coupling of phages.

Biomarker Discovery
The true scientific goal of serum proteomic pattern analysis is in fact biomarker discovery. Since the study by Petricoin et al. (12) on proteomic patterns to detect ovarian cancer, the use of SELDI-TOF-MS protein profiling as a diagnostic tool, has become an important subject of investigation (13). Until now, this approach has been suggested for different diseases, like ovarian (12, 14-18), prostate (9, 19-22)

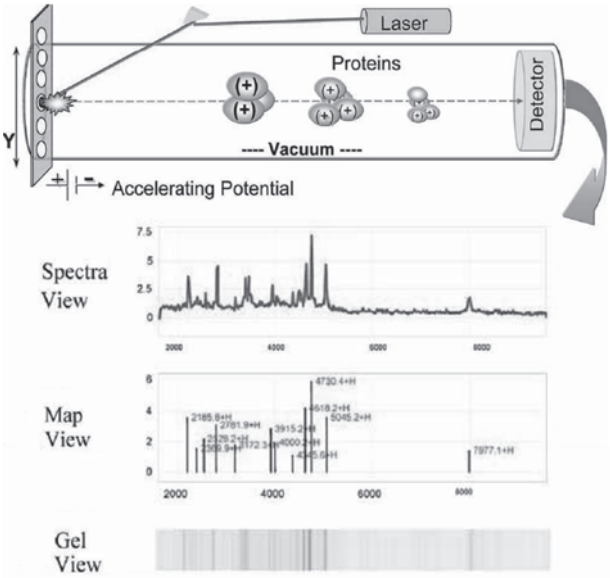

Figure 2. The ions of the molecules in the sample are represented in a spectra, map and gel view with the mass-to-charge ratio (m/z) on the x-axis and the corresponding signal intensity on the y-axis.

and lung (23) cancer, but also for inflammatory diseases (24, 25). Although the first papers on SELDI-TOF-MS seemed very promising, until now it did not yet result in widely applicable new approaches or diagnostic tools. It is with no doubt a promising technique, but further improvement of both reproducibility and sensitivity is required, before the process of translating new markers into clinical laboratory tests can be further developed (26). The vast majority of the currently available data on biomarker detection in cancer, have been produced with SELDI-TOF-MS. As was recently critically discussed by Diamandis (27), two types of data have been reported in the literature, 1) discriminating peaks of unknown identity (increased or decreased) between normal individuals and patients with cancer; and 2) data in which at least some of the peaks have been positively identified. Table 1 shows differences found in prostate and ovarium carcinoma, even in studies using comparable experimental conditions. Some important pre-analytical and analytical aspects will be discussed here.

Pre-analytical aspects

Protein profiling can only become a reliable diagnostic tool when it fulfils the criteria for reproducibility and standardization that are generally accepted for diagnostic tests in clinical chemistry. Therefore, some essential aspects to improve reproducibility and standardization of SELFI-TOF-MS will be discussed here.

Storage effects

To avoid pre-analytical errors, sample collection for proteomic analysis should be accurately described and standardized. Effects of sample storage and the consequences of differences in sample preparation are highly underestimated. We recently compared protein profiles of freshly frozen serum samples with frequently thawed serum samples. The number of freeze-thaw cycles should be perfectly standardized and comparable for control and disease population. It is to be expected that especially in the earlier protein profiling studies archived samples were used for which conditions of control and patient populations were not fully identical. It has now become apparent that both the number of freeze-thaw cycles, freezing temperature and storage time should at least be identical for both study and control population. This can easily be overcome in prospective studies by dividing the samples in aliquots before storage (28).

Serum or plasma

Until now, insufficient information is available to decide whether serum or plasma should be preferred in proteomic studies. Most studies have used serum, but further research on this topic is required (28). It is generally assumed that, more peaks can lead to more significant differences between populations as was the case in our study. Theoretically, however, plasma with protease inhibitors contains more intact proteins not attacked by proteolytic enzymes. Further examinations on the differences between serum and plasma are required.

Sample preparation

Samples can be denatured with urea/CHAPS (9, 14, 16-20, 22), but can also be fractionated with anion exchange chromatography (15). Denaturing conditions allow protein-protein interaction disruption before analysis by SELDI-TOF-MS. With fractionation by anion exchange chromatography the highly abundant proteins such as albumin and immunoglobulins (60-

Table 1. Summary of previous reports on prostate and ovarian cancer with a proteomics approach using SELDI-TOF-MS

Author	Chip	*m/z* values (Da)
Prostate cancer		
Adam (20)	IMAC3-Cu	4475, 5074, 5382, 7024, 7820, 8141, 9179, 9507, 9656
Banez (19)	IMAC3-Cu WCX2	IMAC: 3960, 4469, 9713, 10266, 22832 WCX2: 3972, 8226, 13952, 16087, 25167, 33270
Qu (9)	IMAC3-Cu	PCA vs non/cancer: 9655, 9720, 6542, 6797, 6949, 7024, 8067, 8356, 3963, 4079, 7885, 6991 BPH vs HC: 7820, 4580, 7844, 4071, 7054, 5298, 3486, 6099, 8943
Li (22)	IMAC3-Ni	2680, 10300, 17900
Petricoin (21)	C16 hydrophobic interaction	2029, 2367, 2582, 3080, 4819, 5439, 18220
Ovarian cancer		
Petricoin (12)	C16 hydrophobic interaction	543, 989, 2111, 2251, 2465
Zhang (15)	IMAC3-Cu	3272 (pH 9 fraction) 12828, 28043 (pH 4 fraction)
Rai (18)	IMAC3-Ni	8600, 9200, 19800, 39800, 54000, 60.000, 79.000
Kozak (14)	SAX2	3100, 13900, 21000, 79000, 106700
Ye (17)	IMAC3-Cu	11723
Vlahou (16)	SAX2 IMAC	SAX2 4400, 21500 IMAC 5540, 6650, 11700

80% of total serum protein content), which can interfere with the resolution and sensitivity of the proteome profiling techniques, will be visible in specific fractions. Linke et al. (29) illustrated that fractionation greatly increases the number of peptide and protein ion signals that can be observed by SELDI-TOF-MS, when compared to both unfractionated (only denatured) as well as albumin-depleted samples. By using different denaturing steps or using fractionated samples, other significant peaks resulting in different biomarkers can be detected.

Sampling time
We suggest, according to World Healthy Organization (WHO), anticoagulants in diagnostic laboratory investigations to use a clotting time of 30 minutes at room temperature, spinning for 15 minutes at a minimum speed of 1500g at 4ºC and storage of the samples in aliquots within 1 hour at -80ºC after blood collection. Obviously the consequences of differences in sample characteristics within a study population, but also between study and control population, like for instance use of fasting or non-fasting samples, age-matching of the samples should always be properly standardized.

Patient population
The number of patients and healthy controls in the training and validation sets is very important because the reliability of the results improves with increasing numbers. A clear description of the training and validation population is essential, like the severity of disease. Because SELDI-TOF-MS fingerprinting probably measures peptides present in high abundance in serum (e.g. mg/L to g/L range) the molecules, which are detected, probably originate from common disease mechanisms or general protection mechanisms, i.e. epiphenomena of the diseases, such as acute phase response, cachexia etc. It is clear that the robustness of the technology should be validated by comparing patient groups with comparable disease mechanisms. Method validation should therefore be extended not only to healthy controls, but also to diseases with comparable generalized disease conditions (infection, cachexia etc).

Post-analytical aspects

Bioinformatics and biostatistics
Peak detection, laser settings and data analysis software affect the ultimate *m/z* values found. Different multivariate analysis software can be used to classify different groups. Biomarkers Patterns (Ciphergen Biosystems Inc.) is a decision tree algorithm which is very often used in protein profiling studies. The decision trees can be based on the intensity, S/N ratio or area under the curve (AUC). Propeak, classification and regresion tree (CART), AdaBoost, and principal component analysis (PCA) are other examples of multivariate analysis software programs which can be used to classify the different groups. Some groups develop their own statistical software program by combining more multivariate analysis techniques. It is hard to compare the results of studies when all these different kind of software programs are used to classify groups.
In a recent review we showed that apart from the pre-analytical strategy, the post-analytical strategy also has an enormous impact on the final results. By comparing previous reports on prostate and ovarian cancer we showed large differences in *m/z* values of the biomarkers presented in the different studies, even in studies with comparable patient populations (28). It should be noted that careful and precise selection of the peak labelling settings and normalization of peak intensities are considered critical for biomarker identification and for the efficient and reliable performance of any learning algorithm used in conjunction with the SELDI-TOF-MS system (16).

Quality control
As mentioned before, the effect of pre- and post-analytical variables on protein profiling needs further and more systematic investigation. Therefore, a stringent standardized protocol is needed, not only for pre- and post-analytical aspects, but also for calibration and quality control (QC) performance. The results of the quality control procedure are described in a previous report of Bons et al. (30).
Recently Plebani et al. (31) indicated that only few studies published carefully described their quality control procedures incorporated in proteomic experimental protocols. We developed a well-defined protocol for calibration of the Protein Biosystems IIc (PBS IIc) instrument, to implement QC samples with independent certified standards and to determine acceptance criteria for quality control. Because the QC samples are spotted on a NP20 array, which is a normal phase array, without washing or selective binding steps, only the MALDI-TOF-MS part of the PBS IIc instrument is checked. Stable instrument performance over time is a prerequisite before any proteomic experiments should be performed. The QC procedure described acts prospectively by checking the calibration every week in contrast to some other studies, where QC samples are included in the profiling studies and quality control thus acts retrospectively or where no quality control procedure is performed at all.
Data analysis was performed with in house developed software (ShewhartPlots), which was based on the Shewhart control chart principle (32). *M/z* values, intensities, signal-to noise (S/N) ratios and peak resolutions are imported in this software. Two dimensional Youden plots are made by drawing insulin (x-axis) and apomyoglobin (y-axis) in one plot for all parameters and three dimensional Youden plots are made by drawing insulin (x-axis), apomyoglobin (y-axis) and albumin (z-axis) in one plot for all parameters. Analysis are only performed if minimal quality requirements are fulfilled.

Reproducibility
We showed that variations in the signal of the QC samples can be caused by pipetting variability in the handling of the QC sample, spot and chip variability,

crystallization of the EAM and laser detector variability over time. The reproducibility of serum protein profiling by SELDI-TOF-MS was investigated by spotting one QC sample, consisting of insulin and apomyoglobin on 2 or 4 NP20 chips. Coefficient of variation (CV) values from approximately 10 to 40% were achieved for intensities and signal-to-noise (S/N) ratios. The pooled CV value for the mass accuracy was below 0.1%. The CV values for intensities, S/N ratios and mass accuracy described in the study of Bons et al. were comparable with the CV values reported by Semmes et al. (33). Semmes et al. performed across-laboratory measurement of three *m/z* peaks in a standard pooled serum. This resulted in a 0.1% CV for mass accuracy. The CVs for signal to noise ratio's were 34-40% and the variations in the intensities of the three peaks for all laboratories were 15-36%.

Lee et al. (34) also indicated that it is hard to reproduce experiments. They investigated renal cell carcinoma and included samples from patients with renal cell carcinoma, patients with benign urological diseases and healthy controls in the training set. An initial blind group of samples was used to test the models. Sensitivities and specificities of 81.3-83.3% were achieved. However, subsequent testing 10 months later with a different blind group of samples resulted in much lower sensitivities and specificities (41.0-76.6%).

Potential sources of variability that arise during SELDI-TOF-MS profiling include spot-to-spot variation of chip surfaces, laser detector variability over time, pipetting variability (35) and the crystallization process of the EAM (36, 37). We demonstrated that the reproducibility of the crystallisation process can be increased by using an incubator with a constant temperature of 28°C and a constant atmospheric humidity of 45%. The same QC sample (insulin and apomyoglobin) as described above was used and CV values of 4 to maximal 25% were achieved for intensities and S/N ratios.

Conclusions

Any new technology, particularly one being presented as a potential clinically used diagnostic tool, requires stringent quality control to evaluate analytical performance over time. Instrument performance, however, must be compared not only during one experiment, but also over the course of time. We recently defined a standard protocol for calibration and acceptance criteria for the independent certified QC samples were established (30). Stringent QC as indicated above prevents unreliable data acquisition from the very start.

By introducing standard protocols and strict quality control, the analytical variation of protein profiling experiments can be significantly reduced. However, further optimization of SELDI-TOF-MS is required in order to become a reliable technique for biomarker detection and only if reproducibility of SELDI-TOF-MS protein profiling is significantly improved it can become a valuable diagnostic tool in different diseases.

References

1. Srinivas PR, Srivastava S, Hanash S, Wright GL, Jr. Proteomics in early detection of cancer. Clin Chem 2001; 47 (10): 1901-11.
2. Hutchens TW, Yip T. New Desorption strategies for the mass spectrometric analysis of macromolecules. Rapid Commun Mass Spectrom 1993; 7: 576-80.
3. Bischoff R, Luider TM. Methodological advances in the discovery of protein and peptide disease markers. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 803 (1): 27-40.
4. Issaq HJ, Veenstra TD, Conrads TP, Felschow D. The SELDI-TOF MS approach to proteomics: Protein profiling and biomarker identification. Biochem Biophys Res Comm 2002; 292 (3): 587-92.
5. Caputo E, Moharram R, Martin BM. Methods for on-chip protein analysis. Anal Biochem 2003; 321 (1): 116-24.
6. Aebersold R, Goodlett DR. Mass spectrometry in proteomics. Chem Rev 2001; 101 (2): 269-95.
7. Wiesner A. Detection of tumor markers with ProteinChip(R) Technology. Curr Pharm Biotechnol 2004; 5 (1): 45-67.
8. Shiwa M, Nishimura Y, Wakatabe R, Fukawa A, Arikuni H, Ota H, et al. Rapid discovery and identification of a tissue-specific tumor biomarker from 39 human cancer cell lines using the SELDI ProteinChip platform. Biochem Biophys Res Commun 2003; 309 (1): 18-25.
9. Qu Y, Adam BL, Yasui Y, Ward MD, Cazares LH, Schellhammer PF, et al. Boosted decision tree analysis of surface-enhanced laser desorption/ionization mass spectral serum profiles discriminates prostate cancer from noncancer patients. Clin Chem 2002; 48 (10): 1835-43.
10. Gygi SP, Aebersold R. Mass spectrometry and proteomics. Curr Opin Chem Biol 2000; 4 (5): 489-94.
11. Furuta M, Shiraishi T, Okamoto H, Mineta T, Tabuchi K, Shiwa M. Identification of pleiotrophin in conditioned medium secreted from neural stem cells by SELDI-TOF and SELDI-tandem mass spectrometry. Brain Res Dev Brain Res 2004; 152 (2): 189-97.
12. Petricoin III EF, Mills GB, Kohn EC, Liotta LA. Proteomic patterns in serum and identification of ovarian cancer. The Lancet 2002; 360 (9327): 170-1.
13. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002; 359 (9306): 572-7.
14. Kozak KR, Amneus MW, Pusey SM, Su F, Luong MN, Luong SA, et al. Identification of biomarkers for ovarian cancer using strong anion-exchange ProteinChips: potential use in diagnosis and prognosis. Proc Natl Acad Sci U S A 2003; 100 (21): 12343-8.
15. Zhang Z, Bast RC, Jr., Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res 2004; 64 (16): 5882-90.
16. Vlahou A, Schorge JO, Gregory BW, Coleman RL. Diagnosis of ovarian cancer using decision tree classification of mass spectral data. J Biomed Biotechnol 2003; 2003 (5): 308-14.
17. Ye B, Cramer DW, Skates SJ, Gygi SP, Pratomo V, Fu L, et al. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. Clin Cancer Res 2003; 9 (8): 2904-11.
18. Rai AJ, Zhang Z, Rosenzweig J, Shih Ie M, Pham T, Fung ET, et al. Proteomic approaches to tumor marker discovery. Arch Pathol Lab Med 2002; 126 (12): 1518-26.
19. Banez LL, Prasanna P, Sun L, Ali A, Zou Z, Adam BL, et al. Diagnostic potential of serum proteomic patterns in prostate cancer. J Urol 2003; 170 (2 Pt 1): 442-6.

20. Adam BL, Qu Y, Davis JW, Ward MD, Clements MA, Cazares LH, et al. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. Cancer Res 2002; 62 (13): 3609-14.
21. Petricoin EF, 3rd, Ornstein DK, Paweletz CP, Ardekani A, Hackett PS, Hitt BA, et al. Serum proteomic patterns for detection of prostate cancer. J Natl Cancer Inst 2002; 94 (20): 1576-8.
22. Li J, White N, Zhang Z, Rosenzweig J, Mangold LA, Partin AW, et al. Detection of prostate cancer using serum proteomics pattern in a histologically confirmed population. J Urol 2004; 171 (5): 1782-7.
23. Zhukov TA, Johanson RA, Cantor AB, Clark RA, Tockman MS. Discovery of distinct protein profiles specific for lung tumors and pre-malignant lung lesions by SELDI mass spectrometry. Lung Cancer 2003; 40 (3): 267-79.
24. Poon TC, Hui AY, Chan HL, Ang IL, Chow SM, Wong N, et al. Prediction of liver fibrosis and cirrhosis in chronic Hepatitis B infection by serum proteomic fingerprinting: A pilot study. Clin Chem 2004; 51: 328-35.
25. Zhu XD, Zhang WH, Li CL, Xu Y, Liang WJ, Tien P. New serum biomarkers for detection of HBV-induced liver cirrhosis using SELDI protein chip technology. World J Gastroenterol 2004; 10 (16): 2327-9.
26. Hortin GL, Jortani SA, Ritchie JC, Jr., Valdes R, Jr., Chan DW. Proteomics: a new diagnostic frontier. Clin Chem 2006; 52 (7): 1218-22.
27. Diamandis EP. Serum proteomic profiling by Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry for cancer diagnosis: next steps. Cancer Res 2006; 66 (11): 5540-1.
28. Bons JA, Wodzig WK, van Dieijen-Visser MP. Protein profiling as a diagnostic tool in clinical chemistry: a review. Clin Chem Lab Med 2005; 43 (12): 1281-90.
29. Linke T, Ross AC, Harrison EH. Profiling of rat plasma by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, a novel tool for biomarker discovery in nutrition research. J Chromatogr A 2004; 1043 (1): 65-71.
30. Bons JA, de Boer D, van Dieijen-Visser MP, Wodzig WK. Standardization of calibration and quality control using surface enhanced laser desorption ionization-time of flight-mass spectrometry. Clin Chim Acta 2006; 366: 249-56.
31. Plebani M. Proteomics: the next revolution in laboratory medicine? Clin Chim Acta 2005; 357 (2): 113-22.
32. Westgard JO, Groth T, Aronsson T, de Verdier CH. Combined Shewhart-cusum control chart for improved quality control in clinical chemistry. Clin Chem 1977; 23 (10): 1881-7.
33. Semmes OJ, Feng Z, Adam BL, Banez LL, Bigbee WL, Campos D, et al. Evaluation of serum protein profiling by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. Clin Chem 2005; 51 (1): 102-12.
34. Lee SW, Lee KI, Kim JY. Revealing urologic diseases by proteomic techniques. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 815 (1-2): 203-13.
35. Koopmann J, Zhang Z, White N, Rosenzweig J, Fedarko N, Jagannath S, et al. Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. Clin Cancer Res 2004; 10 (3): 860-8.
36. Jock CA, Paulauskis JD, Baker D, Olle E, Bleavins MR, Johnson KJ, et al. Influence of matrix application timing on spectral reproducibility and quality in SELDI-TOF-MS. Biotechniques 2004; 37: 30-2, 4.
37. Cordingley HC, Roberts SL, Tooke P, Armitage JR, Lane PW, Wu W, et al. Multifactorial screening design and analysis of SELDI-TOF ProteinChip array optimization experiments. Biotechniques 2003; 34: 364-5, 8-73.

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Serum markers in breast cancer: are they of value and will they get better?

M.J. DUFFY

Available serum markers for breast cancer include CA 15-3, BR 27.29 (also known as CA27.29), CEA, tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS) and the shed form of HER-2. Of these, the most widely used are CA 15-3 and CEA (for review, see refs 1, 2). The aim of this presentation is to discuss the present and likely future use of serum markers in breast cancer.

Screening/Aiding early diagnosis

Lack of sensitivity and specificity preclude the use of all existing serum markers for the early detection of breast cancer. Women with apparently localized breast cancer who present with a high preoperative marker level (e.g., 5-10 times the upper limit of normal) are likely to have advanced disease (3) and should undergo appropriate investigations to diagnose or exclude this possibility.

Determining prognosis

A number of studies have shown that elevated preoperative levels of either CA 15-3 or CEA are associated with poor outcome in patients with breast cancer (1). For example, in our study on 600 newly diagnosed breast cancer patients, the prognostic impact of preoperative CA 15-3 levels was independent of tumour size and lymph node status (4). Importantly, the prognostic value of CA 15-3 was also observed in lymph node-negative patients, the subgroup of breast cancer patients in which new prognostic factors are most urgently needed.

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