Application of SELDI-TOF mass spectrometry in clinical evaluation of thrombotic thrombocytopenic purpura

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1. Mass spectrometer as a clinical diagnostic modality

A mass spectrometer is a spectroscopic device used to identify molecules according to mass and charge. Usually, the analytes are ionized and then separated and detected by their mass-to-charge \( (m/z) \) ratios in a magnetic field. There are two broad applications of mass spectrometer based technologies. One area involves biomarker discovery, identification of new drug targets, or amino acid sequence analyses to determine protein identities or structures. This application is currently mostly research oriented, and requires substantial support in resources and personnel expertise. The second area is related to direct
identification and measurement of specific compounds in a sample. This latter application is straightforward measurement of analytes, which is similar to many existing methods used in clinical laboratories. Mass spectrometry has been used considerably in the diagnostic industry in recent decades [1–5].

Early uses of mass spectrometry in clinical laboratories were limited to the measurement of smaller molecules ($M_r < 1000$ daltons) such as analysis of organic acids in urine for a prenatal screen of inborn errors of metabolism. Because of the complexity of sample preparation and the expertise required for interpretation of the results, these tests were primarily performed in specialized reference or university-based laboratories. Over the last two decades, new developments in ion sources have made it possible to analyze a greater variety of molecules including water soluble compounds, peptides, and proteins. In turn, the areas of clinical application of mass spectrometry have been greatly expanded. In particular, when the data from mass spectrometry is coupled with computer control and data analysis software, unique opportunities are created to routinely test for many compounds that have been difficult to measure by other clinical assays. In general, mass spectrometer based methods offer similar test accuracy, precision, and turnaround time when compared to existing clinical analysis techniques. Additionally, the ion peak that gives rise to the readout of a specific analyte is available for separate evaluation when necessary. This practice of manually evaluating the result of a particular analyte can be very useful when a laboratory encounters an instrument error that produces an incorrect reading of test results.

Currently, immuno-assay is the predominant method of testing for many analytes in diagnostic laboratories. However, there are limitations that prevent using antibody based detection for measurement of all analytes. For example, the antibody to some molecules such as steroid hormones may not always be sufficiently specific. In this case, measurement by tandem mass spectrometric assay would be a better method for more accurate testing [6,7]. In other cases, targeted analytes can be immuno-captured by affinity matrix and then subject to MALDI mass spectrometer analysis to evaluate effects due to post-translational modification, proteolysis and genetic variation [8]. For the detection of small molecules, mass spectrometers have proven to be particularly useful in drug screening in such applications as antivirus therapy for HIV [9], drug monitoring [10,11], newborn screening [5], drug pharmacokinetics [12], and toxicology screening [13–15]. These unique applications have revealed the enormous utility of the mass spectrometer in clinical testing and the potential for much wider application in the future.

2. Unique application of SELDI-TOF mass spectrometer

SELDI-TOF mass spectrometry involves the proteinChip Array technology [16]. As a mass spectrometer, SELDI-TOF does not offer the high mass accuracy that is needed for protein identity determination and for protein structural analysis. However, SELDI-TOF does provide adequate sensitivity for separation and measurement of peptides and proteins in biological samples. The most important feature of SELDI-TOF comes from different arrays or chip surfaces that are available for isolating a specific compound or a subset of substances. These arrays allow for selective purification and/or enrichment of target peptides/proteins of interest on the array. Following ionization by a laser beam, the target analyte(s) can be measured by the mass spectrometer. Several kinds of arrays are available, each coated with specific chemical groups such as immobilized metal affinity capture (IMAC) array, cation or anion exchange array, or an array with a hydrophobic property. Therefore, it is possible to pre-select a particular array based on the properties of the target analytes, enrich a target molecule, and then detect it by mass spectrometer.

The readout from the mass spectrometer is relatively easy to interpret. The mass to charge ratio ($m/z$), also described as the peak position, provides the identity of the analyte. The relative abundance of the
analyte can be determined by evaluating the intensity of the peak or peak area. However, it should be made clear that mass spectrometry is not inherently quantitative. When peptides or proteins are ionized, the extent of ionization varies from sample to sample, causing significant inter-assay variation [17,18]. With regard to SELDI-TOF, there are possible heterogeneities in surface chemistry between different array spots or between different batches of protein arrays. These factors mandate inclusion of an internal control for calibration when SELDI-TOF is used for quantification of a compound. Ideally, the internal control is a known compound that interacts specifically with the selected array as the analyte. When the same amount of internal control is added to all the measurements, the quantity of the analyte can be calculated as a ratio to the internal control. This approach minimizes the variations due to chemical matrix of the arrays or due to the detection variations intrinsic to mass spectrometry. Figure 1 illustrates

Fig. 1. Use of internal control in SELDI-TOF based test. Panel A shows the readout of an analyte without including an internal control (IC). Panel B illustrates the readout with an IC. In this case, the analyte is quantified as a ratio to IC, therefore minimizing inter-assay variations caused by experiments, proteinChips, and mass spectrometer.
Fig. 2. Measurement of ADAMTS13 activity. Recombinant vWF73 fusion protein containing the ADAMTS13 cleavage site is cleaved by ADAMTS13, generating a $\sim 7739$ dalton peptide tagged with histidine. This small peptide specifically binds to nickel charged IMAC proteinChip and then is quantified by SELDI-TOF with a proper internal control.

Thrombotic Thrombocytopenic Purpura (TTP) is a life-threatening disorder that is caused by a deficiency of a metalloproteinase, ADAMTS13 (A Disintegrin And Metalloproteinase with a Thrombospondin type 1 motif, member 13). ADAMTS13 cleaves von Willebrand factor (vWF) \textit{in vivo}, which prevents platelet thrombosis [19]. When a vWF fusion protein with histidine tag was used as a substrate for ADAMTS13, a $\sim 7739$ dalton peptide containing a 6 X histidine tag was generated in an amount corresponding to the plasma ADAMTS13 activity in TTP patients [20,21]. This finding creates the preconditions for the design of an assay in which ADAMTS13 cleaved product is selected by IMAC proteinChip and then measured by SELDI-TOF with a proper internal control.
Table 1

Determination of ADAMTS13 autoantibody titer in Bethesda unit (BU)

<table>
<thead>
<tr>
<th>Sample dilution in saline</th>
<th>Sample mix (equal volume)</th>
<th>ADAMTS13 activity</th>
<th>Residual ADAMTS13 activity</th>
<th>BU calculated from the residual activity</th>
<th>Final BU after multiplying by the dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>PNP + saline</td>
<td>50.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiluted</td>
<td>PNP + undiluted patient plasma</td>
<td>1.4% 2.8%</td>
<td>3.1</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>PNP + patient plasma at 1:2 dilution</td>
<td>1.7% 3.4%</td>
<td>3.1</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td>PNP + patient plasma at 1:4 dilution</td>
<td>9.5% 19.0%</td>
<td>2.4</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>1:8</td>
<td>PNP + patient plasma at 1:8 dilution</td>
<td>16.8% 33.5%</td>
<td>1.4</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>1:16</td>
<td>PNP + patient plasma at 1:16 dilution</td>
<td>27.8% 55.7%</td>
<td>0.8</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>1:32</td>
<td>PNP + patient plasma at 1:32 dilution</td>
<td>37.8% 75.5%</td>
<td>0.4</td>
<td>12.8</td>
<td></td>
</tr>
</tbody>
</table>

Average = 12.16

Thus, the capture of 7739 dalton peptide by IMAC proteinChip is relatively specific in a way analogous to the antibody/antigen reaction in the immuno-based assays. Moreover, the mass of the analyte, a 7739 dalton peptide derived from the cleavage of vWF by ADAMTS13, is in the best mass range whereby a compound undergoes adequate ionization by laser beam and then can be effectively detected by the SELDI-TOF mass spectrometer. In our experience, SELDI-TOF appears to offer the best test sensitivity and reproducibility when analyzing peptides with a mass range from 5 to 20 kD. Figure 2 depicts how the ADAMTS13 activity is measured by SELDI-TOF mass spectrometer.

Most cases of TTP are due to production of ADAMTS13 autoantibody that somehow impairs the functional properties of ADAMTS13 in vivo [22,23]. An accurate measurement of antibody activity may be of value in the evaluation of TTP’s clinical course and to help predict the imminent relapse of TTP [22, 24]. In our laboratory, determination of ADAMTS13 autoantibody inhibitory titers is performed using a similar approach as described for the determination of Bethesda unit (BU) in patients with a factor VIII inhibitor [25]. As shown in Table 1, ADAMTS13 activity was determined after pooled normal plasma (PNP) was mixed with an equal volume of patient plasma made at various dilutions. Afterwards, the ADAMTS13 activity from each sample was divided by the ADAMTS13 activity obtained from PNP control to obtain the residual activity. The BU was then extrapolated from the residual activity according to the standard Bethesda graph [26,27]. Next, the BU from each diluted sample was calculated by multiplying sample’s dilution factors. Finally, ADAMTS13 antibody inhibitory titer (BU) in this patient was determined by averaging BU values obtained from three diluted samples that gave rise to a residual activity around 50%.

3. Clinical evaluation of patients with thrombotic thrombocytopenic purpura

TTP is a thrombotic microangiopathy that frequently leads to devastating thrombotic complications and sometimes death [28–32]. ADAMTS13 protease deficiency is a primary risk factor in pathogenesis of TTP [33]. ADAMTS13 is a physiological metalloproteinase that cleaves von Willebrand factor (vWF) multimers and prevents over-polymerization of vWF [34]. In adult patients, TTP is mostly due to the production of ADAMTS13 autoantibodies, which impair ADAMTS13 function leading to an acquired ADAMTS13 deficiency [23,35,36]. In the absence of ADAMTS13, vWF undergoes excessive polymerization that eventually leads to an uncontrolled platelet thrombosis and onset of TTP. With a timely diagnosis, TTP patients usually respond well to daily plasma exchange therapy. However, more than half of these patients experience disease recurrences, resulting in considerable mortality and morbidity.
Altogether, there are two key clinical issues in the management of TTP patients. First, there is a tremendous need for a rapid diagnostic test to help clinicians to rapidly initiate the proper therapy. Second, there is demand for a biomarker that predicts the recurrence of TTP and allows for early prophylactic treatment to improve clinical outcome.

Because the treatment with plasma exchanges (PE) can reduce the mortality of TTP from greater than 80% to less than 20% [37], a rapid diagnostic test is critical for clinicians to initiate timely therapy. In contrast, many patients with other serious medical problems such as complications of bone marrow transplantation, human immunodeficiency virus infection, or disseminated malignancy may present similar clinical manifestations as TTP. Most of these patients however have normal ADAMTS13 function and do not typically respond to PE therapy [38–41]. An adequate measurement of ADAMTS13 activity will help clinicians correctly recognize these complicated clinical conditions, prevent wasting of valuable blood components, and avoid unnecessary complications related to PE therapy and catheter placement [42]. Since the implementation of our SELDI-TOF based method [21], fast turnaround time for the detection of ADAMTS13 activity has greatly helped us in making the correct diagnosis, instituting appropriate therapy, and improving patient outcomes [43–45]. Likewise, several other methodologies to detect ADAMTS13 activity have been reported [20,35,36,46–54].

The current regimen of PE therapy has generally been effective in the induction of clinical remission [37]. However, more than half of TTP patients still experience disease recurrences, resulting in considerable mortality and morbidity [28]. The post-thrombotic sequelae in TTP include chronic renal insufficiency and neurological complications. Clinical biomarkers that predict disease activity and TTP’s recurrences would be of tremendous value, and allow for early prophylactic treatment. In our initial study, we correlated TTP disease activity with ADAMTS13 antibody level in sequentially collected samples from three TTP patients [24]. Interestingly, one of the three patients displays clear discordance between antibody amount and clinical course [24]. This discovery prompted us to use our SELDI-TOF mass spectrometer based method to evaluate the inhibitory titer of ADAMTS13 autoantibody in all samples from this patient. Consistent with our prediction, ADAMTS13 antibody functional activity indeed correlated with the clinical course of the disease [24]. This initial discovery suggests that the functional characteristics or neutralizing activity of the antibody inhibitor may be of value as a surrogate biomarker to evaluate disease activity in TTP and to predict TTP recurrences.

In summary, this review article illustrates a unique application of SELDI-TOF mass spectrometry to measure ADAMTS13 activity and to evaluate ADAMTS13 autoantibody activity. Both of these parameters are keys in the pathobiology and clinical manifestation of TTP. Importantly, this “proof of concept” application highlights a new area of future diagnostic approaches in which key pathogenic enzymes can be rapidly measured by SELDI-TOF based technologies.

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References

REFERENCES


