Detection and Determination of Protein Network Associated with Atrial Fibrillation Phenotypes

Oliver Klein\textsuperscript{1,2}, Thorsten Hanke\textsuperscript{1,3}, Junfeng Yan\textsuperscript{1}, Grit Nebrich\textsuperscript{1}, Sophie Krause\textsuperscript{1}, Herbert Thiele\textsuperscript{1} and Salah A Mohamed\textsuperscript{1,4,5}\footnote{Corresponding author: Salah A Mohamed, Department of Cardic and Thoracic Vascular Surgery, University of Luebeck, Luebeck, Germany, Tel: +49-451-5006425; Email: salah.mohamed@uksh.de} \\
\textsuperscript{1}Department of Cardic and Thoracic Vascular Surgery, University of Luebeck, Luebeck, Germany \\
\textsuperscript{2}Charité-Universitätsmedizin, Berlin, Berlin-Brandenburger Centrum for Regenerative Therapien Campus Virchow-Klinikum, Berlin, Germany \\
\textsuperscript{3}Fraunhofer Institute for Medical Image Computing MEVIS, Bremen, Germany \\
\textsuperscript{4}Both authors have contributed equally to this work \\
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Abstract

Atrial fibrillation (AF) is associated with increased risks of stroke, cardiac failure, and mortality. Since the discrimination of AF phenotype is inadequate, accurate diagnosis remains elusive. Left atrial appendage tissue resected routinely during the maze procedure was collected from patients with paroxysmal, persistent, and long-standing persistent arrhythmia. In situ comprehensive proteomic approaches of matrix-assisted laser desorption/ionization imaging mass spectrometry was used to differentiate and classify the spatial molecular processes in the pathology of AF phenotypes. Using unsupervised computational evaluation strategy, probabilistic latent semantic clustering, and receiver operating characteristic analysis (SCILS Lab), the acquired peptide signatures and characteristic m/z species could be used to assign the AF phenotype. Intensity distribution of the given m/z values, which are discriminative for the considered cluster, was determined to distinguish between paroxysmal and persistent AF (mean, 4.08 ± 1.21 vs 1.59 ± 0.12, p=0.09) and persistent and long-standing persistent AF (1.59 ± 0.12 vs 6.85 ± 3.02, p=0.02). Tissue-based proteomic approach provides clinically relevant information, which may be beneficial in improving risk stratification in AF patients.

Keywords: Atrial fibrillation; MALDI-imaging; Imaging mass spectrometry

Introduction

The development of matrix-assisted laser desorption/ionization (MALDI)-imaging mass spectrometry (IMS) has opened new horizons for mass spectrometry in biology and medicine [1-3]. MALDI imaging has become a mature technology, allowing reproducible high-resolution measurements to localize proteins and smaller molecules for several purposes, particularly to detect and discover new biomarkers, with a major focus in cancer research [4].

MALDI-IMS can determine the spatial distribution of several molecular compounds in a single measurement by collecting mass spectra across a flat sample (e.g., a tissue section, plant tissue, and agar slice). MALDI-IMS allows the reconstruction of molecular images based on the spatial distribution of molecules. Each mass spectrum is measured at a spatial pixel with an assigned pair of spatial coordinates x and y and represents a plot of relative abundances of ionizable molecular compounds along their mass-to-charge (m/z) ratio. MALDI-IMS can determine the molecular masses of unknown compounds with specific spatial localization or establish spatial localization of known molecular compounds with known molecular masses. In proteomics, MALDI-IMS serves as a superior discovery tool to image the spatial distribution of molecular compounds, thus complementing immunohistochemistry or genetics-based methods such as in situ hybridization [5,6]. In metabolomics, MALDI-IMS is used for discovery of antibiotics and imaging of drugs and their metabolites [7,8].

According to Watrous et al., the primary strength of an IMS-based analytical technique is the ability to visualize multiple molecular distributions across the sample surface [9]. The application of this technique does not require chemical labels or antibodies and maintains the morphological and molecular integrity of the measured tissue. Therefore, molecular histology using mass spectrometry-based imaging is a promising new field for biomarker discovery, drug metabolite profiling, lipid analysis, and proteomics. In the last decade, IMS has seen incredible technological advances in its applications to biological samples, e.g., human and animal tissues. Moreover, new samples are continuously being analysed, particularly bacterial films, whole animal body sections, plant tissues, grains, and insect larvae. Therefore, MALDI-IMS was applied for tissue classification and therapy stratification [4,10,11]. This technology is driven by the advances in instrumentation and bioinformatics, thus widening the range of applications to which it can be applied. Here, we present for the first time the use of MALDI-IMS to study the mechanisms of atrial remodelling. The primary advantage lies within the possibility of using adult human tissue. In this study, we hypothesized that our MALDI-IMS workflow is suitable to facilitate the differentiation of AF phenotypes in spatial visualization of atrial remodelling in humans.

Materials and Methods

Sample collection and preparation

The study conforms to the principles outlined in the Declaration of Helsinki for use of human tissue. Patients with paroxysmal (PX), persistent (PE), and long-standing persistent (LP) arrhythmia were
included in this study. The study protocol was approved by the institutional ethics committee (reference number 08-046/08-050), and written informed consent was obtained from all patients. Atrial tissue routinely removed during the maze procedure was collected during surgery and carefully divided into two parts. One part was immediately snap frozen in liquid nitrogen and preserved at -80°C to be used later for gene expression and further protein analyses. The second part was fixed in 4.5% paraformaldehyde, embedded in paraffin, and prepared on slides suitable for MALDI-IMS. Briefly, paraformaldehyde-fixed specimens were dehydrated by washing successively with increasing concentrations of ethanol (70, 80, 96, 100%), cleared in xylene (for 1 and 1.5 h), and embedded in paraffin. Three 7 µm thick sections of each AF phenotype were prepared from the paraffin blocks and transferred onto the surface of the indium-tin oxide-coated glass slide (Bruker Daltonik, Bremen, Germany). The sections were de-waxed twice in xylene for 3 min each and passed through decreasing concentrations of ethanol (2 × 100%, 95%, and 70%) for 1 min each. Endonuclease and trypsin solution were applied directly onto each section using an automated spraying device. The tissue was incubated with trypsin solution for 3 h at 37°C in a moist chamber. Following trypsinization, the matrix solution (α-cyano-4-hydroxycinnamic acid) was applied in using manufacturer’s protocol (Bruker Daltonik, Bremen, Germany). MALDI-IMS data acquisition was performed on an Autoflex III MALDI-TOF/TOF with flexControl 3.0 and flexImaging 3.0 softwares (Bruker Daltonik, Bremen, Germany) in a positive ion reflector mode and in a range of m/z 800-3000 Da and a raster width of 80 µm.

Results

Patients diagnosed with AF were divided into subcategories according to the latest guidelines established by the European Society of Cardiology (ESC) in 2012 [13]. The characteristics of the patients are shown in Table 1.

![Figure 1: Detection average spectra from AF phenotypes.](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Paroxysmal AF (n=3)</th>
<th>Persistent AF (n=3)</th>
<th>Long-standing persistent AF (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>63.67 ± 1.76</td>
<td>71.33 ± 4.70</td>
<td>73.33 ± 3.53</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>1:2</td>
<td>2:1</td>
<td>1:2</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MV-vitium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>33.30%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>MS</td>
<td>33.30%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CMV</td>
<td>33.30%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CHF-NYHA (mean ± SD)</td>
<td>3 ± 0</td>
<td>3.33 ± 0.33</td>
<td>3 ± 0</td>
</tr>
</tbody>
</table>

Table 1: Patient characteristics. AF: Atrial Fibrillation; Age: Years; Gender: Male:Female; MV-vitium: Mitral Valve Vitium; CHF: Chronic Heart Failure; NYHA: New York Heart Association.

![Figure 2: Classification of the mass spectra from AF phenotypes by probabilistic latent semantic analysis.](image)

Detection of peptide signatures in AF phenotypes

The primary proteomic screenings were simultaneously performed using tissue sections from the PX, PE, and LP AF patient groups. Using the segmentation approach, 431 m/z values in a mass range between 800-3000 Da and a raster width of 80 µm.
m/z 800 and 3,000 (threshold: 0.107) were extracted by peak picking and used to compare the PE, PX, and LP AF patient groups using SCiLS Lab software. Figure 1 shows the average spectra for the three patient groups.

Comparison of the mass spectra from AF phenotypes

Peptide signature was detected by pLSA, which allowed the discrimination of PX, PE, and LP AF patients. The pLSA-component 1 (pLSA-C1), pLSA-component 2 (pLSA-C2) and pLSA-component 3 (pLSA-C3), results in discrimination of spectra from PX, PE, to LP (Figure 2A). In particular, the spectra from LP AF tissue are characterized by low values for pLSA-C1 and high values for pLSA-C3 (Figure 2B). Subsequent to pLSA, ROC analysis was used for the automatic identification of discriminating masses (m/z value) between two AF states. ROC analysis judges the performance of an m/z value as a binary classifier based on a discrimination threshold. Pairwise comparison of the AF patient subtypes (PX, PE, and LP) was performed to obtain a peak list of discriminative m/z values. This analysis identified discriminative m/z values for characterizing selected pathophysiological AF subtypes (ROC (AUC)=0.61, <0.4]. Among these were 180 m/z values that discriminate PX and LP AF patients, 119 m/z values that separate PE and LP AF patients and 128 that distinguish PX and PE AF patients (Supplementary Table 1).

For example, the m/z values 1770 ± 0.125% Da, 1547 ± 0.125% Da, and 1696 ± 0.125% Da distinguish PE and PX from LP AF subtypes (Figure 3).

Discussion

MALDI-IMS plays an important role because of its unique advantages of sensitivity, wide dynamic range, molecular specificity, and flexibility to address several varied analytic. AF is the most commonly sustained cardiac arrhythmia and is responsible for stroke and heart failure. The prevalence of this disease is estimated to be more than twice between 2001 and 2050 [14]. The bedlam contraction of the atrium, including supraventricular arrhythmia, is divided into different pathological mechanisms that contribute to the development of AF. This begins with distinguishing the first detectable episode, irrespective of whether it is symptomatic or self-limited. Published guidelines from the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology (ACC/AHA/ESC) conclude that the complex cellular processes and networks involved in the pathological mechanisms that contribute to the development of AF is currently not well understood.

Figure 3: Characteristic m/z values from AF phenotypes. Spatial distribution of characteristic m/z values for paroxysmal (PX), persistent (PE), and long-standing persistent (LP) atrial fibrillation types. The m/z value of 1171.37 showed significantly higher spatial intensities in LP and PE than that in PX. Further, the m/z values 1019.60 indicate lower spatial intensities in PX in comparison to LP and PE. The corresponding m/z values 1532.99 show an increase in spatial intensities in PX in comparison to LP and PE.
and preservation of atrial fibrillation is miR-208a. This miRNA is associated with the origin of arrhythmia and fibroses. It belongs to a family of miRNAs which encode for the myosin heavy chain genes and is intronic miRNA. The Mhy6 gene encodes for a heavy chain of the Myosin. Myosin allows the contraction of the heart. Many miRNAs, including miRNA 208a, can be detected in blood where they are either bound to proteins or enveloped in small membranous particles like exosomes. Giving that fact, that single miRNA may repress the translation of hundreds of proteins; we detected and determined specifically for the first time using the MALDI-IMS technique unique links that indicate the spatial characteristics of pathophysiological processes and is able to support the discrimination of molecular processes of AF phenotypes [11]. By knowing the exact type of AF in the patient, the physician can individualize a proposed tapered strategy more easily and can also report the probable outcome of the planned treatment option to the patient with a higher level of certainty. Furthermore, with the knowledge of the exact AF type, the physician can obtain a thorough insight into the patient's risk of neurological complications, as there is evidence that the rate of strokes is higher in LP AF patients than in PX AF patients.

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References