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Detection and Determination of Protein Network Associated with Atrial Fibrillation Phenotypes

Oliver Klein[2#], Thorsten Hanke[1#], Junfeng Yan[1], Grit Nebrich[2], Sophie Krause[1], Herbert Thiele[3] and Salah A Mohamed[1*]

[1]*Department of Cardic and Thoracic Vascular Surgery, University of Luebeck, Luebeck, Germany*

[2]*Charité-Universitaetsmedizin, Berlin, Berlin-Brandenburger Centrum for Regenerative Therapien Campus Virchow-Klinikum, Berlin, Germany*

[3]*Fraunhofer Institute for Medical Image Computing MEVIS, Bremen, Germany*

[#]*Both authors have contributed equally to this work*

[*]**Corresponding author:** Salah A Mohamed, Department of Cardic and Thoracic Vascular Surgery, University of Luebeck, Luebeck, Germany, Tel: +49-451-5006425; E-mail: salah.mohamed@uksh.de

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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 ionisable 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.

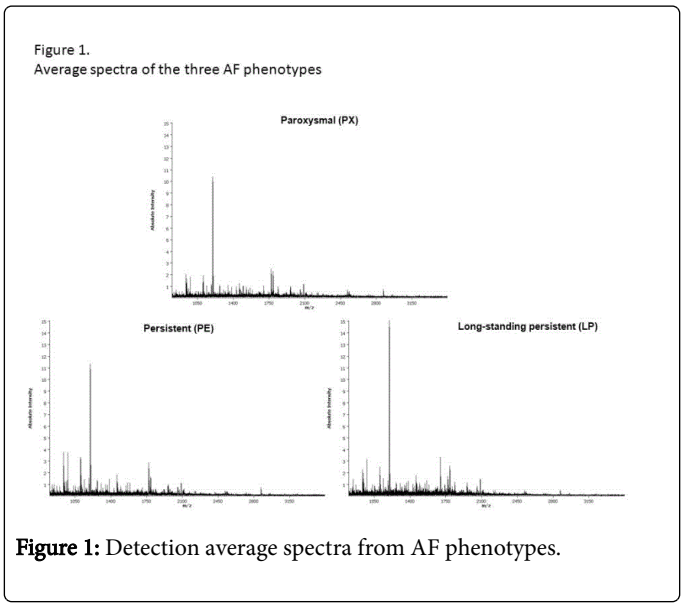

Figure 1: Detection average spectra from AF phenotypes.

Statistics

Statistical analysis was performed using excel/WIN STAT and SPSS. The data obtained from each experiment are presented as mean ± standard deviation. For a priori identification of intratumor biomolecular heterogeneity, the multivariate nature of MALDI imaging data (simultaneous detection of several proteins) was applied using several methods to cover a broad range of information present in the mass spectra of the protein/peptide imaging (e.g., principal component analyses, hierarchical cluster analyses, receiver operating characteristic (ROC) curve, probabilistic latent semantic analysis (pLSA), and colocalization analysis, as detailed by Deininger et al., [12]). Mass spectra for the AF groups (PX, PE, and LP) were extracted by segmentation approach and statistical comparison was performed in the software SCiLS Lab (SCiLS GmbH, Bremen, Germany).

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.

Characteristic	Paroxysmal AF (n=3)	Persistent AF (n=3)	Long-standing persistent AF (n=3)
Age (mean ± SD)	63.67 ± 1.76	71.33 ± 4.70	73.33 ± 3.53
Gender (M:F)	1:02	2:01	1:02
Comorbidities			
MV-vitium			
MI	33.30%	100%	100%
MS	33.30%	0%	0%
CMV	33.30%	0%	0%
CHF-NYHA (mean ± SD)	3 ± 0	3.33 ± 0.33	3 ± 0

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.

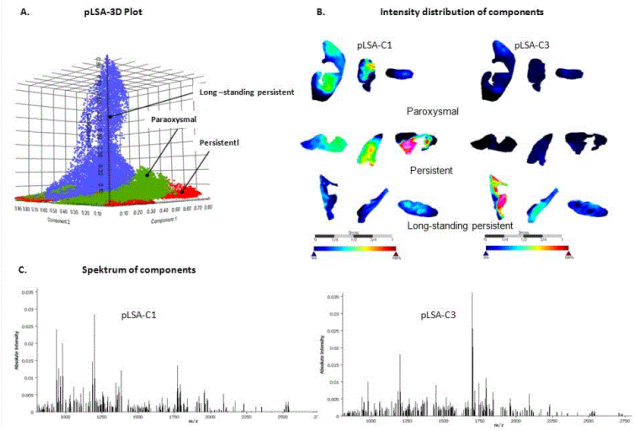

Figure 2: Classification of the mass spectra from AF phenotypes by probabilistic latent semantic analysis. **(A)** pLSA-component 1 (pLSA-C1), pLSA-component 2 (pLSA-C2) and pLSA-component 3 (pLSA-C3) results in discrimination of spectra from PX, PE, to LP. **(B)** In particular, the spectra from LP AF tissue are characterized by low values for pLSA-C1 and high values for pLSA-C3.

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

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).

Detection of the discriminative peptides/protein (m/z values) in AF phenotypes

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).

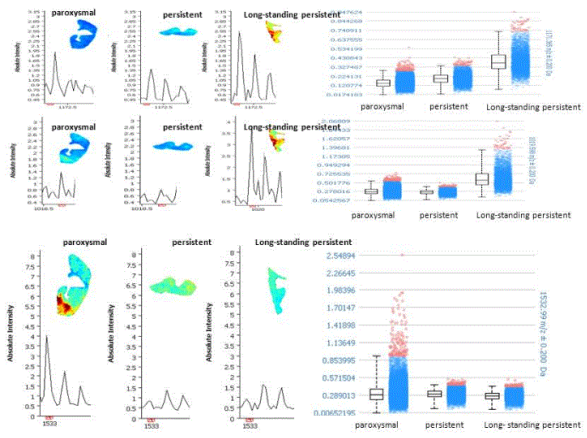

Figure 3: Characteristic m/z values from AF phenotypes. Spatial distribution of characteristic m/z values for paroxysmal (PX), persistent (PE), and long-standing persistent (LP) atrial fibrillation types. The m/z value of 1171.37 showed significantly higher spatial intensities in LP and PE than that 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.

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) committee of experts on the treatment of AF patients recommend a classification comprising the following three patterns: PX AF, episodes terminating spontaneously within 7 days (most episodes last less than 24 h); PE AF, episodes lasting more than 7 days and may require either pharmacologic or direct current intervention to terminate; and LP AF, episodes persisting for more than 1 year, because either cardio version has failed or cardio version has not been attempted [13]. Thus, by implementation of a consensus statement for classification, it was expected that a higher level of exact clinical judgment could be reached. However, in routine clinical practice, there is a high rate of AF misinterpretation, most probably due to patient-specific characteristics, e.g., symptomatic versus asymptomatic episodes. Charitos et al. showed that the rates of misclassification are up to 21% among the AF phenotypes [15]. The differential diagnosis is then based on objective digital visualization methods, which improves the diagnosis accuracy and thereby the individualized planning of treatment. Furthermore, a subjective misinterpretation and accordingly wrong therapies could decrease. In the present study, we highlighted for the first time the use of a comprehensive proteomics, the MALDI-IMS approach, to facilitate better understanding of AF and the complex cellular processes and networks involved in its pathology. AF can be caused by various mechanisms, the precise details of which remain unclear. Basically, the pathophysiology can be roughly divided into two primary mechanisms: ectopy and re-entry. In ectopy, irregular pacemaker cells from the atrium are located in the pulmonary vein. As the so-called triggers, they induce preterm atrial excitation. Re-entry represents the occurrence of circulating excitations in the atrium. These are caused by electric and structural remodelling processes. Typical of electric remodelling are abbreviated action potentials and effective atrial refractory periods of atrial cells. These are due to the intracellular calcium overload caused by a large number of atrial excitations. AF, however, is additionally enhanced and maintained by electric remodelling [16,17]. Besides the electrophysiological changes, AF may also lead to morphologic alterations in the atrial tissue. Fibrosis is the principal characteristic feature of structural remodelling. Further, frequently occurring alterations include atrial myocyte hypertrophy, enlarged nuclei and mitochondria, myocyte degeneration and apoptosis, and glycogen accumulation. These contribute to perturbed excitation and conduction and further influence atrium contractility [18]. Recent studies have highlighted the role of expression changes in microRNAs to support the diagnosis of the severity of AF status [19]. MiRNAs are small RNA molecules which functions in post-transcriptional regulation of gene expression. It is known, that miRNAs can be involved in the origin of atrial fibrillation through the influence of the expression of ion canals and change thus the management of excitement. One of these miRNA which could be involved in the origin

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

1. Caprioli RM, Farmer TB, Gile J (1997) Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. Anal Chem 69: 4751-4760.
2. Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM (2001) Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. Nat Med 7: 493-496.
3. Yanagisawa K, Shyr Y, Xu BJ, Massion PP, Larsen PH, et al. (2003) Proteomic patterns of tumour subsets in non-small-cell lung cancer. Lancet 362: 433-439.
4. Rauser S, Marquardt C, Balluff B, Deininger SO, Albers C, et al. (2010) Classification of HER2 receptor status in breast cancer tissues by MALDI imaging mass spectrometry. J Proteome Res 9: 1854-1863.
5. Rauser S, Deininger SO, Suckau D, Höfler H, Walch A (2010) Approaching MALDI molecular imaging for clinical proteomic research: current state and fields of application. Expert Rev Proteomics 7: 927-941.
6. Franck J, Arafah K, Elayed M, Bonnel D, Vergara D, et al. (2009) MALDI imaging mass spectrometry: state of the art technology in clinical proteomics. Mol Cell Proteomics 8: 2023-2033.
7. Kroiss J, Kaltenpoth M, Schneider B, Schwinger MG, Hertweck C, et al. (2010) Symbiotic Streptomycetes provide antibiotic combination prophylaxis for wasp offspring. Nat Chem Biol 6: 261-263.
8. Yang YL, Xu Y, Straight P, Dorrestein PC (2009) Translating metabolic exchange with imaging mass spectrometry. Nat Chem Biol 5: 885-887.
9. Watrous JD, Alexandrov T, Dorrestein PC (2011) The evolving field of imaging mass spectrometry and its impact on future biological research. J Mass Spectrom 46: 209-222.
10. Meding S, Nitsche U, Balluff B, Elsner M, Rauser S, et al. (2012) Tumor classification of six common cancer types based on proteomic profiling by MALDI imaging. J Proteome Res 11: 1996-2003.
11. Klein O, Strohschein K, Nebrich G, Oetjen J, Trede D, et al. (2014) MALDI imaging mass spectrometry: discrimination of pathophysiological regions in traumatized skeletal muscle by characteristic peptide signatures. Proteomics 14: 2249-2260.
12. Deininger SO, Ebert MP, Fütterer A, Gerhard M, Röcken C (2008) MALDI imaging combined with hierarchical clustering as a new tool for the interpretation of complex human cancers. J Proteome Res 7: 5230-5236.
13. Camm AJ, Lip GY, De CR, Savelieva I, Atar D, et al. (2012) 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation-developed with the special contribution of the European Heart Rhythm Association. Eur Heart J 33: 2719-2747.
14. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, et al. (2001) Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. JAMA 285: 2370-2375.
15. Charitos EI, Purerfellner H, Glotzer TV, Ziegler PD (2014) Clinical classifications of atrial fibrillation poorly reflect its temporal persistence: insights from 1,195 patients continuously monitored with implantable devices. J Am Coll Cardiol 63: 2840-2848.
16. Wakili R, Clauß S, Kääb S (2012) [Molecular mechanisms of atrial fibrillation: potential role of microRNAs as new therapeutic targets and potential biomarkers]. Herz 37: 166-171.
17. Woods CE, Olgin J (2014) Atrial fibrillation therapy now and in the future: drugs, biologicals, and ablation. Circ Res 114: 1532-1546.
18. Zhang Y, Dong D, Yang B (2011) Atrial remodeling in atrial fibrillation and association between microRNA network and atrial fibrillation. Sci China Life Sci 54: 1097-1102.
19. Radtke A, Hanke T, Yan J, Godau B, Cordes J, et al. (2014) MicroRNA 208 in Atrial Fibrilation. J Clin Exp Cardiolog 5: 325.