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Review article

Utility of mass spectrometry for the diagnosis of the unstable coronary plaque

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ABSTRACT
Mass spectrometry is a powerful technique that is used to identify unknown compounds, to quantify known materials, and to elucidate the structure and chemical properties of molecules. Recent advances in the accuracy and speed of the technology have allowed data acquisition for the global analysis of lipids from complex samples such as blood plasma or serum. Here, mass spectrometry as a tool is described, its limitations explained and its application to biomarker discovery in coronary artery disease is considered. In particular an application of mass spectrometry for the discovery of lipid biomarkers that may indicate plaque morphology that could lead to myocardial infarction is elucidated.

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INTRODUCTION

Mass spectrometry is an indispensable tool for chemical analysis owing to its speed, sensitivity, and versatility. The last two decades have seen rapid and significant developments in MS instrumentation, experimental methods and data analysis approaches.[1–3] These developments have led to the possibility of lipidomic analyses where thousands of lipid ions are measured. Software suited to analyzing mass spectrometry data sets can identify the lipids, determine their relative concentrations between samples and statistically analyze significant differences. This ability has led to this procedure being used for complex samples such as can be found in biological fluids, tissues and cell cultures.[4,5] Therefore the field of lipidomics can now be applied to a number of applications related to understanding mechanisms of disease and in the determination of prognostic markers of disease.[5] This article provides a summary of the use of mass spectrometry in the field of biomarker discovery in coronary artery disease (CAD) using the lipidomics platform.

VASCULAR BIOLOGY OF CORONARY ATHEROGENESIS

The first step to atherogenesis is lipoprotein accumulation in the arterial intima due to a high cholesterol and saturated fat diet.[6] Leukocyte recruitment and accumulation also occurs early in lesion generation and move through the endothelial layer into the intima. The leukocytes then accumulate lipids and become foam cells.[7] These foam cells are also rich in proinflammatory mediators and these mediators promote inflammation in the plaque.[8,9] The innate immune system is also known to respond to the lesion by the recognition of danger-associated molecular patterns found on oxidized-low density lipoproteins and apoptotic cells that provide a continuous trigger for inflammatory responses.[10] There is also evidence of adaptive immunity involvement in plaque progression with candidate antigens including modified lipoproteins.[11] Following atheroma initiation, smooth muscle cells (SMC) accumulate in the advancing atheroma after recruitment from the underlying media into the intima and become morphologically distinct from the less mature phenotype seen in the normal arterial medial layer.[12] These SMCs undergo cell replication and cell death and add to the complication of the atherosclerotic plaque. Extracellular matrix, such as collagen, secreted by the SMCs then builds up to make up much of the volume of the advanced plaque and forms a fibrous cap over the growing plaque. The plaque also develops areas of calcification.

Initially the plaque grows outward in an abluminal direction; however after the plaque burden exceeds approximately 40% of the cross-sectional area of the artery, luminal stenosis starts to occur. But rather than the progressive growth of the intimal lesion to a critical stenosis, thrombosis of a not necessarily occlusive plaque most often causes episodes of unstable angina and MI. Thrombosis occurs either by fracture of the fibrous cap or superficial erosion of the intima.

Fracture of the fibrous cap accounts for the cause of two-thirds of acute MI. The strength of the plaque's fibrous cap undergoes dynamic regulation and thinning occurs perhaps as a result of reduced collagen synthesis and increased degradation. The site of the fracture is usually poor in SMCs responsible for collagen synthesis and rich in macrophages which secrete matrix-degrading enzymes that break down collagen. In addition, the rupture site is rich with foam cells. Superficial erosion of the intima accounts for one quarter of acute MIs that result in sudden death. The pathobiology of superficial erosion is not understood well. However, desquamation of epithelial cells and degradation of nonfibrillar collagen found in the basement membrane are thought to contribute. Repeated cycles of plaque disruption, thrombosis *in situ* and healing are thought to contribute to lesion evolution and plaque growth.[13]

Insufficient control of risk factors associated with coronary artery disease (CAD) such as obesity, smoking, hypertension, diabetes mellitus, dietary patterns and psychosocial factors cause a high rate of hospital admissions due to acute myocardial infarction (MI).[14] Strategies to detect MI before it occurs could aid healthcare professionals to prevent the occurrence of the MI event in susceptible patients. This task is made difficult by the fact that MI and sudden cardiac death are the first manifestations of coronary atherosclerosis in the majority of patients.[15,16] Therefore a safe and low cost prognostic technology for the detection of MI in asymptomatic patients is required.

PREDICTING RISK FOR CORONARY ARTERY DISEASE IN ASYMPTOMATIC PERSONS

Current risk stratification strategies for CAD involve exercise stress testing with electrocardiography or with radionucleotides and echocardiography. This assessment works well for the evaluation of patients

with symptoms and known coronary heart disease, however, it has low predictive value in asymptomatic patients.[17] Risk modeling based on epidemiological studies to estimate the probability of future death or myocardial infarction (MI) in an individual suffer from several flaws, in particular the inability to incorporate new risk factors, the impact of diet, estrogens, personality traits and physical activity.[17] Coronary artery calcium (CAC) scanning by computed tomography without the use of a contrast agent is a recently approved technique for estimating the risk of MI.[18,19] Another potential strategy is to test for a biochemical signature to indicate the presence of CAD.

ASSESSMENT OF CORONARY PLAQUE VULNERABILITY BY COMPUTED TOMOGRAPHY ANGIOGRAPHY

Coronary computed tomography (CT) angiography (CCTA) permits the non-invasive detection of vulnerable plaques which may help to identify patients at risk for developing acute coronary events.[20] Plaques prone to rupture – also termed thin cap fibroatheroma (TCFA) – have a large necrotic core (area $> 1.0\,mm^2$ in 80% of cases) with a thin fibrous cap (thickness $< 65\,\mu m$).[21] Unfortunately, the limited CT spatial resolution and plaque detection threshold ($> 1\,mm$) precludes the assessment of fibrous cap thickness and necrotic core area by current CT scanners.[22]

CCTA enables quantification of total coronary plaque burden and allows accurate and reproducible measurement of coronary atheroma volume especially with automated 3D quantification software.[23] The culprit plaques in patients with acute coronary syndrome (ACS) have a larger volume than stable plaques ($193\,mm^3$ vs. $104\,mm^3$ respectively, $p = 0.001$).[24] Moreover, a direct relation has been observed between increased coronary plaque volume and subsequent adverse cardiac events in a systematic review of six serial intravascular ultrasound (IVUS) trials; each standard deviation increase in atheroma volume had a 1.3-fold higher risk for a major cardiac adverse events, myocardial infarction, or coronary revascularization.[25]

Coronary artery calcification (CAC) is a surrogate for coronary atherosclerosis and closely correlates with the extent and severity of atherosclerosis.[26–28] Calcium increases the shear stress and hence the risk of rupture of coronary plaques. CAC is considered present if a minimum of three contiguous pixels with an attenuation of ≥ 130 Hounsfield Units (HU) are detected along the course of a coronary artery. Several studies have reported that spotty calcification is highly prevalent in vulnerable plaques associated with ACS and is associated with increased adverse cardiac events and accelerated progression of coronary atherosclerosis.[29–31] The CCTA cut-off to define a small calcification as spotty is $< 3\,mm$, however micro-calcifications in approximately two thirds of ruptured plaques cannot be detected by current CT scanners.[20] In addition, current scoring techniques make several assumptions related to calcium density, location of calcium, spatial distribution of calcium and micro-calcification that may affect the prediction of a future cardiac event.[32]

CT also allows the identification of individual plaque components with correlation to virtual histology IVUS (VH-IVUS).[23] Plaques can be classified into calcified (plaques with more than 50% calcium), mixed (plaques with less than 50% calcium), and non-calcified plaques (NCPs) (Figure 1).[33] NCPs are further classified into low CT attenuation "lipid-rich" plaques and those with predominantly fibrous tissue on the basis of CT attenuation values. Low CT attenuation (defined as < 30 HU) has been identified in 88% of ruptured plaques compared with 18% of the stable lesions ($P < 0.001$).[34] In addition, a ring-like CT attenuation pattern of NCP - a central area of low CT attenuation and a surrounding ring like higher attenuation "napkin ring sign" - may indirectly indicate unstable plaque with large lipid-rich necrotic core.[35]

Intravenous injection of novel contrast agents may be an adjunct to detect inflammatory cells within coronary plaques with CT scanners in several animal studies. Macrophages in atherosclerotic plaques of rabbits can be detected with CT after the intravenous injection of iodinated nanoparticles dispersed with surfactant.[36] In addition, multicolour CT detected gold-labelled high density lipoproteins (HDL) nanoparticles targeted to activated macrophages in a mouse model.[37]

CCTA is a promising technique for the detection of unstable plaque. However, the cost related to running the test routinely in patients showing low- or no-risk is not feasible. In addition if contrast reagents are used the potential side-effects of the applied radiation are a deterring factor. Therefore, a safer potential strategy is to test for a biochemical signature to indicate the presence of CAD. A biochemical signature that can be detected by sampling peripheral blood, although invasive, does not involve the potential side effects of CCT thereby avoiding high cost and unwarranted risks for asymptomatic patients.

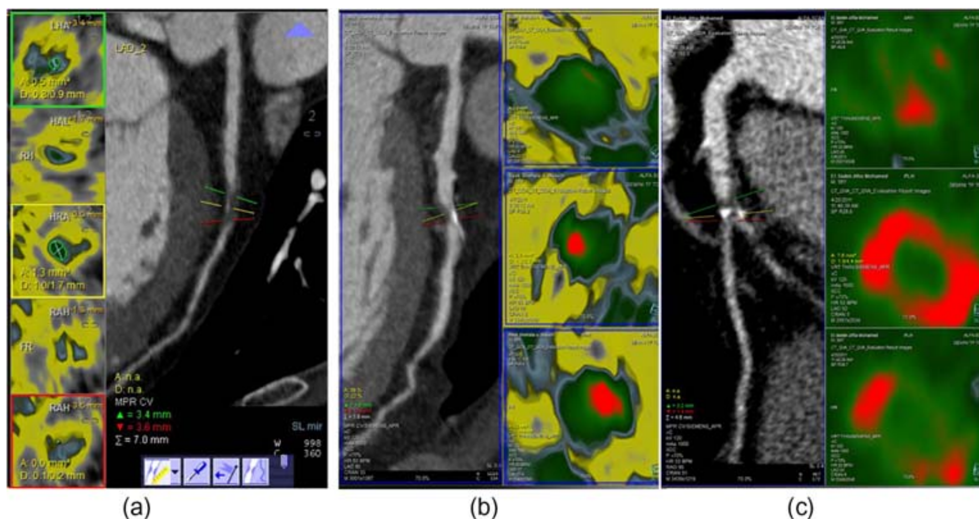

Figure 1. **Coronary Plaque Characterization by MDCT: Non-calcified plaque (a), Mixed plaque (b), Calcified plaque (c). Red colour: calcium.**

MASS SPECTROMETRY FOR BIOCHEMICAL ANALYSIS

The ‘omics fields, including genomics, transcriptomics, proteomics, lipidomics and metabolomics are associated fields in systems biology where a complex interaction between biological systems is studied (Figure 2). Metabolomics is primarily concerned with the high-throughput identification and quantification of small metabolites that are less than 1500Da. Lipidomics is considered a subset of metabolomics and attempts to describe a complete lipid profile. Proteomics is the study of the entire set of proteins produced or modified by an organism. Both the proteome and metabolome of any organism varies with time and under distinct conditions such as disease states.[38] A common instrument used for the analysis of samples for proteomic, lipidomic and metabolomic studies is the mass spectrometer (MS).

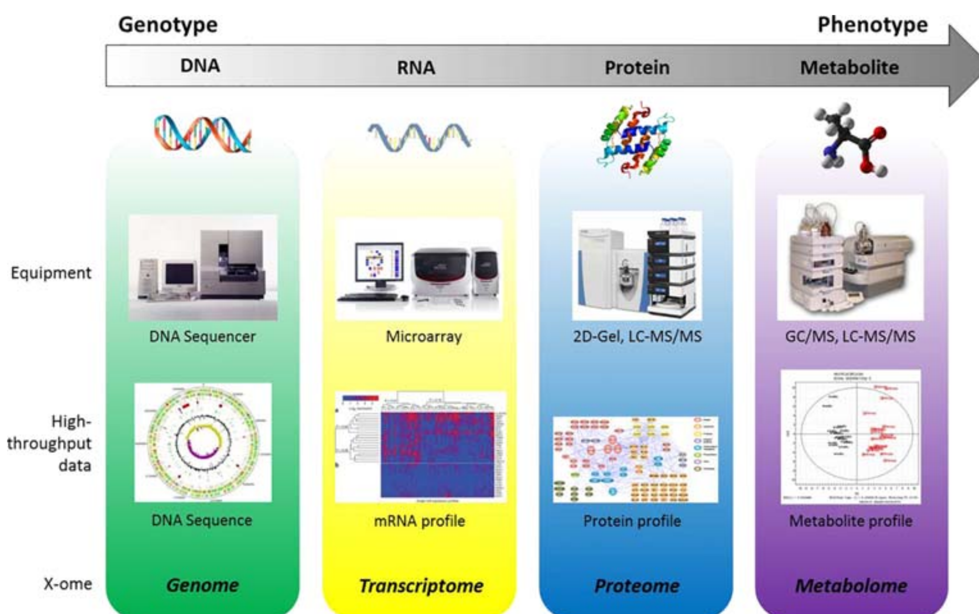

Figure 2. **The interconnection of systems biology showing the equipment used for analysis and the data generated.**

The incorporation of novel biomarkers to improve current models of prediction of preclinical CAD has been an on-going area of research. The use of two well-known biomarkers, Cardiac Troponin and Creatine-Kinase-MB isoform, used for the diagnosis of MI, has revolutionized the management of

patients presenting with chest pain. Due to the late-rise of troponin, a number of new candidate biomarkers are also being tested for the diagnosis of acute coronary syndrome.

INVESTIGATIVE BIOMARKER RESEARCH FOR CORONARY ARTERY DISEASE

The global analysis of lipids by mass spectrometry is an untargeted form of chemical analysis where experimental hypotheses are not yet formed. The workflow – from sample collection to results – is simplified in Figure 3. It requires unbiased acquisition of data in the hope that trends can be discerned

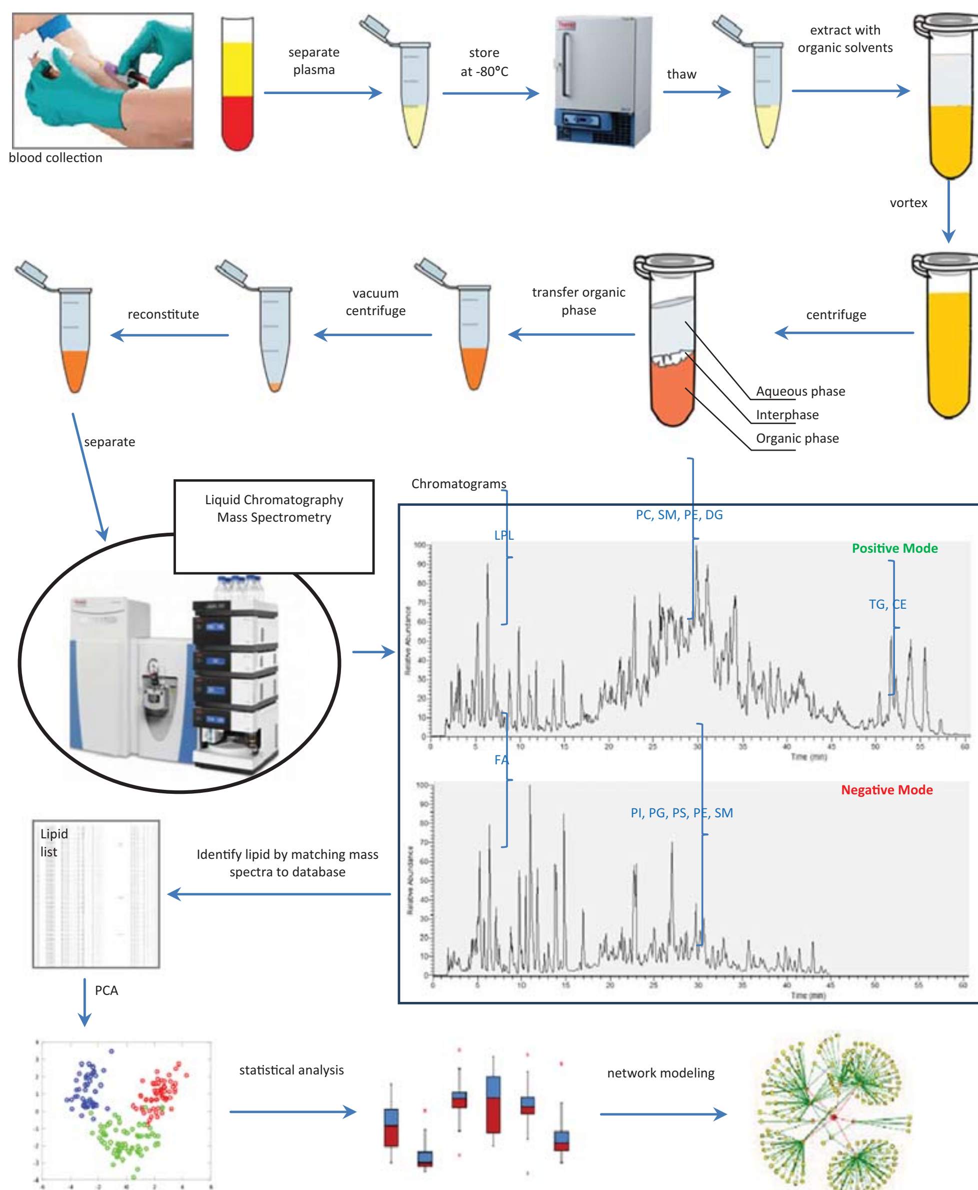

Figure 3. A typical workflow of a lipidomics experiment. Blood is separated and stored as plasma. On the day of the analysis the required volume of plasma is extracted with organic solvents and the organic phase concentrated and injected on a liquid chromatography mass spectrometry system. The liquid phase is ionised with the application of positive and negative voltages and corresponding chromatograms of detected ions are recorded. The classes of lipids commonly seen in blood plasma isolate to certain regions of the chromatogram. The data is matched against a database to identify the lipids. Once samples are analyzed, statistical analysis commonly performed are principal components analysis (PCA) of samples from patients at different stages of disease. Network modeling is used to identify metabolic pathways that have changed.
Abbreviations: FA-fatty acid, LPL-lysophospholipid, PC-phosphatidylcholine, PI-phosphatidylinositol, PG-phosphatidylglycerol, PS-phosphatidylserine, PE-phsophatidylethanolamine, SM-sphingomyelin, MG-monoglycerol, DG-diglycerol, TG-triglycerol, CE-cholesteryl ester.

based on known sample variability. Lipidomic approaches to the identification of disease biomarkers rely principally on the comparative analysis of lipids in normal and diseased patients, animal models or cell cultures to identify aberrant concentration changes that may represent new lipid biomarkers or elucidate a disease mechanism. The global analysis of lipids using mass spectrometry has proven to be a useful tool in the study of cardiac diseases.

Lipidomics for the prediction of CAD

The eukaryotic lipidome might comprise of 10,000 to 100,000 individual species of lipids originating from a few hundred lipid classes.[39–41] These lipids are distributed as part of the biological membrane, energy storage substances and sometimes function as signal transducers.[42–44] Lipidomics relies on tandem mass spectrometry performed with on-line liquid chromatography to identify the individual fatty acid chains and any polar head groups. Due to the structure of the aliphatic fatty acid chains present at varied lengths in lipids, any lipid having two or more fatty acids are difficult to identify as there are several possible isomers that are isobaric with identical molecular elements. However with the speed and mass accuracy of current high resolution tandem mass spectrometers, fragmentation of these isobaric compounds leads to correct identification of the exact molecular species within each lipid class (Figure 4).

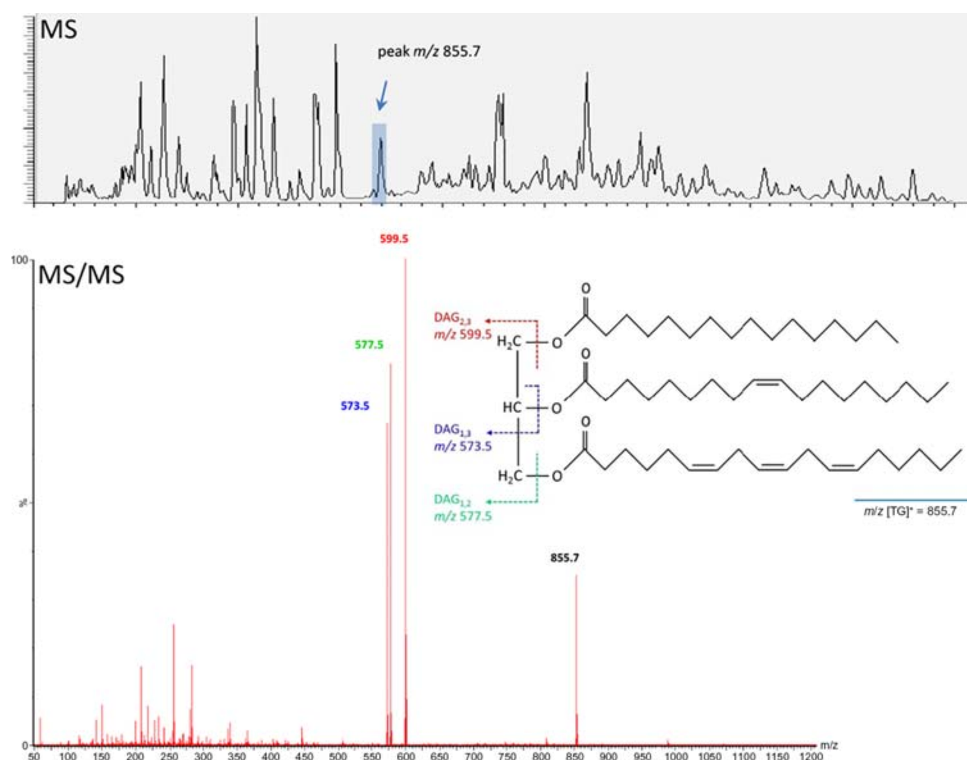

Figure 4. The identification of TG(16:0/18:1/18:3) by tandem mass spectrometry (MS/MS). Peaks corresponding to lipids in the chromatogram are identified by triggering tandem mass spectrometry (MS/MS). When a peak is detected in MS, the ions are selectively fragmented by collision-induced dissociation (CID). During CID, TG breaks characteristically as shown thereby allowing the deduction of the number of carbons and double bonds in each fatty acid chain. The lipid is measured with m/z 855.7 and its fragments due to collision-induced dissociation (CID) are *m/z* 599.5, 577.5, 573.5.

Altered lipid metabolism and dyslipidemia in the context of inflammation and oxidative stress are driving forces in the transition from stable to unstable plaques.[45] A number of studies have highlighted a characteristic lipid signature within unstable human plaques and also in the circulating blood plasma. One particular triglyceride (TAG) with low carbon number and double-bond content, TAG(54:2), is associated with CVD[45] suggesting that the relevance of particular TAGs has been underestimated by unwarranted focus on total triglycerides. It is still uncertain whether the TAG levels are related to proatherogenic lipoprotein dynamics or lipoprotein retention in the vessel wall, plaque instability and

thrombogenecity. Lipidomic profiling by mass spectrometry of plaques obtained from endarterectomies show certain cholesteryl esters, in particular CE(16:1), CE(18:1) and CE(18:3), are detectable in only advanced atherosclerotic plaques but not in normal arteries.[46] The plasma lipidomic profile of the same patients showed that the dominant CEs present in the plaque were also present in the plasma making these potential biomarkers that can easily be tested for. A plasma lipid profiling study of 220 subjects was able to identify 102 differentiated lipids between patients with stable CAD and healthy control individuals and 50 lipids between stable CAD and unstable CAD.[47] Adding these lipids to a model and also taking into consideration traditional risk factors and the concentration of C-reactive protein, made a marked improvement in the C-statistic which measures how well the model can discriminate between observations at different levels of the outcome.[47,48] Current testing for CAD is related to the class of lipids rather than the bioactivity of a single lipid species. These studies show that actual species composition of lipid classes is likely to be an atherogenic factor and are therefore potential biomarkers.

Limitations of lipidomic high-throughput analytical mass spectrometry

Mass spectrometry-based quantitative techniques allow the analysis of numerous biomarkers in parallel. However the technique faces its own challenges. Firstly, since the chemical properties of the different classes of lipids vary considerably, only a portion of the lipidome can be examined at one time with one analytical technique. Attempting to cover the entire range of lipids will require several experiments. Secondly, due to the complexity of biological samples, sample preparation techniques to decrease the complexity of sample prior to analysis needs to be used but can result in the loss or poor recovery of particular lipids resulting in an underestimation of concentration values.[49] If these sample preparation steps are not undertaken, the selectivity of the assay is compromised as isobaric analytes with similar *m/z* can appear co-eluting with the marker of interest resulting in an overestimation of the marker concentration. For these reasons protein changes related to atherosclerosis, such as allosteric or conformational changes in apolipoprotein molecules that render it antigenic, cannot be detected by a lipidomics platform. Lastly, the reproducibility of results between laboratories is essential and recent studies comparing the correlation of variation of results obtained from the analysis of the same sample in different laboratories with the same technique have proven to be adequate.[50]

CONCLUSION

Mass spectrometry has been widely used to analyze biological samples and has evolved into an indispensable tool for research. In the attempt to fully understand human physiology, advanced technologies that push the boundary of mass spectrometry capabilities has allowed the technique to address an ever-increasing array of biological questions. Advancements in the global lipidomic analysis of samples for the discovery of novel biomarkers are made possible in the last decade due to these instrument improvements. The technique demonstrates enormous potential for the determination of lipid biomarkers indicative of plaque instability in asymptomatic patients as a screening test.

REFERENCES

[1] Angel TE, Aryal UK, Hengel SM, Baker ES, Kelly RT, Robinson EW, Smith RD. Mass spectrometry-based proteomics: existing capabilities and future directions. *Chem Soc Rev*. 2012;41(10):3912–3928.
[2] Niessen WM. Progress in liquid chromatography-mass spectrometry instrumentation and its impact on high-throughput screening. *J Chromatogr A*. 2003;1000(1-2):413–436.
[3] Smith RD. Trends in mass spectrometry instrumentation for proteomics. *Trends Biotechnol*. 2002;20(12 Suppl):S3–S7.
[4] Griffin NM, Yu J, Long F, Oh P, Shore S, Li Y, Koziol JA, Schnitzer JE. Label-free, normalized quantification of complex mass spectrometry data for proteomic analysis. *Nat Biotechnol*. 2010;28(1):83–89.
[5] Issaq HJ, Fox SD, Chan KC, Veenstra TD. Global proteomics and metabolomics in cancer biomarker discovery. *Journal of Separation Science*. 2011;34(24):3484–3492.
[6] Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is Impaired by a high–saturated fat diet but not by a high-carbohydrate diet. *Arteriosclerosis, thrombosis, and vascular biology*. 2005;25(6):1274–1279.
[7] Muller WA. Mechanisms of transendothelial migration of leukocytes. *Circulation Research*. 2009;105(3):223–230.
[8] Hartvigsen K, Chou MY, Hansen LF, Shaw PX, Tsimikas S, Binder CJ, Witztum JL. The role of innate immunity in atherogenesis. *Journal of Lipid Research*. 2009;50(Supplement):S388–S393.
[9] Libby P, Ridker PM, Hansson GK. Inflammation in AtherosclerosisFrom Pathophysiology to Practice. *Journal of the American College of Cardiology*. 2009;54(23):2129–2138.

[10] Miller YI, Choi SH, Wiesner P, Fang L, Harkewicz R, Hartvigsen K, Boullier A, Gonen A, Diehl CJ, Que X, Montano E, Shaw PX, Tsimikas S, Binder CJ, Witztum JL. Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circulation Research*. 2011;108(2):235–248.

[11] Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. *Clinical Immunology*. 2010;134(1):33–46.

[12] Mulvihill ER, Jaeger J, Sengupta R, Ruzzo WL, Reimer C, Lukito S, Schwartz SM. Atherosclerotic plaque smooth muscle cells have a distinct phenotype. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2004;24(7):1283–1289.

[13] Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. *European heart journal*. 2013;34(10):719–728. doi:10.1093/eurheartj/ehs411

[14] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *The Lancet*. 2004;364(9438):937–952.

[15] Nabel EG, Braunwald E. A tale of coronary artery disease and myocardial infarction. *New England Journal of Medicine*. 2012;366(1):54–63.

[16] Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *New England Journal of Medicine*. 2013;368(21):2004–2013.

[17] Pitt B, Rubenfire M. Risk stratification for the detection of preclinical coronary artery disease. *Circulation*. 1999;99(20):2610–2612.

[18] Taylor AJ, Taylor AJ, Cerqueira M, Hodgson JM, Mark D, Min J, O'Gara P, Rubin GD. ACCF/SCCT/ACR/AHA/ASE/ASNC/NASCI/SCAI/SCMR appropriate use criteria for cardiac computed tomography: a report of the American college of cardiology foundation appropriate use criteria task force, the society of cardiovascular computed tomography, the American college of radiology, the American heart association, the American society of echocardiography, the American society of nuclear cardiology, the north American society for cardiovascular imaging, the society for cardiovascular angiography and interventions, and the society for cardiovascular magnetic resonance. *Journal of the American College of Cardiology*. 2010;56(22):1864–1894.

[19] Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JMcB, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK. 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American Society of Echocardiography, American Society of Nuclear Cardiology, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance. *Journal of the American College of Cardiology*. 2010;56(25):e50–e103.

[20] Maurovich-Horvat P, Ferencik M, Voros S, Merkely B, Hoffmann U. Comprehensive plaque assessment by coronary CT angiography. *Nature Reviews Cardiology*. 2014;11(7):390–402. doi:10.1038/nrcardio.2014.60

[21] Achenbach S, Can CT. detect the vulnerable coronary plaque? *The international journal of cardiovascular imaging*. 2008;24(3):311–312.

[22] van der Giessen AG, Toepker MH, Donelly PM, Bamberg F, Schlett CL, Raffle C, Irlbeck T, Lee H, vanWalsum T, Maurovich-Horvat P, Gijsen FJ, Wentzel JJ, Hoffmann U. Reproducibility, accuracy, and predictors of accuracy for the detection of coronary atherosclerotic plaque composition by computed tomography: an ex vivo comparison to intravascular ultrasound. *Investigative radiology*. 2010;45(11):693–701.

[23] Boogers MJ, Broersen A, van Velzen JE, de Graaf FR, El-Naggar HM, Kitslaar PH, Dijkstra J, Delgado V, Boersma E, de Roos A, Schuijf JD, Schalij MJ, Reiber JH, Bax JJ, Jukema JW. Automated quantification of coronary plaque with computed tomography: comparison with intravascular ultrasound using a dedicated registration algorithm for fusion-based quantification. *European heart journal*. 2012;33(8):1007–1016.

[24] Pflederer T, Marwan M, Schepis T, Ropers D, Seltmann M, Muschiol G, Daniel WG, Achenbach S. Characterization of culprit lesions in acute coronary syndromes using coronary dual-source CT angiography. *Atherosclerosis*. 2010;211(2):437–444.

[25] Nicholls SJ, Hsu A, Wolski K, Hu B, Bayturan O, Lavoie A, Uno K, Tuzcu EM, Nissen SE. Intravascular ultrasound-derived measures of coronary atherosclerotic plaque burden and clinical outcome. *Journal of the American College of Cardiology*. 2010;55(21):2399–2407.

[26] Greenland P, Bonow RO, Brundage BH, Budoff MJ, Eisenberg MJ, Grundy SM, Lauer MS, Post WS, Raggi P, Redberg RF, Rodgers GP, Shaw LJ, Taylor AJ, Weintraub WS. ACCF/AHA clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography. *Journal of the American College of Cardiology*. 2007;49(3):378–402.

[27] Choi YH, Hong YJ, Park IH, Jeong MH, Ahmed K, Hwang SH, Lee MG, Park KH, Sim DS, Kim JH, Ahn Y, Cho JG, Park JC, Kang JC. Relationship between coronary artery calcium score by multidetector computed tomography and plaque components by virtual histology intravascular ultrasound. *Journal of Korean medical science*. 2011;26(8):1052–1060.

[28] Nicholls SJ, Tuzcu EM, Wolski K, Sipahi I, Schoenhagen P, Crowe T, Kapadia SR, Hazen SL, Nissen SE. Coronary artery calcification and changes in atheroma burden in response to established medical therapies. *Journal of the American College of Cardiology*. 2007;49(2):263–270.

[29] Motoyama S, Kondo T, Sarai M, Sugiura A, Harigaya H, Sato T, Inoue K, Okumura M, Ishii J, Anno H, Virmani R, Ozaki Y, Hishida H, Narula J. Multislice computed tomographic characteristics of coronary lesions in acute coronary syndromes. *Journal of the American College of Cardiology*. 2007;50(4):319–326.

[30] Ehara S, Kobayashi Y, Yoshiyama M, Shimada K, Shimada Y, Fukuda D, Nakamura Y, Yamashita H, Yamagishi H, Takeuchi K, Naruko T, Haze K, Becker AE, Yoshikawa J, Ueda M. Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction an intravascular ultrasound study. *Circulation*. 2004;110(22):3424–3429.

[31] Kataoka Y, Wolski K, Uno K, Puri R, Tuzcu EM, Nissen SE, Nicholls SJ. Spotty calcification as a marker of accelerated progression of coronary atherosclerosis: insights from serial intravascular ultrasound. *Journal of the American College of Cardiology*. 2012;59(18):1592–1597.
[32] Alluri K, Joshi PH, Henry TS, Blumenthal RS, Nasir K, Blaha MJ. Scoring of Coronary Artery Calcium Scans: History, Assumptions, Current Limitations, and Future Directions. *Atherosclerosis*. 2015;239(1):109–117. doi:10.1016/j.atherosclerosis.2014.12.040
[33] Achenbach S, Moselewski F, Ropers D, Ferencik M, Hoffmann U, MacNeill B, Pohle K, Baum U, Anders K, Jang IK, Daniel WG, Brady TJ. Detection of calcified and noncalcified coronary atherosclerotic plaque by contrast-enhanced, submillimeter multidetector spiral computed tomography a segment-based comparison with intravascular ultrasound. *Circulation*. 2004;109(1):14–17.
[34] Ozaki Y, Okumura M, Ismail TF, Motoyama S, Naruse H, Hattori K, Kawai H, Sarai M, Takagi Y, Ishii J, Anno H, Virmani R, Serruys PW, Narula J, Coronary CT. angiographic characteristics of culprit lesions in acute coronary syndromes not related to plaque rupture as defined by optical coherence tomography and angioscopy. *European heart journal*. 2011;32(22):2814–2823.
[35] Maurovich-Horvat P, Hoffmann U, Vorpahl M, Nakano M, Virmani R, Alkadhi H. The napkin-ring sign: CT signature of high-risk coronary plaques? *Journal of the American College of Cardiology: Cardiovascular Imaging*. 2010;3(4):440–444.
[36] Hyafil F, Cornily JC, Feig JE, Gordon R, Vucic E, Amirbekian V, Fisher EA, Fuster V, Feldman LJ, Fayad ZA. Noninvasive detection of macrophages using a nanoparticulate contrast agent for computed tomography. *Nature medicine*. 2007;13(5):636–641.
[37] Cormode DP, Roessl E, Thran A, Skajaa T, Gordon RE, Schlomka JP, Fuster V, Fisher EA, Mulder WJ, Proksa R, Fayad ZA. Atherosclerotic Plaque Composition: Analysis with Multicolor CT and Targeted Gold Nanoparticles 1. *Radiology*. 2010;256(3):774–782.
[38] Vasan RS. Biomarkers of Cardiovascular Disease: Molecular Basis and Practical Considerations. *Circulation*. 2006;113(19):2335–2362.
[39] van Meer G. Cellular lipidomics. *The EMBO journal*. 2005;24(18):3159–3165.
[40] VanMeer G, Voelker D, Feigenson G. Membrane lipids: where they are and how they behave. *Nature Reviews Molecular Cell Biology*. 2008;9:112–124.
[41] Yetukuri L, Ekroos K, Vidal-Puig A, Oresic M. Informatics and computational strategies for the study of lipids. *Molecular BioSystems*. 2008;4(2):121–127.
[42] Brown DA, London E. Structure and function of sphingolipid-and cholesterol-rich membrane rafts. *Journal of Biological Chemistry*. 2000;275(23):17221–17224.
[43] Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. *Nature Reviews Cancer*. 2003;3(8):582–591.
[44] Gupta N, DeFranco AL. Lipid rafts and B cell signaling. *Seminars in Cell & Developmental Biology*. 2007;18(5):616–626.
[45] Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck Study. *Circulation*. 2014;129:1799–1803.
[46] Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, Baumert M, Allen M, Davies AH, Monaco C, Smith A, Xu Q, Mayr M. Comparative lipidomics profiling of human atherosclerotic plaques. *Circulation: Cardiovascular Genetics*. 2011;4(3):232–242.
[47] Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, MacIntosh GL, Goudey B, Stern L, Kowalczyk A, Haviv I, White AJ, Dart AM, Duffy SJ, Jennings GL, Kingwell BA. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011;31(11):2723–2732.
[48] LaValley MP. Logistic regression. *Circulation*. 2008;117(18):2395–2399.
[49] Keshishian H, Addona T, Burgess M, Mani DR, Shi X, Kuhn E, Sabatine MS, Gerszten RE, Carr SA. Quantification of cardiovascular biomarkers in patient plasma by targeted mass spectrometry and stable isotope dilution. *Molecular & cellular proteomics*. 2009;8(10):2339–2349.
[50] Addona TA, Abbatiello SE, Schilling B, Skates SJ, Mani DR, Bunk DM, Spiegelman CH, Zimmerman LJ, Ham AJ, Keshishian H, Hall SC, Allen S, Blackman RK, Borchers CH, Buck C, Cardasis HL, Cusack MP, Dodder NG, Gibson BW, Held JM, Hiltke T. Multi-site assessment of the precision and reproducibility of multiple reaction monitoring–based measurements of proteins in plasma. *Nature biotechnology*. 2009;27(7):633–641.