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The clinical translation of eicosanoids and other oxylipins, although challenging, should be actively pursued

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Although the potential of lipidomics for disease diagnosis, prognosis and treatment is recognized, its translation into the clinic remains challenging. Awareness of both the potential and challenges of lipidomics has been boosted by large international consortia (e.g., *International Lipidomics Society* and the *European COST Network "EpiLipidNet"*) that are facilitating structural development of the field. Specific lipid classes, such as ceramides, are on the way to adoption in clinics for routine use [1]. Hopefully, similar paths will be followed by other lipid classes that have shown high clinical potential, such as eicosanoids and other oxylipins. Oxylipins are a specific class of lipid metabolites derived from the oxygenation of polyunsaturated fatty acids (PUFAs) that are ubiquitous in human biofluids and tissues. Other than cholesterol and triglycerides, the oxylipins have shown the most striking clinical utility of all lipid classes in the past decades. Indeed, drugs that target oxylipin pathways have been used for more than a century to alleviate pain, swelling, fever and asthmatic conditions; aspirin being the oldest of the numerous NSAIDs that have experienced commercial and clinical success [2].

Oxylipins include hundreds of molecules having a wide range of structures, polarities and concentrations, depending on the substrate PUFA and the biosynthesis pathway (Fig. 1). Although substrate availability and enzyme affinities may influence the profiles of oxylipins produced in different cells types or tissues, oxylipins can theoretically be produced from any type of PUFA (i.e., short chain or long chain omega 6 and omega 3 PUFAs). Regarding biosynthesis pathways, the oxylipins are generated by the coordinated action of over 50 unique and cell-specific enzymes, including phospholipase A2 that releases the PUFA from membrane phospholipids and the primary oxidative enzymes, namely cyclooxygenases, lipoxygenases and cytochrome P450 epoxygenases/hydrolases (see [3] for details). Moreover, oxylipins can also be produced *via* free radical mediated reactions (i.e., autooxidation) that notably generate isoprostanoids (see [4] for details). Once released, free oxylipins signal by binding to G protein-coupled receptors or interacting with intracellular pathways, such as peroxisome proliferator-activated receptors (PPARs) or nuclear factor kappa B (NFκB) [5]. They can also be rapidly incorporated into more complex lipids, such as phospholipids, thereby leading to the generation of phospholipid-esterified oxylipins that remain cell bound when exerting their actions [6]. Esterified oxylipins are also found in abundance in phospholipids, triglycerides and cholesterol esters of lipoproteins, to which they confer a large part of their bioactivity towards immune cells and vasculature [7]. The structural diversity of oxylipins leads to a wide array of biological functions, many of which remain to be elucidated. Relevant to clinical research, oxylipins are potent regulators of inflammation, vascular tone, endothelial function and platelet aggregation, and many of them also inform on oxidative stress [8]. Comprehensive oxylipin profiling, therefore, has the potential to provide a wealth of information regarding the underlying mechanisms of various complex diseases, and to provide new tools and/or therapeutic targets for diagnosis, prognosis and treatment. Several large lipidomic studies have recently highlighted this potential. For instance, untargeted oxylipin profiling in large cohort studies, namely FINRISK 2002 and the Framingham Heart Study, identified and validated a strong association between free oxylipins in plasma and blood pressure in the general population [9].

Another relevant example of the potential of oxylipins in the field of cardiovascular diseases arises from a large multicentric study showing

Abbreviations: CVD, cardiovascular disease; CE, cholesteryl ester; LDL, low density lipoprotein; NFκB, nuclear factor kappa B; oxCE, oxidized CE; oxLDL, oxidized LDL; oxTG, oxidized TG; PC, phosphatidylcholine; PL, phospholipid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; TG, triglyceride.

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that serum levels of specific free oxylipins are associated with the onset of acute myocardial infarction [10]. The plasma signature of free oxylipins also shows strong potential for the discovery of new biomarkers in liver diseases, as recently exemplified in patients with non-alcoholic steatohepatitis [11] and acutely decompensated cirrhosis [12]. Finally, a recent cohort study suggests that the profiling of free oxylipins in urine could provide a non-invasive approach for the diagnosis and monitoring of asthma in adolescent and adults [13]. These encouraging results need to be fully validated in large replication studies. Of note, although esterified oxylipins are the major form of circulating oxylipins, the vast majority of human studies have focused on free oxylipins. Future studies should include both pools (i.e., free and esterified oxylipins) to provide a more realistic and comprehensive view of the associations between circulating oxylipins and health.

A first challenge in the translation of oxylipins to the clinic is related to harmonization of the procedures for sampling and sample preparation. These vary widely between laboratories, as highlighted in the studies briefly described above. This point is particularly important in the field of oxylipins, as we know that depending on the type of biological sample used (i.e., plasma, serum or urine), and the type of oxylipins one is focusing on (i.e., free, esterified or total), the oxylipin patterns are very different and hardly comparable [8]. This prevents the generation of large, harmonized databases, which are crucially needed to establish meaningful and consistent links between oxylipins and disease.

Another challenge associated with lipids, in general, and with oxylipins, in particular, is related to their stability during storage and preparation. This must be well understood and controlled to ensure reliable and reproducible oxylipin profiles. Concerning total oxylipins in plasma (i.e., free and esterified), we have recently shown that most are robust at −80 °C for up to 15 months, while their stability during sample preparation is ensured by the addition of the radical scavenger, butylated hydroxytoluene [14].

The clinical translation of oxylipins also depends on technical and biological variabilities, which, if too high, could compromise the detection of pathological changes based on the oxylipin signature profiles. We have recently shown that the analysis of total oxylipins in plasma can be achieved with relatively low variability [15]. Moreover, provided that a standardized and harmonized protocol is followed, and a common reference material is used, we have also shown that it is possible to generate reproducible and comparable oxylipin concentrations in independent laboratories – another crucial point for clinical translation. Finally, most lipidomic studies have taken a disease-based approach from which promising candidate biomarkers, or new potential therapeutic targets, have been identified. However, to utilize this information in diagnosis, prognosis and treatment, it will be crucial to establish reference values and the ranges of natural variability in a healthy population.

In comparison to free oxylipins, analysed directly or after hydrolysis of oxidized complex lipids, the direct analysis of oxidized complex lipids, including oxygenated phospholipids (PLs), cholesteryl esters (CE) and triglycerides (TG), remains much less developed. However, considering that the majority of oxylipins, at least in blood plasma, are found to be esterified to complex lipids, identification and quantification of oxidized complex lipids could provide additional information on their origin, distribution and metabolism. The role of complex oxidized lipids in disease pathogenesis is probably most well-studied for atherosclerosis [16]. Atherosclerosis, a major factor underlying development of cardiovascular diseases (CVDs), is characterized by the accumulation of apoB-containing lipoproteins in arterial walls that disturbs laminar blood flow and is associated with non-resolving low-grade, chronic inflammation. Oxidized complex lipids, and their protein adducts on low density lipoprotein (LDL), have been identified as major atherogenic factors initiating increased endothelial permeability, accumulation of immune cells in the arterial intima media, enhanced uptake of oxidized LDL (oxLDL) by macrophages at the site of atherosclerotic lesions, transformation of the macrophages into foam cells, and formation of atherosclerotic plaques. Oxidized cholesteryl esters (oxCE) within oxLDL are considered to be a primary contributor to this process. OxCE, often measured as a sum of all oxidized species, are enriched in atherosclerotic plaques and blood plasma of diseased individuals [17]. Some reports provided targeted MS analysis of oxCE species detecting CE 18:2, CE 20:4, and CE 22:6 oxidized derivatives [18–20]. Phospholipid oxidation is associated with the development of atherosclerosis, CVDs and metabolic diseases as well; phosphatidylcholines (PC), the major phospholipid class, have been reported as oxidation targets in a variety of pathological conditions, including diabetes mellitus, chronic kidney disease, and several CVDs including atherosclerosis [19,21]. Oxidative truncation of polyunsaturated acyl chains in PC lipids leads to the formation of reactive electrophilic moieties (e.g., carbonyls) capable of reacting with nucleophilic substrates, including basic amino acid residues of lipoproteins. Availability of natural IgM antibodies capable of recognizing the head group of oxidized PC lipids, allowed for the development of a sensitive ELISA method for the detection of oxPC-protein adducts [16]. An assay for oxidized PC adducts on apoB-containing lipoproteins was introduced by BostonHeart Diagnostics for

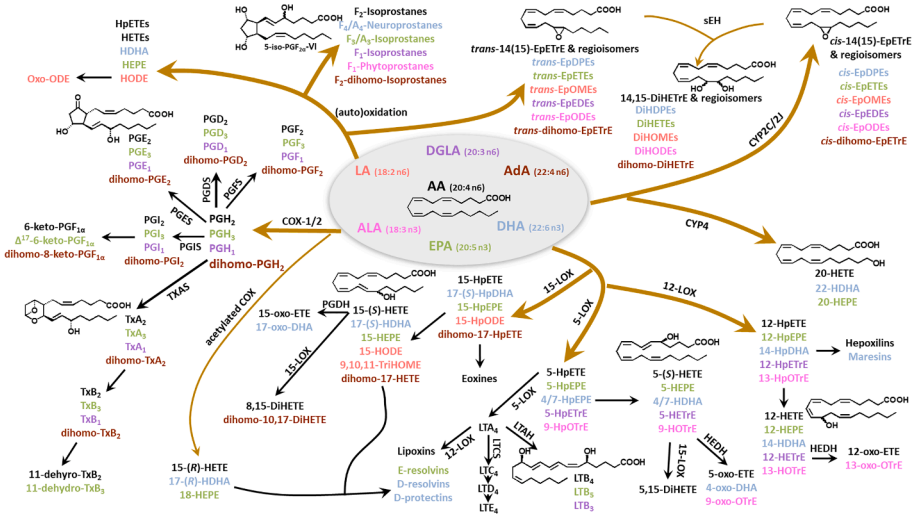

Fig. 1. Overview of the main eicosanoids and other oxylipins produced from PUFAs through COX, LOX, CYP and free-radical mediated pathways (Adaptation from [8]).

clinical use in August 2020. Based on 42 clinical studies, clinicians are advised to use oxPL-apoB levels in a precision medicine approach to classify patients into higher or lower CVD risk categories (https://bostonheartdiagnostics.com/test/oxpl-apob-c-2/).

Overall, distribution of oxidized complex lipids in blood plasma is much less studied compared to free oxylipins (measured directly or after hydrolysis). Detection of oxidized complex lipids in biological samples has two particular analytical limitations – low natural abundance and extremely high structural diversity of possible molecular species. While 100 known oxylipin species will translate into 100 oxCE species (keeping the cholesterol moiety constant, unless oxysterols are considered), the variability of PL head groups (at least 6) and second fatty acyl chains (for instance, the 10 most abundant PUFAs) give 6 000 potential oxPL molecular species. For oxidized TG (oxTG), with two additional fatty acyl moieties, the potential number of oxTG species could be in the range of 10 000, if oxidation of only one acyl chain is considered. Furthermore, whereas a large number of labelled standards are commercially available for the absolute quantification of free oxylipins, standard availability for complex oxidized lipids is the complete opposite. Currently, fewer than two dozen complex oxidized lipid standards are commercially available. Considering the significance of chemically defined standard compounds for method development, as well as application as internal standards to support absolute quantification, the paucity of standards remains the main limitation in analysis of oxidized complex lipids. Importantly, development of harmonized analytical workflows addressing the stability of oxidized complex lipids, as well as their intra- and inter-individual variability are required to support potential clinical translation.

Despite their complexity, the clinical translation of free and esterified oxylipins should be actively pursued as they have the potential not only to provide new insights in the pathogenesis of various complex diseases, but also to improve disease management by allowing for earlier diagnosis, a better stratification of risk, and the identification of new therapeutic targets promoting advancement towards precision medicine. Paving the way for the clinical translation of oxylipins will require intense, worldwide collaboration between researchers, clinicians and industry in order to (i) provide harmonized procedures allowing robust and reproducible oxylipin profiling, (ii) conduct large cohort replication studies to validate the consistency of the identified candidate oxylipins, (iii) establish the boundaries of a healthy oxylipin signature, (iv) produce new standards, especially for the oxidized complex lipids, and (v) develop bioinformatic tools facilitating clinical interpretation. The newly established EU COST Action EpiLipidNET (Pan-European Network in Lipidomics and EpiLipidomics; https://www.epilipid.net/) and Clinical Lipidomics Interest group within ILS both aim to address these challenges via community driven efforts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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