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Perspective

Metabolomics as a tool to improve decision making for the vascular surgeon – wishful thinking or a dream come true?

Developing a better understanding of disease pathophysiology is central to the advancement of modern medicine. Omics technologies such as genomics, transcriptomics and proteomics, have all been successfully employed but have limitations [1]. Metabolomics is defined as the '*quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification*'.[2] This represents a new frontier in omics disciplines, enabling identification and quantification of end products of cellular metabolism to a level of detail superior to that provided by proteomic techniques. Nuclear Magnetic Resonance (NMR) Spectroscopy and Mass Spectrometry (MS) coupled techniques can be employed via untargeted approaches to provide information on metabolites in a given sample, or via targeted approaches to quantify their respective concentrations. This permits the objective quantification of metabolites at the end of biological pathways, taking into account both innate and acquired factors, and thus characterise the effect of disease processes at the cellular level. However, whilst there are a multitude of potential applications of this technology, questions remain regarding its translatability to clinical practice given existing technical and economic challenges.

The gap between bench and bedside has already been bridged in some specialties. The intelligent knife (iKnife) can detect cancerous tissue by analyzing the composition of the diathermy plume during electrocautery via MS techniques; this technology is currently being piloted for breast and colorectal cancer procedures, and could potentially revolutionise cancer treatment [3]. Separately, large scale epidemiological metabolic phenotyping studies have identified novel biomarkers that can be utilized for the diagnosis and prognostication of diseases such as hypertension [4,5].

Vascular disease is an area where metabolomics can play a role, particularly due to its increasing health burden and the need for improved diagnostic, prognostic and therapeutic interventions. In venous disease, deep venous thrombosis (DVT), is the single, most common cause of preventable hospital acquired mortality, and can be difficult to diagnose clinically. The current DVT biomarker, D-dimer, is poorly specific and only moderately sensitive [6]. Furthermore, in the first two weeks following clot development, thrombolysis can be employed to lyse the clot, but this hinges on being able to estimate what the age of the clot is, which is often difficult in poor historians or in DVTs that initially develop subclinically. As such, there is need for improved diagnosis, ageing and prognostication of disease; preliminary data employing metabolomic platforms to this end has already been published. Animal work has shown increased levels of citrate, malate, fumarate and α-ketoglutarate in serum in a porcine model with DVT; these are accumulated Krebs cycle intermediates caused by reduced oxidative phosphorylation in hypoxic conditions [7]. Murine studies revealed differences in inflammatory cells, fibrotic changes, tissue remodelling and collagen deposition in DVT animals, associated with thrombus ageing [8]. A separate study found elevated carnitine levels in plasma when DVT is present; these are necessary to convert free fatty acids to acylcarnitine [9] In human studies, acylcarnitines have been found to be reduced in patients with venous thrombosis [10] and in patients at high risk of pulmonary embolism [11]. This suggests that carnitine metabolism may be dysregulated in VTE and provides evidence that metabolomic platforms have the potential to be used to risk stratify patients with this condition.

Chronic venous disease has also been explored with metabolic phenotyping studies, alongside other omics disciplines [12–14]. There is preliminary data suggesting that metabolic profiling of chronic venous ulcers at baseline is predictive of healing status at 20 weeks [15]. Metabolites associated with lipid metabolism dysregulation and inflammation are particularly important, potentially playing a role in ulcer prognostication and revealing biological pathways that can be acted upon via topical or systemic supplements to help improve healing outcomes [15]. Imaging mass spectrometry not only permits identification of metabolite presence, but also their spatial distribution; this has revealed lipid accumulation patterns specific to varicose veins compared to controls, particularly around the valve leaflet, highlighting the importance of topographical assessment [16].

Aortic disease is an area of interest in metabolomics research; aortic aneurysms can be asymptomatic until the moment of rupture and death; as such, the national aneurysm screening programme has helped reduce rupture related mortality [17], however this is targeted to men and relies on ultrasound scanning which is dependent on operator skill and patient factors. Several omics studies have focused on aneurysms, providing valuable results such as proteomic biomarkers of aneurysms or genetic predisposition to aortic diseases [18–20]. However, further studies are required in order to translate those findings into clinical applications. Metabolomics is also showing promising results in aneurysm biomarker identification, including aminomalonic acid, guanidinosuccinic acid and glycerol [21]. Metabolomics techniques have found aortic dissection to be associated with aberrant lipid metabolism, including increased sphingomyelin and reduced ceramide levels, lipids important in tissue regeneration and cell signaling [22]. Changes in glycerophosphocholine and ergothioneine levels were also associated with aortic dissection and therefore could be used to diagnose or evaluate the treatment effectiveness [23]. In atherosclerosis, studies have linked some novel biomarkers to the disease, both in blood serum and from the gut microbiota [24–26]. Metabolite variations in eicosanoid and β-oxidation pathways have been observed, which can lead to a

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variety of diseases, including stroke [27]. These biomarkers therefore could be utilized to improve interventions in arterial disease. However, their role in risk stratifying patients or diagnosis is yet to be elucidated.

Despite the obvious potential, the application of metabolomics to vascular disease presents a number of challenges. From a practical perspective, sample collection, preparation and experimental analysis must be reproducible, streamlined and based upon robust standard operating procedures to minimize the introduction of bias. Metabolomics technologies are extremely sensitive and therefore small changes in sample collection or analysis can have a significant impact on the findings.

From a pragmatic perspective, the hypothesis-generating approach of early untargeted metabolomics work is unlikely to yield clear translational benefits. Metabolite identification at a single point in time provides limited information on complex, dynamic processes. Serial measurements are therefore key to establish context [28]. Furthermore, an individual metabolite may play a role in multiple pathologies, reducing their ability to be specific to a single disease. This is something that affects many omics studies; for example, interleukins and matrix metalloproteinases are dysregulated in both arterial and venous disease [29]. However, the quantification of a panel of metabolites and their relationship, or ratio, may be effective in differentiating different pathological processes.

A further challenge relates to confounding factors: [30] the presence of comorbidities, age, the type of diet, the individual's genome and microbiome can all cause systemic metabolic alterations, including circadian rhythm [31]. All of these variables make it challenging to set universal reference ranges for use in diagnostic tests. To overcome this obstacle, research groups are collaborating to create cumulative and reliable databases such as the human metabolome database [32], as well as performing metabolic phenotyping of large populations, via epidemiological studies, to gain information on the distribution of metabolites in different patient populations [33]. To minimize the risk of confounding factors it is important to validate any findings in separate cohorts or utilising distinct platforms such as polymerase chain reaction or enzyme linked immunosorbent assays to provide complementary data.

Metabolomics studies in vascular disease have therefore provided valuable results [1]. However, it is difficult to interpret them in isolation. As such, all steps of the omics hierarchy play a valuable role in elucidating the mechanistic pathways of disease and determining which molecules have the best potential to be useful diagnostic, prognostic or therapeutic tools. Proteomics studies, for instance, have identified extracellular matrix (ECM) composition remodelling in different types of cardiovascular disease associated with matrix metalloproteinase activity [34,35,36] Genome-wide association studies have linked genomic variations to vascular diseases and shown that environmental risk factors may have different impacts based on differences in genomic background [37,38]. However, unlike the genome, proteins and metabolites undergo drastic changes in disease; as such genomics studies provide limited insight for translational purposes [39] Several transcriptomics studies have shown differences in gene expression in disease, such as using microarray applications to show mRNA deregulation in venous thromboembolism [40] or differences in miRNA expression in serum to diagnose AAA [41]. Despite the significant body of work, cross – omics studies with validation via different experimental approaches are still lacking in the literature. These are necessary to fully explore pathways of disease and characterise the ‘interactome’.

Metabolic phenotyping has demonstrated clear potential for translational application in vascular disease. Data provided by metabolomics studies need to be integrated with other omics research to gain an understanding of the mechanisms of disease. Nonetheless, the journey from bench to bedside is ongoing. Further studies are required to explore this potential in greater detail; robust experimental designs, internal and external validation, clear and reproducible sampling and experimental protocols, metabolite quantification and suitably sized populations are necessary to infer meaningful mechanistic conclusions and develop technology with realistic clinical applications [42].

Conflict of interest

The Authors declare that there is no conflict of interest.

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Kirlikaya B., Langridge B., Davies A.H.*, Onida S.
Academic Section of Vascular Surgery, Department of Surgery and Cancer, Imperial College London, UK
E-mail addresses: a.h.davies@imperial.ac.uk (A.H. Davies), s.onida@imperial.ac.uk (S. Onida).

* Corresponding author at: Academic Section of Vascular Surgery, Department of Surgery and Cancer, Imperial College London, 4th Floor, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK.