Clinical prediction of wound re-epithelisation outcomes in non-severe burn injury using the plasma lipidome

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Abstract

Impaired wound healing in burn injuries can lead to complications such as skin graft loss, infection, and increased risk of scarring, which impacts long-term patient outcomes and quality of life. While wound

repair in severe burns has received substantial research attention, poor wound outcomes in cases of non-severe burns (classified as <20% of the total body surface area (TBSA)) remain relatively understudied despite also having considerable physiological impact and constituting the majority of hospital admissions for burns. Predicting outcomes in the early stages of healing would decrease financial and patient burden, and aid in preventing long-term complications from poor wound healing. Lipids have been implicated in inflammation and tissue repair processes and may play essential roles in burn wound healing. Longitudinal plasma samples were collected from patients (n=20) with non-severe (<15% TBSA) flame or scald burns over a 6-week period including timepoints pre- and post-surgical intervention. Samples were analysed using liquid chromatography-tandem mass spectrometry and nuclear magnetic resonance spectroscopy to detect 850 lipid species and 112 lipoproteins. Statistical analyses, including orthogonal projection to latent structures-discriminant analysis was performed to identify changes associated with either re-epithelialisation or delayed wound re-epithelisation.

The results demonstrated that the plasma lipid and lipoprotein profiles at admission could predict wound re-epithelisation outcomes at two weeks post-surgery, and that these discriminatory profiles were maintained over a 6-week period. Triacylglycerides, diacylglycerides (DAG) and low density lipoprotein (LDL) subfractions were associated with delayed wound closure, with DAG(18:2_18:3) and LDL/High density lipoprotein (HDL) ratio having the most influence (p-value < 0.02, Cliff's delta > 0.7), while HDL subfractions, phosphatidylinositols, phosphatidylcholines (PC), and phosphatidylserines were associated with re-epithelisation at two weeks post-surgery, with PC(16:0_18:1) and HDL-2 apolipoprotein-A1 showing the greatest influence on the model (p-value < 0.01 , Cliff's delta < -0.7). We demonstrate clinical prediction of wound re-epithelisation in non-severe burn patients using lipid and lipoprotein profiling. Further validation of the models will potentially lead to personalised intervention strategies to enhance injury outcomes, reducing the risk of chronic complications post-burn injury.

Keywords

Non-severe burns; Burn injury; Wound healing; Longitudinal; Early prediction; Re-epithelisation outcomes; Patient stratification; Lipid profiling; Lipoproteins; Metabolic phenotyping

Highlights

- Demonstration of wound healing prediction from time of hospital admission for non-severe burns.
- Plasma lipid and lipoprotein profiles within 48 hours of admission to hospital with non-severe burn injury are distinctly different between patients whose wounds re-epithelialized within two weeks and those with delayed re-epithelisation.
- Patients with delayed wound re-epithelisation have a persistent lipid and lipoprotein signature from burns admission up to six weeks post-injury.

Introduction

Burn injuries represent a significant global health burden and pose considerable challenges to clinicians in achieving successful wound healing[1,2]. While severe burns receive considerable research into improvements of clinical management[3–5], the majority of burn injuries requiring hospitalisation are clinically classed as "non-severe" defined as <20% total burn surface area (TBSA)[6]. Despite a non-severe classification, such injuries can still result in significant physiological damage and can compromise skin barrier functionality[7]. Due to the heterogeneity of non-severe burn injury, prediction of wound outcomes is challenging, with clinical classification and subsequent management reliant on visual wound observation, which has been reported to only be 60-75% accurate[2,8,9].

Due to the complex nature of wound healing, any imbalances or dysregulation in the associated pathways can lead to impaired wound healing and chronicity[11]. Several issues can contribute to poor wound healing, including but not limited to, increased oxidative stress, biofilm formation, cytokine, chemokine and growth factor imbalances, leaky blood vessels and excessive inflammation[11–13]. Impaired wound healing can result in short- and long-term health complications. In both severe and non-severe burns, a significant issue with delayed wound healing is the risk of graft failure[14], increased susceptibility to infection[15] and scar formation[16]. Scarring, particularly hypertrophic scars, can leave patients with long-term mobility issues, and chronic itch, discomfort and/or painful sensations[16]. Even without visible scars, an estimated 70% of burns patients will experience

dysesthesia during their recovery, which can result in chronic pain, hyper- or hypo-sensitivity and temperature fluctuations[17]. Cumulatively, these can negatively impact mental health of patients, increasing the risk of depression and anxiety[18] and affecting their quality of life.

Despite significant innovations in burn wound management and treatment, such as skin replacements[19], hydrogels[19], cell suspension spray[19] and surgical excision[20], complications with wound repair still occur following a non-severe burn and can lead to secondary patient morbidities[16–18,21]. Therefore, a comprehensive understanding of the underlying mechanisms involved in non-severe burn wound healing is crucial for improving patient outcomes and reducing the trauma of burn injuries.

In recent years, there has been significant progress in elucidating these mechanisms, contributing to the emergence of biomarkers that could predict patient mortality and outcomes in severe burns. Biomarkers such as bilirubin[22], creatinine[22], and C-reactive protein[23] have been monitored longitudinally and show promise as prognostic markers of mortality. However, predictive research has mostly focused on mortality[20–22] and other severe complications like sepsis[24], due to the life-threatening nature of severe burns, with minimal research on the wound healing process[25]. Targeting wound healing is critical to the post-burn recovery process, as it can prevent infections and prolonged inflammation which are common causes of morbidity and mortality in burn patients[4,25,26]. Predicting wound healing outcomes in the early stages of a burn provides a potential opportunity for improving patient outcomes. Early prediction can lead to increased effectiveness of time-critical treatments, such as wound debridement, that when performed late has been shown to delay wound closure and is associated with increased hospitalisation duration and infections[20,27]. Additionally, early prediction can stratify patients into risk groups for wound healing outcomes, which can guide clinicians into different treatment regimens for better patient recoveries, reducing health burden and financial cost[28]. To date, minimal research has been conducted on predicting wound healing outcomes in non-severe burns[8], despite accounting for up to 90% of the patient population in developed countries[29].

To research heterogenic conditions like wound healing, studies have moved away from traditional single biomarkers to panels of biomarkers or metabolic phenotypes, as the metabolome is directly

influenced by the physiological state and vice versa[30]. Novel analytical techniques such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance spectroscopy (NMR), are able to analyse small molecules within the metabolome, shedding light on perturbed metabolism that may be associated with disease[31–34]. These techniques have emerged as powerful tools for analysing metabolic phenotypes and identifying metabolic signatures that could predict patient outcomes in the early stages of disease[33]. New studies have demonstrated the predictive ability of metabolic phenotyping models, particularly with lipid[33] and lipoprotein[35,36] signatures that could stratify SARS-CoV-2 infections from other symptomatically similar infections[33,35] and their recovery trajectories post-infection with high accuracy and precision[36]. Lipid and lipoprotein metabolism has been associated with numerous inflammatory mechanisms and previous research into non-severe burns has identified dysregulation in these signatures three years post-burn injury[37]. No other studies have investigated lipid and lipoprotein metabolism longitudinally in non-severe burns to assess its ability to predict patient outcomes, even though it shows potential in severe burns[38,39] and other inflammatory conditions like ulcerative colitis[40].

We have previously demonstrated that lipid and lipoprotein metabolism measured by liqui chromatography-tandem mass spectrometry (LC-QQQ-MS) and NMR spectroscopy, respectively, in this non-severe burn cohort was perturbed up to six weeks post-injury when compared to non-burn controls[31]. The study suggests that patients were experiencing persistent inflammation that could impact long-term recovery[11]. As lipid and lipoprotein changes were apparent at burn admission, we investigated if these signatures could predict the re-epithelisation of non-severe burn wounds at two weeks post-surgery, a common wound healing parameter assessed by clinicians when monitoring wound recovery. By employing the same analytical techniques, this study aimed to identify whether monitoring lipid and lipoprotein profile trajectories could effectively predict wound healing outcomes in the early stages of non-severe burn injury and potentially be translated clinically to stratify patients at risk of poor wound healing.

Materials and methods

Reagents and chemicals

LC-MS grade water was produced from a Milli-Q® IQ 7000 (Merck Millipore) and Optima LC-MS grade organic solvents were purchased from Thermo Fisher Scientific (Malaga, WA, Australia). Phosphate buffer was acquired from Bruker (Bruker BioSpin, Billerica, Massachusetts, USA). Isotopically labelled lipid standards were obtained from the Lipidyzer™ Internal Standards Kit from SCIEX (MA, USA), and SPLASH LipidoMIX™ internal standards, lysoPG 17:1 and lysoPS 17:1 were purchased from Avanti Polar Lipids (Sigma-Aldrich, North Ryde, NSW, Australia).

Study design and biological samples

Non-severe burn plasma samples were acquired from the placebo arm of a randomised placebo-controlled trial of Celecoxib for Acute Burn Inflammation and Fever (CABIN Fever Trial), registered with the Australian New Zealand Clinical Trials Registry (Registration number: ACTRN12618000732280). The use of plasma samples and subsequent data received ethical approval by the South Metropolitan Health Service Ethics Committee (Approval number: RGS731). Non-severe burns patients (n=20; total samples = 66) who suffered flame or scald burns that were less than 15% total burn surface area (TBSA), aged between 18 to 65 years of age and gave consent were recruited in the State Adult Burns Unit at Fiona Stanley Hospital (Western Australia) within 48 h of hospital admission. Collection of whole blood in lithium heparin tubes was scheduled on study enrolment (n=18) and the day of surgery (for burn wound debridement)(n=10), as well as two days (n=11), two weeks (n=11), and six weeks post-surgery (n=16). Samples were centrifuged and stored at -80C within 4 hours of collection. At two weeks post-surgery, patient burn wounds were assessed for dressing cessation if the wound was re-epithelialized or dressing continuation until wound closure (**Figure 1**). Mean (± SD) days from burn injury at the six weeks post burns surgery timepoint was 45.64 (± 7.5) days, TBSA was 4.0% (± 3.6%) and age at time of collection was 39.87 (± 16.73) years. Additional demographic information of the non-severe burn cohort is tabulated in **Table 1**.

Table 1. Demographics and outcome parameters collected of the total non-severe burn patients recruited into the CABIN Fever trial, divided into overall and re-epithelisation groups. For each

characteristic, p-values were computed using Wilcoxon rank sum test to observe any demographic differences.

Characteristic	**Overall**, N = 20[1]	**Re-epithelisation**, N = 10[1]	**Delayed re-epithelisation**, N = 10[1]	**p-value**[2]
Sex				>0.9
Male	17 (85%)	8 (80%)	9 (90%)	
Female	3 (15%)	2 (20%)	1(10%)	
Age	40 (25, 50)	42 (25, 57)	34 (26, 45)	0.6
Days from burn injury to enrolment	2.54 (1.82, 3.72)	2.03 (1.89, 3.97)	2.70 (1.77, 3.31)	0.37
TBSA%	3.55 (1.43, 6.12)	5.05 (2.86, 6.54)	1.85 (0.70, 4.20)	0.089
Burn source				0.057
Flame	13 (65%)	9 (90%)	4 (40%)	
Scald	7 (35%)	1 (10%)	6 (60%)	
First aid before admission	14 (70%)	6 (60%)	8 (80%)	0.6
Hospital length of stay in days	5 (2.75, 7)	5 (5, 7)	3 (1.25, 6)	0.12
POSAS scores	34 (27, 42)	38 (27, 40)	30 (26, 42)	0.5
Post-surgery in hospital days	3 (0, 4)	3 (3, 4)	0.50 (0, 2.50)	
Infection present	1 (5.0%)	1 (10%)	0 (0%)	0.092
Antibiotics given	2 (10%)	1 (10%)	1 (10%)	>0.9
Surgery type				>0.9
Graft	11 (55%)	6 (60%)	5 (50%)	>0.9
Cell autograft	6 (30%)	3 (30%)	3 (30%)	
Non-operative	3 (15%)	1 (10%)	2 (20%)	
Scars requiring laser	1 (5.0%)	1 (10%)	0 (0%)	
Re-graft required	1 (5.3%)	1 (11%)	0 (0%)	>0.9
Skin phenotype				0.5
I	11 (55%)	4 (40%)	7 (70%)	
II	3 (15%)	2 (20%)	1 (10%)	0.7
III	3 (15%)	2 (20%)	1 (10%)	
IV	2 (10%)	1 (10%)	1 (10%)	
V	1 (5.0%)	1 (10%)	0 (0%)	

[1] n (%); Median (IQR)
[2] Fisher's exact test; Wilcoxon rank sum test; Wilcoxon rank sum exact test

Abbreviations: TBSA – Total body surface area, POSAS – Patient and observer scar assessment scale.

Pooled quality control samples

To ensure reproducibility and quality during the analytical runs, commercially available pooled human plasma (BioIVT, Westbury, NY, USA) was purchased and sub-aliquoted prior to LC-QQQ-MS and ^{1}H NMR spectroscopy analysis. Pooled plasma samples were used as quality control (QC) samples throughout the runs between every tenth study sample.

Lipid profiling

A comprehensive targeted lipid profiling approach was employed using a previously published LC-QQQ-MS method[41] to detect 1163 lipid species from 20 lipid subclasses in plasma and was used without any modifications (**Figure 1**). Briefly, using a Biomek i5 liquid handling system (Beckman Coulter, Mount Waverley, Victoria 3149, Australia) for automation, 90 µL of stable isotope labelled (SIL) internal standards diluted in IPA was added to 10 µL of plasma. Following chilling and centrifuging, 50 µL of supernatant was transferred to a 350 µL 96-well plate (Eppendorf, Macquarie Park, NSW) for same-day LC-QQQ-MS analysis. Targeted lipid profiling was performed on a ExionLC™ coupled to a 6500+ QTRAP (SCIEX, Concord, CA).

^{1}H NMR spectroscopy

^{1}H NMR spectroscopy analysis of plasma samples was conducted using a published method[42]. Briefly, plasma samples were centrifuged, and the supernatant combined 1:1 with phosphate buffer (75 mM Na_2HPO_4, 2 mM NaN_3, and 4.6 mM sodium trimethylsilyl propionate-[2,2,3,3- $2H_4$] (TSP) in D_2O, pH 7.4 ± 0.1) and mixed. 600 µL was then transferred into 5 mm outer diameter SampleJet NMR tubes and sealed with POM balls. Analysis was completed a 600 MHz Bruker Avance III HD spectrometer (Bruker BioSpin, Billerica, Massachusetts, USA) using the Bruker *in vitro* Diagnostics research (IVDr) methods. A standard ^{1}H 1D experiment with solvent pre-saturation was run (32 scans, 98304 data points,18028.85 Hz spectral width)[43] with an acquisition time of 4 min.

Lipoprotein subfraction analysis

The Bruker IVDr Lipoprotein Subclass Analysis (B.I.-LISA™) generates 112 lipoprotein parameters for each plasma sample using the standard ^{1}H 1D experiment. This quantifies the −CH2 peak at δ = 1.25 and the −CH3 peak at δ = 0.80 in the normalised 1D spectrum (completed within Bruker QuantRef manager in Topspin) using a PLS-2 regression model [44]. The lipoprotein classes assessed included very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), intermediate-density lipoproteins (ILDLs), and high-density lipoproteins (HDLs). VLDL subfractions were categorised into six density classes (0.950-1.006 kg/L) as VLDL-1, VLDL-2, VLDL-3, VLDL-4, VLDL-5, and VLDL-6. LDL subfractions were assigned to six density classes as LDL-1 (1.019-1.031 kg/L), LDL-2 (1.031-1.034 kg/L), LDL-3 (1.034-1.037 kg/L), LDL-4 (1.037-1.040 kg/L), LDL-5 (1.040-1.044 kg/L), and LDL-6 (1.044-1.063 kg/L). HDL subfractions were classified into four density classes as HDL-1 (1.063-1.100 kg/L), HDL-2 (1.100-1.125 kg/L), HDL-3 (1.125-1.175 kg/L), and HDL-4 (1.175-1.210 kg/L). Additional details on lipoproteins can be found in the Supplementary Information (**Table S1**).

Mass Spectrometry Data integration

Raw spectra were imported into Skyline 21.1 (MacCoss, WA, USA) software for peak integration and data pre-processing and filtering was performed in R (version 4.4.1, r foundation, Vienna, Austria) in R Studio[45] (version 1.4.1, R Studio, Boston, MA, USA). Data pre-processing consisted of samples/lipid species with >50% missing values being filtered out, imputation of minimum value/2 in samples/lipid species with <50% missing values and filtering features with >30% relative standard deviations in plasma quality control (QC) samples. Each feature underwent signal drift correction using the Random Forrest method in the *statTarget* R package[46] with pooled plasma quality control (QC) samples.

Statistical analysis

Principal component analysis (PCA), orthogonal projection to latent structures-discriminant analysis (OPLS-DA) models and projections were created using the *metabom8* R package (V0.2) from https://github.com/tkimhofer (**Figure 1**). Additionally using *metabom8*, the eruption plot[47] was produced by calculating univariate effect size, Cliff's delta, on the x-axis and the OPLS-DA predictive

loadings on the y-axis, with each point coloured by Mann-Whitney U test p-value. The training OPLS-DA model using samples from the time of burn admission was cross-validated using the Monte Carlo method on 2/3 of the dataset and 1/3 used to predict, with k set to 200. Univariate statistics and visualisation plots were generated in R (version 4.4.1, r foundation, Vienna, Austria) in R Studio[45] using the *ggplot2* (V3.4.2) R package from https://github.com/tidyverse/ggplot2.

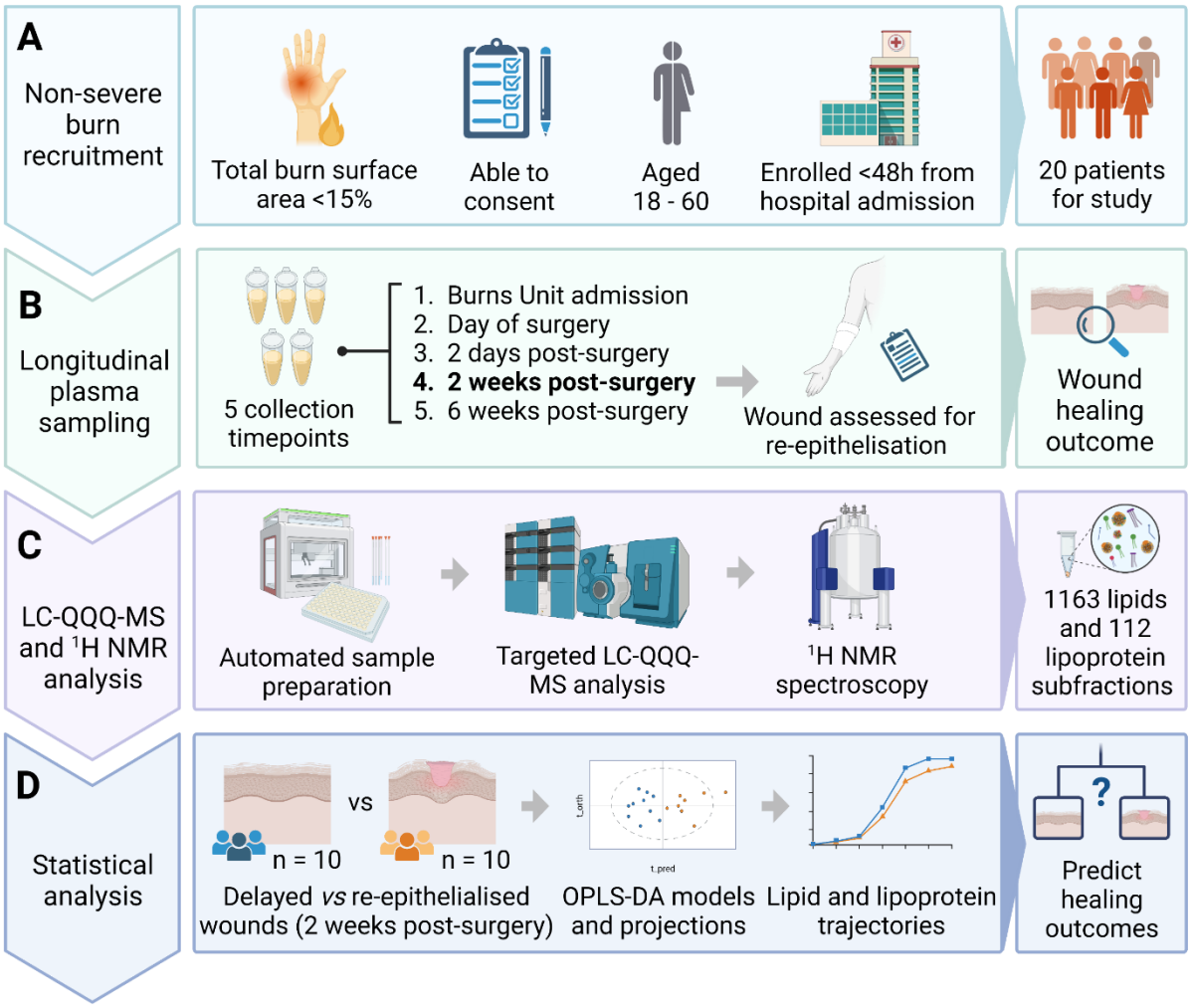

Figure 1. Generalised workflow of patient recruitment to the CABIN Fever trial, sample collection, lipid profiling, ^{1}H NMR spectroscopy and statistical analysis for the study. A) Enrolment criteria for patients with non-severe burns into the CABIN Fever study, with final patient number used in this study (n=20). **B)** Longitudinal plasma sampling of patients, with plasma collected at trial enrolment, day of wound debridement surgery, two days, two weeks and six weeks post-surgery

and burn wounds assessed at two weeks post for re-epithelisation. **C)** Simplified lipid profiling analysis using LC-QQQ-MS to detect 1163 lipid species spanning 20 subclasses and ^{1}H NMR spectroscopy using 1D experiments to measure 112 lipoprotein subfractions. **D)** Statistical analysis comparing lipid profiles of patients with wound re-epithelisation (RE) (n=10) and delayed wound re-epithelisation (DRE) (n=10) at two weeks post-surgery using OPLS-DA modelling and lipid trajectories to predict wound healing outcomes pre-surgery.

Results

Data robustness

From pre-processing data acquired from LC-QQQ-MS, 850 lipid species passed the quality control checks of <50% missing values and <30% relative standard deviations across intermittently analysed pooled QC plasma. The lipid profiling and lipoprotein datasets were combined and underwent PCA modelling of study samples (n=66) and QC plasma samples (n=18) to evaluate robustness (**Figure S1**). QC plasma samples clustered in principal components 1 and 2 indicating minimal variability compared with study samples subfractions across the analytical run.

Clinical parameters

No significant differences were observed between the two groups at admission in sex, age and burn TBSA (**Table 1**). The mean days from injury was not significantly different between re-epithelisation groups at burns admission (**Figure S2**), however all participants who presented to the Burns Unit more than 6 days since the initial injury were in the delayed re-epithelisation (DRE) group. It should be noted that it was not feasible to collect plasma at every scheduled time point for each of the non-severe burn patients (n=20) and a total of 66 samples were analysed in this study. A comprehensive list of de-identified patient numbers at each collection timepoint is provided in **Table S2**.

Plasma lipidome and lipoproteins predicts 2 week wound re-epithelisation outcomes for non-severe burns at hospital admission

Lipid and lipoprotein data from the admission timepoint were used to construct an OPLS-DA (**Figure 2A**) model for classification of re-epithelialisation (RE) *vs* DRE at the 2-week clinic visit. Model scores were R^2X = 0.11, area under receiver operating characteristics (AUROC) = 1, and cross validation-AUROC (CV-AUROC) = 0.75 using the Monte Carlo method on 2/3 of the dataset and the remainder as predictor, with k set to 200. For the projected models, the day of surgery had a sensitivity of 0.67 and a specificity of 1.0 (**Figure 2B**) using a confusion matrix, 2 days post-surgery (**Figure 2C**; sensitivity = 0.83, specificity = 1.0), 2 weeks post-surgery (**Figure 2D**; sensitivity = 1.0, specificity = 0.67), and 6 weeks post-surgery (**Figure 2E**; sensitivity = 1.0, specificity = 0.75).

To visualise OPLS-DA loadings from the OPLS-DA classifier, an eruption plot[47] was produced with significant lipid and lipoproteins coloured by Mann-Whitney U p-value (red for p-values < 0.05; **Figure 3**). For patients with DRE at two weeks post-surgery, key lipid species influencing the admission model were from the triacylglyceride (TAG) and diacylglyceride (DAG) subclasses, with phosphatidylglycerol (PG) PG(20:0_20:3) having the most influence (p-value = 0.012, Cliff's delta = 0.70, OPLS-DA predictive component = 0.056), followed by DAG(18:2_18:3) (p-value = 0.019, Cliff's delta = 0.65, OPLS-DA predictive component = 0.051). Within the fatty acyl (FA) sidechains of the significant TAGs and DAGs, a recurring trend of FA(18:2), FA(18:3) and FA(20:4) was seen. In the RE wound group, the main lipid subclasses driving separation were phosphatidylinositols (PIs), phosphatidylcholines (PCs) and phosphatidylserines (PSs), with PC(16:0_18:1) (p-value = 0.009, Cliff's delta = -0.73, OPLS-DA predictive component = 0.81) and PC(16:0_16:0) (p-value=0.007, Cliff's delta = -0.75, OPLS-DA predictive component = 0.078) showing the greatest influence on the model.

For lipoproteins, the RE group showed higher levels of high-density lipoproteins (HDL) subfractions, particularly HDL subfraction 2 apolipoprotein-A1 (H2A1) (p-value = 0.007, Cliff's delta = -0.75, OPLS-DA predictive component = 0.083) and HDL-2 phospholipids (H2PL) (p-value = 0.005, Cliff's delta = -0.78, OPLS-DA predictive component = 0.085). On the contrary, the DRE group had increased levels of low-density lipoprotein (LDL) subfractions, with the ratio between LDL/HDL (LDHD) exerting the most influence (p-value = 0.004, Cliff's delta = 0.80, OPLS-DA predictive component =

0.077), followed by the apolipoprotein-B100/apolipoprotein-A1 (ABA1) ratio (p-value = 0.015, Cliff's delta = 0.7, OPLS-DA predictive component = 0.067).

Model validation using longitudinal data

To validate the model, data acquired from samples collected at surgery (**Figure 2B**), two days (**Figure 2C**), two weeks and six weeks post-surgery (**Figure 2E**) were projected onto the classification model. Resultant scores plots correctly predicted the DRE and RE patient groups through the timepoints, indicating a distinct multivariate signature of RE and DRE that is consistent over six weeks. The exception was three participants with RE who moved closer to the DRE space at the six weeks post-surgery timepoint and one at two weeks post, indicating a shift in their lipid and lipoprotein signature. Additionally, two DRE patients clustered with the RE group at surgery and one at two days post-surgery. Further investigation into the clinical outcome of the four incorrectly classified participants revealed no clinical abnormalities, whilst participant 14 experienced an adverse inflammatory event independent to the burn.

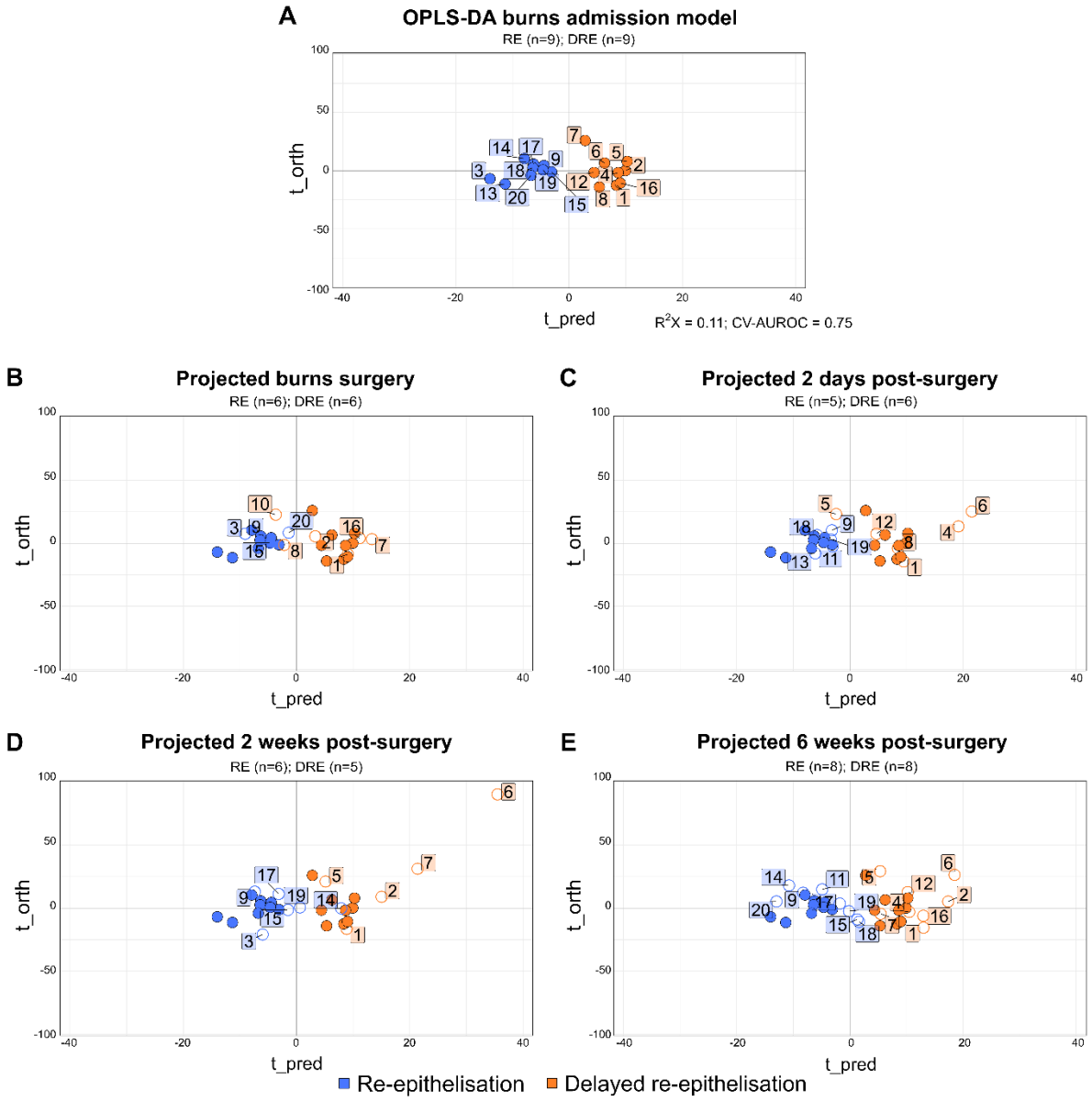

Figure 2. Supervised orthogonal projection to latent structures-discriminant analysis (OPLS-DA) modelling of re-epithelialisation (RE) and delayed re-epithelisation (DRE) groups at burn admission, with projected timepoints of surgery, two days, two weeks and six weeks post-surgery. A) OPLS-DA model of admission (R^2X = 0.11, AUROC = 1, CV-AUROC = 0.75) comparing RE (n=9; coloured blue) and DRE (n=9; coloured orange) using 850 lipid species and 112 lipoprotein subfractions. Each sequential plot (B-E) has predicted OPLS-DA scores of each timepoint projected on the admission model (coloured open circle) with an identifying patient number and sample sizes for RE and DRE groups are provided in the subtitle of each plot.

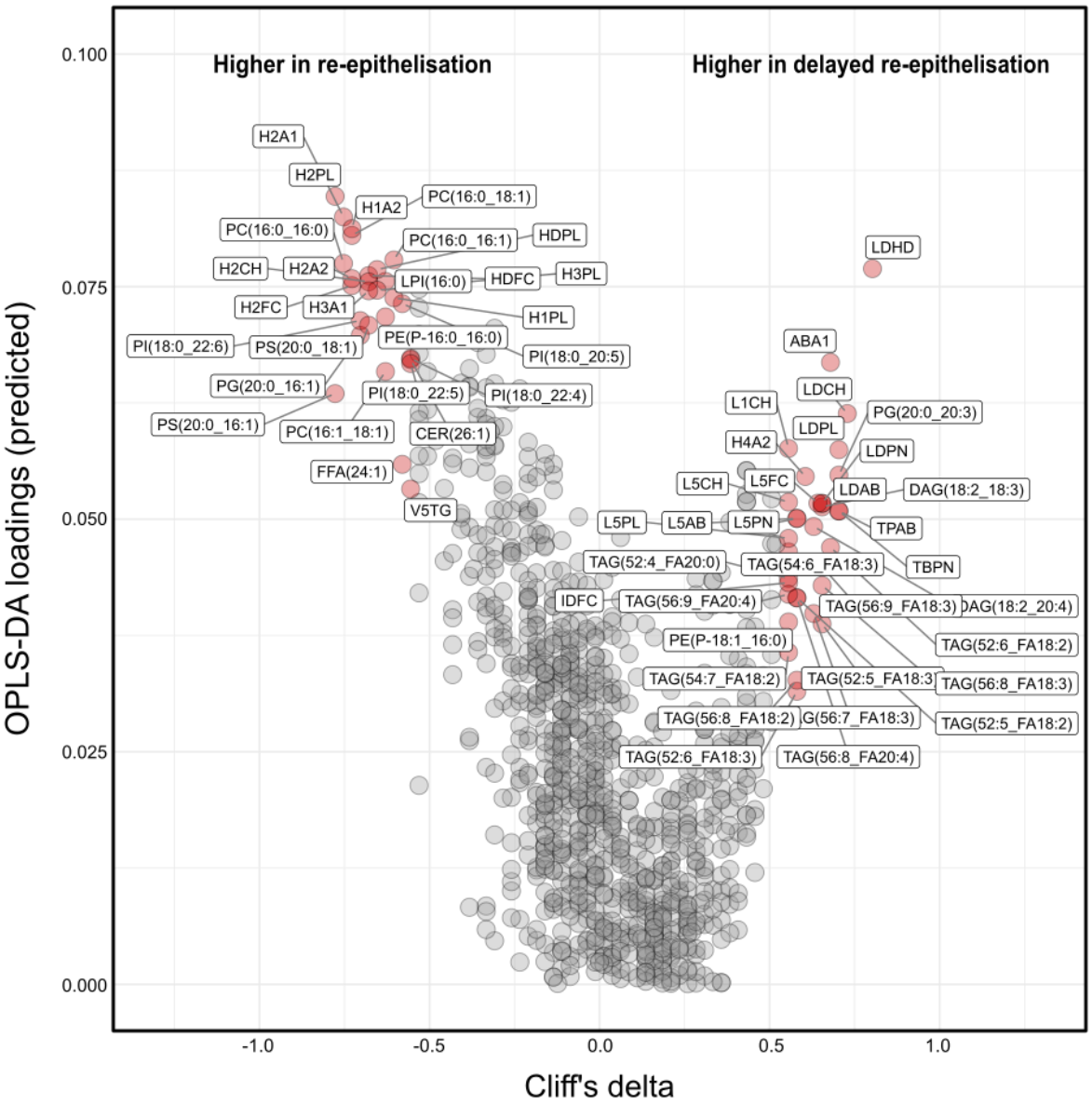

Figure 3. Eruption plot of the modelling of re-epithelisation versus delayed re-epithelisation groups at admission. Significant lipid species and lipoprotein subfractions from Mann-Whitney U analysis are highlighted in red (p-value <0.05) and corresponding Cliff's delta direction for re-epithelisation groups are labelled (left – RE; right – DRE).

Differing lipidomic and lipoprotein signatures of re-epithelisation groups from burns admission to six weeks post-injury.

To observe longitudinal trajectories of specific metabolites, concentration values for the key lipids and lipoprotein subfractions highlighted in the eruption plot (Cliff's delta > 0.5 or <-0.5 and p-value <0.05) (**Figure 3**) were plotted across the five timepoints (**Figure 4**). Concentrations of PC(16:0_16:0), PS(20:0_18:1), PI(18:0_20:5) and PS(20:0_16:1) were higher in the RE patients compared to the DRE group at nearly all timepoints except for surgery. Lysophosphatidylinositol (LPI) LPI(16:0) had similar patterns with the greatest difference in concentration at admission for RE and DRE, but converged at two days post-surgery and then RE slightly increasing over time above DRE. Lipoprotein subfractions H2A1, H2PL, HDL-1 apolipoprotein-A2 (H1A2), HDL-2 free cholesterol (H2FC) and HDL-2 apolipoprotein-A2 (H2A2) followed similar trends as the other lipid species, being higher in RE at all timepoints except for H2FC that converges at surgery.

DRE lipid trajectories for DAG(18:2_18:3), TAG(54:6_FA18:3), TAG(56:9_FA18:3), TAG(56:8_FA18:3) and DAG(18:2_20:4) were similar, having higher concentrations than the RE but converging at two days post-surgery and spiking at two weeks post-surgery before returning to a similar concentration as at admission. For the lipoprotein subfractions, concentrations and ratios all were higher at all time points for LDHD, ABA1, LDL cholesterol (LDCH), LDL phospholipids (LDPL) and LDL subfraction 1 cholesterol (L1CH), except for LDPL and L1CH that had similar median concentrations in RE at two weeks post-surgery. Interestingly, trajectories for both wound outcome groups for the significantly different lipid species had comparable median concentrations between their own admission and six weeks post-surgery.

Even though PC(16:0_16:1), PC(16:0_18:1), PG(20:0_16:1), PG(20:0_20:3), PI(18:0_22:6) and PC(16:1_18:1) lipid species were considered significant from univariate Mann-Whitney U test and had stronger effect sizes at admission (**Figure 3**), trajectories over time for both re-epithelisation groups appeared to be random (**Figure S3**) indicating these lipids could only be useful at burns admission for predicting wound healing and not post-surgery.

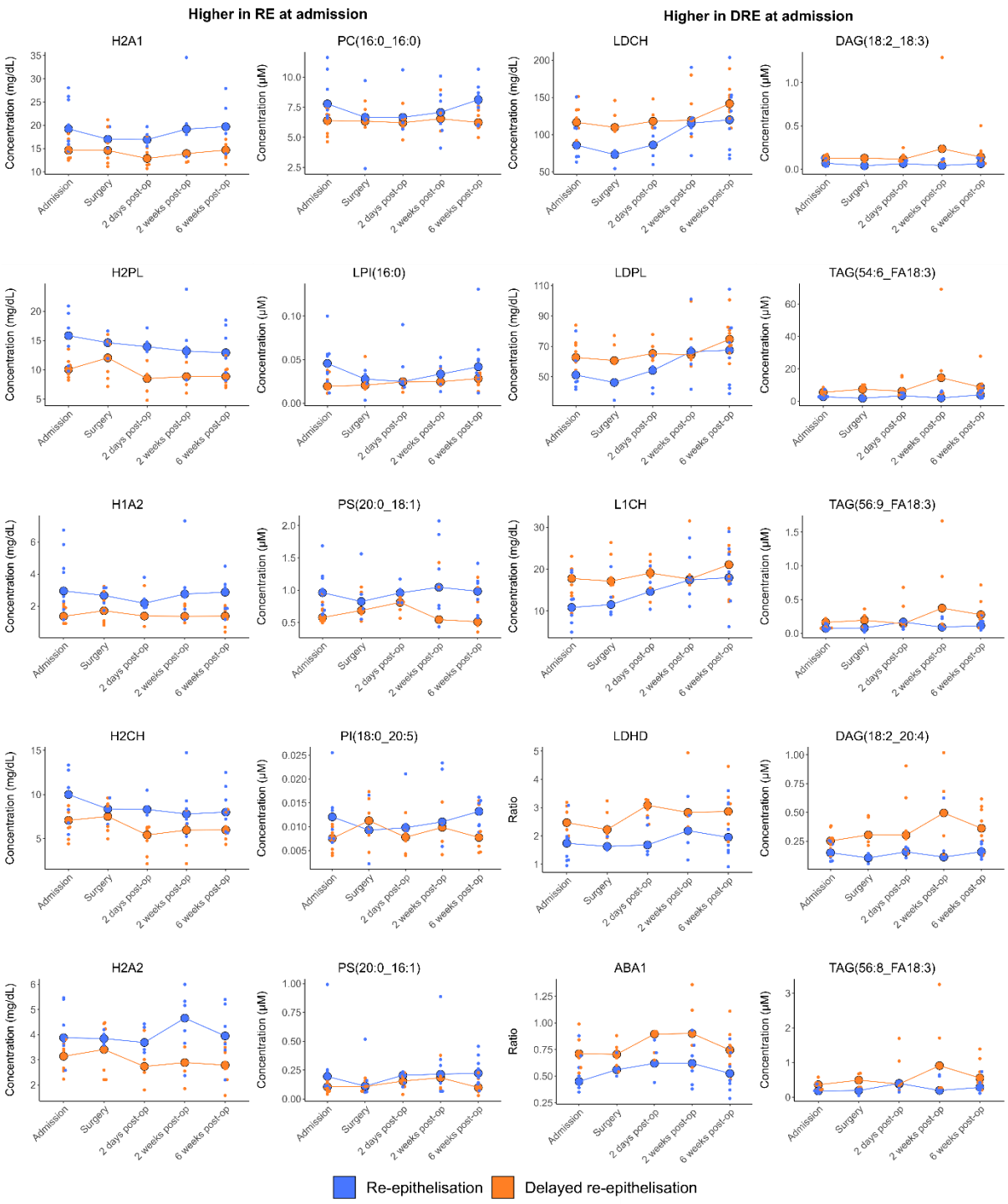

Figure 4. Trajectory dot plots of five significant and main separation driver lipids and lipoprotein subfractions from the eruption plot and OPLS-DA modelling for both wound outcome groups over time. Plots display lipid and lipoprotein subfraction concentrations of each patient with median trendline of both RE (n=10; coloured blue) and DRE (n=10; coloured orange) at burn admission, surgery, two days, two weeks, and six weeks post-surgery.

Discussion

Using samples collected at hospital admission following a burn injury, we were able to classify individuals who experienced delayed re-epithelialisation (DRE) and required extra wound care from patients who had wound re-epithelialisation (RE) at two weeks post burn surgery.

RE and DRE wound outcome groups had distinct lipid and lipoprotein signatures at admission, seen in the separation of the OPLS-DA model (**Figure 2A**). At surgery and two days post-surgery (**Figure 2B, 2C**), the OPLS-DA model was able to predict both groups accurately, except for patient 8 and 10 in the DRE group who clustered with the RE. This could infer that the debridement surgery of their burn wounds may have improved lipid and lipoproteins signatures in these patients to reflect more of the signatures seen in RE in the short-term. However, these patients re-clustered with the DRE group at two weeks post-surgery, except for patient 5, where variations in patient trajectories become more apparent and a greater degree of separation in the model occurred (**Figure 2C, Figure 4**). The predictive model accuracy for two weeks post-surgery was similar to the previous timepoints, only with patient 14 from the RE group moving into the DRE space, most likely due to their adverse inflammatory event experienced aside from the burn injury. At six weeks post-surgery, the model was still able to accurately classify both groups, except for RE patients 18 and 15 profiles moving towards the DRE side. The accuracy of the OPLS-DA model and consistent separation of RE and DRE group infers that patients who experienced delayed wound closure at two weeks post-surgery had a distinct lipid and lipoprotein profile that occurred shortly after the initial burn injury and persisted up to six weeks post-surgery.

For the DRE group, lipid and lipoprotein profiles may be indicating a divergent systemic response to injury and an underlying lipid-mediated inflammatory mechanism compared to the RE group (**Figure 2, Figure 4**). Patients with delayed wound closure at two weeks post-surgery had lipid species predominantly from the TAG and DAG subclasses with FA sidechains of 18:2, 18:3 and 20:4 and LDL lipoprotein subfractions (**Figure 3**). Analysing the trajectories of the most significant TAGs and LDL subfractions (p-value < 0.02), the DRE group had higher concentrations than the RE group across all timepoints with a spike at two weeks post-surgery for some lipid species (**Figure 4**). To our knowledge, no research has explored the role of TAGs or DAGs in the wound healing process, but increased

concentrations of circulating TAGs have previously been detected in severe burns[48]. LDLs carrying apolipoprotein-B100 and the apolipoprotein-B100/apolipoprotein-A1 (ABA1) ratio have previously been shown to reflect inflammatory processes that are associated with atherosclerosis and atherosclerotic risk[34,49,50], suggesting the DRE group may be experiencing an elevated inflammatory response at admission and up to six weeks post-injury compared to RE.

Plasma lipid and lipoprotein profiles from non-severe burn patients with RE were primarily comprised of phospholipids, specifically PSs, PIs and PCs (**Figure 3**). *In vivo*, phospholipids can inhibit pro-inflammatory processes to modulate inflammation[51], for example PSs after skin injury have been observed to bind to annexins on damaged cells to promote apoptotic cell clearance and coagulation during the healing process[52]. Additionally, higher concentrations of HDLs were observed, which are related to phospholipids, as the main constituent of HDL particles are phospholipids (60-70% by mass)[53]. The HDL phospholipid subfraction was also one of the main drivers of separation in the model. The anti-inflammatory properties of HDLs, such as suppressing tumour necrosis factor-α, have been extensively researched in cardiovascular disease[54]. From these results, having a mainly anti-inflammatory lipid and lipoprotein profile from admission to six weeks post-surgery may assist in the regulation of the pro-inflammatory response post non-severe burns in the acute phase and facilitate movement towards wound closure and remodelling (**Figure 2, 4**).

In a study we previously conducted with the same non-severe burn cohort[31], we identified that non-severe burn injury may induce an inflammatory lipid and lipoprotein signature that persisted from admission to six weeks post-surgery when compared to non-burn controls. This initial study was crucial in providing insight into the impact on lipid and lipoprotein metabolism induced by the burn injury and underlying inflammatory mechanisms. Due to this, we found that the lipid species and lipoprotein subfractions that were main drivers of separation between non-severe burns and non-burn controls minimally overlapped with those observed between DRE and RE wound groups, confirming that these profiles are associated with the wound healing process. With this information, clinical stratification and prediction for wound healing outcomes is more attainable as the OPLS-DA model that is based on burn

admission shows a DRE lipid and lipoprotein signature that is not solely due to the impact of the initial injury.

Currently, monitoring burn wound healing is challenging as there is no standardised quantitative tool that can measure healing parameters. Assessment of burn wounds relies mostly on visual observation by clinicians, which can include calculating percentage of the total body surface area (TBSA) burn from charts and depth of injury that is mainly determined by the skin colour and elasticity[55]. As this is visual, there is a degree of subjectivity between clinicians even when specialised and trained, with a study reporting accuracy in burn wound assessment only 60-70% of the time[9]. Furthermore, wound dressings tend to be left on the skin between 2 to 5 days to facilitate healing, which limits opportunities for visual assessment[8]. New studies in non-severe burns have presented technologies that have potential to monitor burn wounds and predict patient outcomes regardless of dressing application[8,56]. Kenworthy et al. have reported that bioimpedance spectroscopy (BIS), the measure of the body's inter-compartmental fluid volumes, correlated with healing rates in limb burns and is non-invasive[8]. Similarly, Khani et al. used wavelet Shannon entropy of terahertz time-domain waveforms to measure the rate of re-epithelisation with high accuracy in predicting wound healing outcomes in pigs[56]. However, these studies were conducted on the local wound rather than using biofluids, in which analysing biofluids, even though invasive, may be more reflective of wound healing outcomes as burn injuries cause a systemic response and numerous processes are involved in tissue repair[11,37]. Predictive modelling using metabolic phenotyping has had success in clinical translation, a recent study has developed an interactive framework that can accurately predict SARS-CoV-2 infection recoveries and stratify patients into risk groups based on their metabolic signatures[36].

Monitoring plasma lipid and lipoprotein profiles after a non-severe burn from admission to hospital could be valuable for predicting wound healing outcomes and guiding treatment options[57]. Patients who experienced DRE at two weeks post-surgery presented with differing plasma profiles from patients with RE, with profiles dominated by TAGs, DAGs and LDL subfractions instead of phospholipids and HDL subfractions (**Figure 2**). Patients at risk of poor re-epithelisation outcomes from hospital admission may benefit from additional lipid-based treatments, such as liposome hydrogels[58] or lung

surfactant[59], both of which have shown promise in accelerating wound healing by supplying the wound directly with phospholipids to support cellular repair[58,59]. Moreover, if evidence of impaired TAG cycling to free fatty acids (FFAs) is observed, balanced supplementation with FFAs, particularly ω-3 FFAs (e.g. α-linolenic acid), to modulate the inflammatory process should be considered as it has shown to improve patient outcomes in severely burn injured patients[60]. With the time related information shown in lipid profiles in **Figure 4**, it is apparent that the TAG, DAG and LDL subfraction predominant lipid and lipoprotein profiles in the delayed re-epithelisation group remained constant after surgical wound debridement and became more pronounced at two weeks post-surgery. This could infer that if these lipid profiles at admission remain constant after surgery that potentially other wound-based treatments could intervene earlier than two weeks post-surgery to improve healing outcomes. The proof of concept shows promise in using lipid and lipoprotein signatures to predict healing outcomes as early as admission and will require further validation in larger non-severe burn cohorts to gain power for clinical translation.

Study limitations

Due to the nature of the non-severe burn trial, we acknowledge there are limitations in this study mainly in sampling duration, sample size and distribution of sex. We acknowledge that the sample size of RE and DRE groups were small, particularly at surgery (**Figure 2, Table S1**). These small sample sizes could result in type II errors and further research with larger sample sizes is needed to confirm the clinical differences observed in the lipid and lipoprotein profiles of the RE and DRE groups[61]. Furthermore, the trajectories cease at the 6-week post-surgery timepoint where the key influencing lipid and lipoprotein concentrations appeared to be returning to similar concentrations as seen at admission (**Figure 4**). Extending the timepoints past this 6-week mark may be beneficial when monitoring trajectories to visualise whether these lipid and lipoprotein profiles change after re-epithelisation of the DRE group and if they differ from hospital admission.

Lastly, the patients recruited into the trial were unequal in terms of sex distribution. To observe any effect of sex on the key influencing lipids and lipoproteins identified driving re-epithelisation groups (**Figure 4**), univariate tests (Mann-Whitney U) were applied to both sexes using lipid and lipoprotein

concentrations at admission (the basis for the OPLS-DA model) (**Figure S3**). No significant differences were seen in the key lipid and lipoproteins, except for the ABA1 ratio where females had higher ABA1 ratios on average at admission (p-value = 0.035).

Conclusion

At study enrolment, the plasma lipidome appeared to be different between patients who had re-epithelialized or who had delayed re-epithelisation of non-severe burn wounds at two weeks post-surgery. These distinctive profiles were maintained over the 6-week recovery period of monitoring with OPLS-DA modelling accurately predicting re-epithelisation outcomes at each of the sample collection timepoints. For patients with re-epithelialized wounds, lipid profiles were driven by phospholipids, specifically PGs, PIs and PCs determined to be the key univariately significant lipid species when compared to the DRE group. Of the lipoprotein subfractions, HDLs, particularly HDL-2 subfractions were drivers of separation for the RE group. Opposing this, patients with delayed re-epithelialized wounds displayed lipid profiles dominated by mainly TAGs and some DAGs with univariately significant lipid species. Additionally, LDL subfractions, such as LDCH and LDPL, were predominant in the lipoprotein profiles. With the higher amount of TAG species present in the DRE group and FFA lipid mediators within the sidechains, the burn injury may potentially be impacting hydrolysis of TAGs to FFAs and contributing to the delay in re-epithelisation. The increase in the LDHD and ABA1 ratios, previously seen to be associated with inflammation and atherosclerosis, may suggest more of a persistent inflammatory response in patients with DRE compared to the RE group. Furthermore, lipid and lipoprotein subfraction trajectories of the phospholipid species and HDL subfractions remained higher in the RE than the DRE group across all timepoints, potentially suggesting that an anti-inflammatory lipid response after a non-severe burn injury may be contributing to proper wound closure and re-epithelisation. These findings exemplify the value in monitoring plasma lipid and lipoprotein signatures after a non-severe burn and that wound healing outcomes can potentially be predicted at admission to hospital but necessitates subsequent validation in larger cohorts of non-severe burns to facilitate clinical implementation.

Declarations

Ethics approval

The randomised placebo-controlled trial of Celecoxib for Acute Burn Inflammation and fever was registered with the Australian New Zealand Clinical Trials Registry (Registration number: ACTRN12618000732280). Ethical approval for the use of the retrospectively collected samples for this study was granted by the South Metropolitan Health Service Health Research Ethics Committee (Approval number: RGS731).

Availability of data and materials

The datasets used and/or analysed in the current study are available from the corresponding author upon request.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Authors contributions

ER, MWF and FMW designed, led, and provided the samples for the CABIN Fever study. MR, GLM, ER, MWF, FMW, EH, JKN, LW and NG contributed to study concept and design. MR analysed the samples by mass spectrometry and drafted the manuscript. SL and PN analysed the samples by nuclear magnetic resonance spectroscopy. RM, SL, PN, JW and JKN curated the lipoprotein dataset and

provided statistical/interpretation input. MR and LW performed statistical analysis of the combined dataset. MR, ER, GLM, MWF, FMW, RM, SL, PN, JW, EH, JKN, NG and LW edited and critically revised the manuscript. ER, FMW, EH and JKN provided funding support. All authors read and approved the final manuscript.

References

1. Greenhalgh DG. Management of Burns. N Engl J Med. 2019;380:2349–59.

2. Markiewicz-Gospodarek A, Kozioł M, Tobiasz M, Baj J, Radzikowska-Büchner E, Przekora A. Burn Wound Healing: Clinical Complications, Medical Care, Treatment, and Dressing Types: The Current State of Knowledge for Clinical Practice. International Journal of Environmental Research and Public Health. 2022;19:1338.

3. Stander M, Wallis LA. The Emergency Management and Treatment of Severe Burns. Emerg Med Int. 2011;2011:161375.

4. Wang Y, Beekman J, Hew J, Jackson S, Issler-Fisher AC, Parungao R, et al. Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring. Advanced Drug Delivery Reviews. 2018;123:3–17.

5. Li Z, Maitz P. Cell therapy for severe burn wound healing. Burns Trauma. 2018;6:13.

6. Osborne T, Edgar D, Gittings P, Wood F, Le Huray T, Allan B, et al. A prospective pilot study of the energy balance profiles in acute non-severe burn patients. Burns. 2022;48:184–90.

7. Shpichka A, Butnaru D, Bezrukov EA, Sukhanov RB, Atala A, Burdukovskii V, et al. Skin tissue regeneration for burn injury. Stem Cell Res Ther. 2019;10:94.

8. Kenworthy P, Phillips M, Grisbrook TL, Gibson W, Wood FM, Edgar DW. Monitoring wound healing in minor burns—A novel approach. Burns. 2018;44:70–6.

9. Monstrey S, Hoeksema H, Verbelen J, Pirayesh A, Blondeel P. Assessment of burn depth and burn wound healing potential. Burns. 2008;34:761–9.

10. Li Z, Maitz P. Cell therapy for severe burn wound healing. Burns & Trauma [Internet]. 2018 [cited 2023 May 24];6. Available from: https://doi.org/10.1186/s41038-018-0117-0

11. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. Open Biology. 2020;10:200223.

12. Dhall S, Wijesinghe DS, Karim ZA, Castro A, Vemana HP, Khasawneh FT, et al. Arachidonic acid-derived signaling lipids and functions in impaired healing. Wound Repair Regen. 2015;23:644–56.

13. Schierle CF, De la Garza M, Mustoe TA, Galiano RD. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair and Regeneration. 2009;17:354–9.

14. Plichta JK, Holmes CJ, Gamelli RL, Radek KA. Local Burn Injury Promotes Defects in the Epidermal Lipid and Antimicrobial Peptide Barriers in Human Autograft Skin and Burn Margin: Implications for Burn Wound Healing and Graft Survival. J Burn Care Res. 2017;38:e212–26.

15. Duke JM, Randall SM, Wood FM, Boyd JH, Fear MW. Burns and long-term infectious disease morbidity: A population-based study. Burns. 2017;43:273–81.

16. Finnerty CC, Jeschke MG, Branski LK, Barret JP, Dziewulski P, Herndon DN. Hypertrophic scarring: the greatest unmet challenge after burn injury. Lancet. 2016;388:1427–36.

17. Hamed K, Giles N, Anderson J, Phillips JK, Dawson LF, Drummond P, et al. Changes in cutaneous innervation in patients with chronic pain after burns. Burns. 2011;37:631–7.

18. Frescos N. Assessment of pain in chronic wounds: A survey of Australian health care practitioners. International Wound Journal. 2018;15:943–9.

19. Chogan F, Chen Y, Wood F, Jeschke MG. Skin Tissue Engineering Advances in Burns: A Brief Introduction to the Past, the Present, and the Future Potential. Journal of Burn Care & Research. 2023;44:S1–4.

20. Fear VS, Poh W-P, Valvis S, Waithman JC, Foley B, Wood FM, et al. Timing of excision after a non-severe burn has a significant impact on the subsequent immune response in a murine model. Burns. 2016;42:815–24.

21. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clinics in Dermatology. 2007;25:19–25.

22. Yoon J, Kym D, Hur J, Won JH, Yim H, Cho YS, et al. Time-varying discrimination accuracy of longitudinal biomarkers for the prediction of mortality compared to assessment at fixed time point in severe burns patients. BMC Emergency Medicine. 2021;21:1.

23. Song J, Ozhathil DK, El Ayadi A, Golovko G, Wolf SE. C-reactive protein elevation is associated with increased morbidity and mortality in elderly burned patients. Burns. 2023;49:806–12.

24. Muñoz B, Suárez-Sánchez R, Hernández-Hernández O, Franco-Cendejas R, Cortés H, Magaña JJ. From traditional biochemical signals to molecular markers for detection of sepsis after burn injuries. Burns. 2019;45:16–31.

25. Rittenhouse BA, Rizzo JA, Shields BA, Rowan MP, Aden JK, Salinas J, et al. Predicting wound healing rates and survival with the use of automated serial evaluations of burn wounds. Burns. 2019;45:48–53.

26. Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, et al. Burn wound healing and treatment: review and advancements. Crit Care. 2015;19:243.

27. Xiao-Wu W, Herndon DN, Spies M, Sanford AP, Wolf SE. Effects of Delayed Wound Excision and Grafting in Severely Burned Children. Archives of Surgery. 2002;137:1049–54.

28. Mathur S, Sutton J. Personalized medicine could transform healthcare. Biomed Rep. 2017;7:3–5.

29. Duke J, Wood F, Semmens J, Spilsbury K, Edgar DW, Hendrie D, et al. A 26-year population-based study of burn injury hospital admissions in Western Australia. J Burn Care Res. 2011;32:379–86.

30. Buergel T, Steinfeldt J, Ruyoga G, Pietzner M, Bizzarri D, Vojinovic D, et al. Metabolomic profiles predict individual multidisease outcomes. Nat Med. 2022;28:2309–20.

31. Ryan M, Raby E, Masuda R, Lodge S, Nitschke P, Maker G, et al. Metabolic consequences of non-severe burn injury are associated with increased plasma markers of inflammation and cardiovascular disease risk. 2023.

32. Yau A, Fear MW, Gray N, Ryan M, Holmes E, Nicholson JK, et al. Enhancing the accuracy of surgical wound excision following burns trauma via application of Rapid Evaporative IonisationMass Spectrometry (REIMS). Burns. 2022;48:1574–83.

33. Gray N, Lawler NG, Zeng AX, Ryan M, Bong SH, Boughton BA, et al. Diagnostic potential of the plasma lipidome in infectious disease: Application to acute sars-cov-2 infection. Metabolites. 2021;11.

34. Lodge S, Nitschke P, Kimhofer T, Wist J, Bong S-H, Loo RL, et al. Diffusion and Relaxation Edited Proton NMR Spectroscopy of Plasma Reveals a High-Fidelity Supramolecular Biomarker Signature of SARS-CoV-2 Infection. Anal Chem. 2021;93:3976–86.

35. Masuda R, Lodge S, Nitschke P, Spraul M, Schaefer H, Bong S-H, et al. Integrative Modeling of Plasma Metabolic and Lipoprotein Biomarkers of SARS-CoV-2 Infection in Spanish and Australian COVID-19 Patient Cohorts. J Proteome Res. 2021;20:4139–52.

36. Ruffieux H, Hanson AL, Lodge S, Lawler NG, Whiley L, Gray N, et al. A patient-centric modeling framework captures recovery from SARS-CoV-2 infection. Nat Immunol. 2023;24:349–58.

37. Begum S, Johnson BZ, Morillon A-C, Yang R, Bong SH, Whiley L, et al. Systemic long-term metabolic effects of acute non-severe paediatric burn injury. Sci Rep. 2022;12:13043.

38. Qi P, Abdullahi A, Stanojcic M, Patsouris D, Jeschke MG. Lipidomic analysis enables prediction of clinical outcomes in burn patients. Sci Rep. 2016;6:38707.

39. Kamolz L-P, Andel H, Mittlböck M, Winter W, Haslik W, Meissl G, et al. Serum cholesterol and triglycerides: potential role in mortality prediction. Burns. 2003;29:810–5.

40. Bjerrum JT, Wang Y, Zhang J, Riis LB, Nielsen OH, Seidelin JB. Lipidomic Trajectories Characterize Delayed Mucosal Wound Healing in Quiescent Ulcerative Colitis and Identify Potential Novel Therapeutic Targets. International Journal of Biological Sciences. 2022;18:1813–28.

41. Ryan MJ, Grant-St James A, Lawler NG, Fear MW, Raby E, Wood FM, et al. Comprehensive Lipidomic Workflow for Multicohort Population Phenotyping Using Stable Isotope Dilution Targeted Liquid Chromatography-Mass Spectrometry. J Proteome Res. 2023;

42. Dona AC, Jiménez B, Schäfer H, Humpfer E, Spraul M, Lewis MR, et al. Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. Anal Chem. 2014;86:9887–94.

43. Nicholson JK, Foxall PJ, Spraul M, Farrant RD, Lindon JC. 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Anal Chem. 1995;67:793–811.

44. Jiménez B, Holmes E, Heude C, Tolson RF, Harvey N, Lodge SL, et al. Quantitative Lipoprotein Subclass and Low Molecular Weight Metabolite Analysis in Human Serum and Plasma by 1H NMR Spectroscopy in a Multilaboratory Trial. Anal Chem. 2018;90:11962–71.

45. R Core Team. R: A Language and Environment for Statistical Computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2022. Available from: https://www.R-project.org/

46. Luan H, Ji F, Chen Y, Cai Z. statTarget: A streamlined tool for signal drift correction and interpretations of quantitative mass spectrometry-based omics data. Anal Chim Acta. 2018;1036:66–72.

47. Kimhofer T, Lodge S, Whiley L, Gray N, Loo RL, Lawler NG, et al. Integrative Modeling of Quantitative Plasma Lipoprotein, Metabolic, and Amino Acid Data Reveals a Multiorgan Pathological Signature of SARS-CoV-2 Infection. Journal of Proteome Research. 2020;19:4442–54.

48. Jeschke MG, Gauglitz GG, Kulp GA, Finnerty CC, Williams FN, Kraft R, et al. Long-Term Persistance of the Pathophysiologic Response to Severe Burn Injury. PLoS One. 2011;6:e21245.

49. Kaneva AM, Potolitsyna NN, Bojko ER, Odland JØ. The Apolipoprotein B/Apolipoprotein A-I Ratio as a Potential Marker of Plasma Atherogenicity. Disease Markers. 2015;2015:e591454.

50. Lorey MB, Öörni K, Kovanen PT. Modified Lipoproteins Induce Arterial Wall Inflammation During Atherogenesis. Front Cardiovasc Med. 2022;9:841545.

51. Bretscher P, Egger J, Shamshiev A, Trötzmüller M, Köfeler H, Carreira EM, et al. Phospholipid oxidation generates potent anti-inflammatory lipid mediators that mimic structurally related pro-resolving eicosanoids by activating Nrf2. EMBO Molecular Medicine. 2015;7:593–607.

52. Kreft S, Klatt AR, Straßburger J, Pöschl E, Flower RJ, Eming S, et al. Skin Wound Repair Is Not Altered in the Absence of Endogenous AnxA1 or AnxA5, but Pharmacological Concentrations of AnxA4 and AnxA5 Inhibit Wound Hemostasis. Cells Tissues Organs. 2016;201:287–98.

53. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome1. J Lipid Res. 2013;54:2950–63.

54. Jia C, Anderson JLC, Gruppen EG, Lei Y, Bakker SJL, Dullaart RPF, et al. High-Density Lipoprotein Anti-Inflammatory Capacity and Incident Cardiovascular Events. Circulation. 2021;143:1935–45.

55. Flanagan M. Wound measurement: can it help us to monitor progression to healing? J Wound Care. 2003;12:189–94.

56. Khani ME, Osman OB, Harris ZB, Chen A, Zhou J-W, Singer AJ, et al. Accurate and early prediction of the wound healing outcome of burn injuries using the wavelet Shannon entropy of terahertz time-domain waveforms. JBO. 2022;27:116001.

57. Meikle TG, Huynh K, Giles C, Meikle PJ. Clinical lipidomics: realizing the potential of lipid profiling. Journal of Lipid Research. 2021;62:100127.

58. Homann H-H, Rosbach O, Moll W, Vogt PM, Germann G, Hopp M, et al. A Liposome Hydrogel With Polyvinyl-Pyrrolidone Iodine in the Local Treatment of Partial-Thickness Burn Wounds. Annals of Plastic Surgery. 2007;59:423.

59. Mirastschijski U, Schwab I, Coger V, Zier U, Rianna C, He W, et al. Lung Surfactant Accelerates Skin Wound Healing: A Translational Study with a Randomized Clinical Phase I Study. Sci Rep. 2020;10:2581.

60. Tihista S, Echavarría E. Effect of omega 3 polyunsaturated fatty acids derived from fish oil in major burn patients: A prospective randomized controlled pilot trial. Clinical Nutrition. 2018;37:107–12.

61. Nayak BK. Understanding the relevance of sample size calculation. Indian J Ophthalmol. 2010;58:469–70.