

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Targeted lipidomics reveals extensive changes in circulating lipid mediators in patients with acutely decompensated cirrhosis

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Lopez-Vicario C., Checa A., Urdangarin A., Aguilar F., Alcaraz-Quiles J., Caraceni P., et al. (2020). Targeted lipidomics reveals extensive changes in circulating lipid mediators in patients with acutely decompensated cirrhosis. JOURNAL OF HEPATOLOGY, 73(4), 817-828 [10.1016/j.jhep.2020.03.046].

Availability:

This version is available at: <https://hdl.handle.net/11585/804300> since: 2021-02-23

Published:

DOI: <http://doi.org/10.1016/j.jhep.2020.03.046>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

Targeted Lipidomics Reveals Extensive Changes in the Blood Lipid Mediator Profile in Acutely Decompensated Cirrhosis

Cristina López-Vicario^{1,2}, Antonio Checa³, Arantxa Urdangarin⁴, Ferran Aguilar¹, José Alcaraz-Quiles², Paolo Caraceni⁵, Alex Amorós¹, Marco Pavesi¹, David Gómez-Cabrero⁴, Jonel Trebicka^{1,6}, Karl Oettl⁷, Richard Moreau^{1,8}, Núria Planell⁴, Vicente Arroyo¹, Craig E. Wheelock³ and Joan Clària^{1,2,9}

¹European Foundation for the Study of Chronic Liver Failure (EF-Clif) and Grifols Chair, Barcelona, Spain; ²Biochemistry and Molecular Genetics Service, Hospital Clínic-IDIBAPS and CIBERehd, Barcelona, Spain; ³Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden; ⁴Translational Bioinformatics Unit, NavarraBiomed, Pamplona, Spain; ⁵Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; ⁶J.W. Goethe University Hospital, Frankfurt, Germany; ⁷Institute of Physiological Chemistry, Center of Physiological Medicine, Medical University of Graz, Graz, Austria; ⁸Inserm, U1149, Centre de Recherche sur l'Inflammation (CRI), UMRS1149; Université Paris Diderot-Paris 7, Paris, France; ⁹Department of Biomedical Sciences, University of Barcelona, Barcelona, Spain

Corresponding author: Joan Clària, Hospital Clínic, EF-Clif, Travessera de Gràcia 11, 08021 Barcelona (Spain), tel. +34 932 271 400, e-mail: jclaria@clinic.cat

Keywords: Lipidomics; decompensated cirrhosis; systemic inflammation; lipid mediators

Electronic word count: 5975

Abstract word count: 273

Number of figures: 6

Number of tables: 1

Conflict of interest statement: None

Financial support: Supported by MEC (SAF15-63674-R) and EF-Clif, a non-profit private organization that receives unrestricted donations from Cellex Foundation, Grifols and the European Union's Horizon 2020 research and innovation programme. Our laboratory is a

1 Consolidated Research Group recognized by the Generalitat de Catalunya (2017SGR1449).
2 CIBERehd is funded by the Instituto de Salud Carlos III. C.L-V received funding from the
3 Horizon 2020 grant agreement N° 825694.
4
5

6 **Author Contributions:** Study concept and design (CLV, JC, RM, VA); acquisition of
7 lipidomics data (CLV, AC, CEW); bioinformatics and statistical analysis (AU, FA, AA, MP,
8 DGC, NP); integration of clinical and biological results and interpretation of data (CLV, JC,
9 AC); drafting of the manuscript (CLV, JC); writing of the final manuscript (CLV, JC, RM);
10 critical revision of the manuscript for important intellectual content (JAQ, PC, JT, KO, VA);
11 study supervision (CLV, JC).
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

1
2 Acute-on-chronic liver failure (ACLF) is a newly described syndrome, which develops in
3
4 patients with acutely decompensated cirrhosis, and is characterized by intense systemic
5
6 inflammation, multi-organ failure and high short-term mortality. The profile of circulating
7
8 lipid mediators, which are endogenous signaling molecules generated from polyunsaturated
9
10 fatty acids released from membrane phospholipids that play a major role in inflammation and
11
12 immunity, is poorly characterized in ACLF. In the current study, we assessed the profile of
13
14 lipid mediators by liquid chromatography coupled to tandem mass spectrometry in plasma
15
16 from patients with acutely decompensated cirrhosis, with (n=119) and without (n=127)
17
18 ACLF, and from healthy subjects (HS, n=18). Measurements were prospectively repeated in
19
20 191 patients with acutely decompensated cirrhosis during a 28-day follow-up period. Fifty-
21
22 nine lipid mediators (out of 100) were detected in plasma from cirrhotic patients, of which,
23
24 16 were significantly associated with the disease status. Among these, 11 lipid mediators
25
26 distinguished patients at any stage from HS, whereas two lipid mediators (leukotriene [LT] E₄
27
28 and 12-hydroxyheptadecatrienoic acid, both derived from arachidonic acid) shaped a minimal
29
30 plasma fingerprint that discriminated patients with ACLF from those without. Levels of LTE₄
31
32 distinguished ACLF grade 3 from ACLF grades 1 and 2, followed the clinical course of the
33
34 disease (increased with worsening and decreased with improvement) and positively
35
36 correlated with markers of inflammation and non-apoptotic cell death. Moreover, LTE₄
37
38 together with LXA₅ (derived from eicosapentaenoic acid) and EKODE (derived from linoleic
39
40 acid) associated with short-term mortality. Interestingly, LXA₅ and EKODE formed a
41
42 signature profile associated with coagulation and liver failures. Taken together, these findings
43
44 uncover specific lipid mediator profiles associated with severity and prognosis of patients
45
46 with acutely decompensated cirrhosis.
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Lay summary

1
2 Acute-on-chronic liver failure (ACLF) is characterized by intense systemic inflammation,
3
4 multi-organ failure and high short-term mortality. In the current study, we assessed by
5
6 targeted lipidomics using a LC-MS/MS-based platform the plasma profile of 100 bioactive
7
8 lipid mediators, which are endogenous signaling molecules generated from polyunsaturated
9
10 fatty acids that play a major role in inflammation and immunity. We identified lipid mediator
11
12 signatures associated with inflammation and non-apoptotic cell death that discriminate
13
14 disease severity and evolution, short-term mortality and organ failures.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

1
2 Patients with acutely decompensated (AD) cirrhosis frequently succumb to the onset of multi-
3 organ failure, a syndrome known as acute-on-chronic liver failure (ACLF) (1,2). ACLF is
4 closely associated with recurrent infections and high short-term mortality and is mostly
5 driven by dysfunctional innate immune system leading to exacerbated systemic
6 inflammation, immune paralysis and tissue immunopathology (3). Indeed, plasma cytokines
7 are unusually elevated in ACLF patients and their levels directly correlate with ACLF
8 severity (3). Moreover, a blood metabolite fingerprint specific for ACLF closely associated
9 with the levels of inflammatory markers and the presence of organ failures has recently been
10 identified (4). Overall, these studies reinforce the concept that systemic inflammation and
11 tissue/organ injury in ACLF is triggered by the concerted actions of cytokines/chemokines
12 and amino acid-derived factors that act as metabotoxins. However, the contribution of lipid
13 mediator species in the pathogenesis of systemic inflammation and development of organ
14 failures in ACLF remains at present unexplored.

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36 Lipid mediators are signaling molecules with potent and diverse actions on blood and tissue
37 homeostasis and responses to stress and injury. These compounds comprise a vast number of
38 species, whose biosynthetic pathways form a complex network of multiple substrates
39 transformed via multiple enzymes (5,6). In general, several enzymes can act on a single
40 substrate, and conversely, multiple substrates can be metabolized by the same enzyme. The
41 majority of the bioactive lipid mediators are intracellularly produced from polyunsaturated
42 fatty acids (PUFAs) released from membrane phospholipids by the action of phospholipase
43 A₂ (5). After the release of free PUFAs to the cytosol, they are rapidly metabolized by three
44 enzymatic families: the cyclooxygenases (COXs), the lipoxygenases (LOXs) and the
45 cytochrome P450 epoxygenases (CYP450), to produce a large array of lipid mediators
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

(**Supplementary Figure 1A**) (5,6). The most common substrate for these enzymes is arachidonic acid (AA), an omega-6-PUFA that is the precursor of eicosanoids such as prostaglandins (PGs), leukotrienes (LTs), thromboxane A₂ (TXA₂) and lipoxins (LXs) (5,6). With the exception of LXs, eicosanoids are considered to be proinflammatory, and some members such PGs and TXA₂ are targeted by NSAIDs (7). In addition to AA, the same enzymes can effectively metabolize its parent precursor, linoleic acid (LA) (**Supplementary Figure 1A**), which is abundant in low-density lipoproteins and inner mitochondrial membrane phospholipids (8). LA derivatives are traditionally considered to exert detrimental actions on renal, respiratory and cardiovascular systems (9). In contrast, the omega-3-PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids give rise to an array of lipid mediators such as 18-HEPE, 17-HDoHE and 14-HDoHE that are involved in the resolution of inflammation (Supplementary Figure 1B) (10). Finally, all PUFAs are susceptible to non-enzymatic oxidation, yielding epoxides, ketones and hydroxylated derivatives, which in general are considered oxidative stress markers (11).

36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

In view of the magnitude and diversity of the lipid mediator network, the analysis and identification of these molecules in complex diseases requires an “omics” approach. This study reports the profiling of 100 lipid mediators using LC-MS/MS in 246 patients with AD cirrhosis of whom 127 did not have ACLF and 119 had ACLF (hereafter called patients with ACLF). Measurements were prospectively repeated in 191 patients during a 28-day follow-up period.

Patients and methods

1
2 The investigation was performed in plasma samples from 246 patients with acutely
3
4 decompensated cirrhosis of whom 119 had ACLF (57 with ACLF-1, 44 with ACLF-2 and 18
5
6 with ACLF-3) from the CANONIC cohort (1). In 191 out of the 246 patients with AD
7
8 cirrhosis, there was availability of plasma samples with enough volume to perform the
9
10 measurements during the 28-day follow-up. All these individuals or their legal
11
12 representatives and the ethics committee of each hospital involved in the study gave informed
13
14 consent for omics investigations in the biobanked material. A flow chart of the patients from
15
16 the CANONIC study included in the targeted lipidomics is shown in **Supplementary Figure**
17
18
19 **2.** The investigation also included 18 healthy subjects (HS, age: 45-65 years).
20
21
22
23
24
25

Analysis of lipid mediators by targeted LC-MS/MS

26
27
28 Plasma levels of 100 lipid mediators were determined by LC-MS/MS. Common and
29
30 systematic nomenclature of these lipid mediators are detailed in **Supplementary Table 1**.
31
32
33
34
35

36 *Analysis of PUFA, isolation of leukocytes, analysis of gene expression by TaqMan low-*
37
38 *density arrays and measurement of cytokines, chemokines and oxidative stress and cell death*
39
40 *(keratin 18 [K18] and caspase-cleaved K18 [cK18]) markers.* See **Supplementary Material**
41
42 **and references 3, 12 and 13.**
43
44
45
46
47

Statistical analysis

48
49
50 Among the 100 lipid mediators screened (**Supplementary Table 1**), 39 were below the
51
52 detection limits of the method in the three study groups. Among the 61 lipid mediators
53
54 detected, two were excluded (5-iso-prostaglandin F2 α -VI and 12-KETE) because they did not
55
56
57
58
59
60
61
62
63
64
65

meet the quality control criteria. Therefore, the final analysis included a total of 59 lipid mediators. See **Supplementary Material**.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Results

1
2 Baseline clinical and standard laboratory data are given in **Supplementary Table 2**. C-
3
4 reactive protein levels were higher in patients with AD relative to HS and much higher in
5
6 those with ACLF. White blood cell count was significantly increased in patients with ACLF
7
8 as compared to those with AD. Platelet count and serum albumin were significantly reduced
9
10 in patients with AD and ACLF. There were significant differences between patients with AD
11
12 and ACLF in serum bilirubin and creatinine levels. Among patients with ACLF, 57 (47.9%)
13
14 had ACLF grade 1 (one organ failure), 44 (36.9%) had ACLF grade 2 (two organ failures),
15
16 and 18 (15.1%) had ACLF grade 3 (three organ failures or more). The frequency of failing
17
18 organs in these patients is also shown in **Supplementary Table 2**. Patients with ACLF had
19
20 higher MELD and CLIF organ failure and Child-Pugh scores and greater 28-day mortality
21
22 than patients with AD.
23
24
25
26
27
28
29
30

Patients with AD and those with ACLF have increased plasma levels of AA and higher AA/EPA ratio

31
32 The final database obtained from the targeted LC-MS/MS analysis included a total of 59 lipid
33
34 mediators (see Patients and methods for the criteria used in the selection of compounds). The
35
36 identity of each lipid mediator was assessed by both the selected reaction monitoring (SRM)
37
38 transition and comparison of retention time to that of authentic standards (**Supplementary**
39
40 **Tables 3 and 4**). Annotated lipid mediators were mostly derived from PUFAs of the omega-6
41
42 (AA and its precursor LA) and omega-3 (DHA and EPA) families (Figure 1A). As shown in
43
44 **Figure 1B**, plasma levels of free PUFA were similar across the three study groups, except for
45
46 the AA, which levels were slightly increased in patients with AD and in those with ACLF.
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1 were considerably reduced in patients with AD and also in patients with ACLF (**Figure 1B**).
2 In both cases (free and total PUFA), the AA/EPA ratio, which is a surrogate marker of
3 systemic inflammation (14), was significantly increased in both groups of patients with
4 cirrhosis (Figure 1C). This finding was consistent with the fact that patients with ACLF
5 exhibited an increased systemic inflammatory burden, as reflected by the presence of
6 augmented plasma levels of cytokines/chemokines (**Supplementary Table 5**). Genes related
7 to the desaturation and elongation of fatty acids were not dramatically altered in leukocytes
8 from patients with cirrhosis, except for *SCD1* and *ELOVL6*, which were up- and down-
9 regulated, respectively (Figure 1D).

24 **Distinct plasma distribution of lipid mediators in patients with AD and in those with** 25 **ACLF**

26 The biosynthesis of lipid mediators from PUFA involves a complex network of LOX, COX
27 and CYP450 enzymes (see **Supplementary Figure 1** for an overview of the biosynthetic
28 pathways and **Supplementary Tables 6-8** for a comprehensive classification of each lipid
29 mediator according to its biosynthetic precursor and enzymatic route). **Figure 2A** shows a
30 graphical representation of the plasma abundance of lipid mediators categorized into two
31 families (omega-6 and omega-3) and classified by each enzymatic pathway. Circles represent
32 the absolute amount of lipid mediators within the pathway (upper panel), and the box plots
33 represent individual values for each subject included in the analysis (lower panel). The most
34 abundant lipid mediators in HS were derived from CYP450 and LOX pathways followed by
35 non-enzymatic metabolites and minor quantities of COX derivatives. The abundance of
36 CYP450-derived lipid mediators of the omega-6 family significantly decreased in patients
37 with AD and ACLF whereas those derived from LOX remained steady. The amount of COX-
38 derived lipid mediators slightly increased in patients with cirrhosis, but changes did not reach
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 statistical significance. Notably, the levels of lipid mediators produced from non-enzymatic
2 routes (i.e. free radical lipid oxidation) abruptly increased in patients with AD and culminated
3 in those with ACLF. This increase was predominantly in the omega-6 family and in particular
4 in the LA-derived lipid mediator EKODE (Supplementary Table 7). The increased levels of
5 these non-enzymatic products were consistent with the presence of an intense degree of
6 systemic oxidative stress, as reflected by the plasma levels of HNA1 and HNA2
7 (Supplementary Table 5), which are established markers of systemic oxidative stress in
8 patients with cirrhosis (15).
9
10
11
12
13
14
15
16
17
18
19
20
21

22 **Leukocyte gene expression of lipid mediator-generating enzymes differs between** 23 **patients and HS**

24 We next investigated the expression of genes coding for enzymes responsible for the
25 conversion of PUFA precursors to the individual lipid mediators in leukocytes from patients
26 and from HS. **Figure 2B** shows the relative distribution of each of the three enzymatic
27 pathways (i.e. CYP450, LOX and COX) and **Figure 2C** shows the expression of individual
28 representative genes among these pathways in leukocytes from HS, patients with AD and
29 patients with ACLF. In agreement with results described earlier, the expression of the main
30 CYP450 enzyme involved in PUFA metabolism, CYP2C8, was markedly down-regulated in
31 patients with AD and in those with ACLF, relative to HS. In contrast, the expression of
32 LOXs, specifically ALOX5, which codes for the 5-LOX enzyme involved in the production of
33 inflammatory LTs, was remarkably up-regulated in patients with AD and in those with
34 ACLF. Expression of enzymes of the COX pathway, especially COX-2 (PTGS2) and
35 mPGES-1 (PTGES1), was also up-regulated in patients relative to HS.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Distinct profile of lipid mediators in patients with AD and in those with ACLF relative to HS

Next, we grouped the 59 lipid mediators detected in the plasma of patients according to their cognate chemical families and calculated for each family the fold changes in AD vs HS and in ACLF vs HS. Finally, we ranked fold changes from the highest to the lowest values and the results were visualized in a Cleveland plot (**Figure 3A**). This analysis revealed that LTs, epoxy-keto fatty acids, AA/DHA epoxides, PGs and TX were increased in patients (either AD or ACLF) as compared to HS. In contrast, LXs, which are anti-inflammatory and pro-resolving lipid mediators, LA diols and LA epoxides were remarkably reduced in cirrhosis.

We then calculated fold changes for each individual lipid mediator in AD vs HS and in ACLF vs HS and ranked and plotted the results in a Cleveland plot (**Figure 3B**). This analysis uncovered that 24 out of a total of 59 lipid mediators were significantly increased (fold change >1.5) in AD as compared to HS, of which 4 were further increased in ACLF as compared to AD (**Figure 3B** and **Supplementary Table 9**). On the other hand, the plasma levels of 9 lipid mediators were significantly decreased (fold change <0.5) in AD as compared to HS and none of them was further reduced in ACLF as compared to AD. A complete list of fold-changes between ACLF and AD for all the 59 lipid mediators included in the analysis is provided in **Supplementary Table 9**. Changes in circulating levels of lipid mediators in patients affected indistinctly all PUFA families as shown on the left side of **Figure 3B**, where each lipid mediator is color coded by its biosynthetic precursor.

We next plotted in volcano plots the fold changes in the levels of lipid mediators in patients with AD and in those with ACLF relative to HS, taking into account the statistical significant differences (P values). As shown in **Figure 3C**, this analysis identified increased levels of 8-

1
2
3 HETE, 14,15-DiHETrE, 12,13-epoxy-9-keto-10(trans)octadecenoic acid (EKODE), 11,12-
4 DiHETrE, 8-HETrE, 13-HOTrE γ , LTE₄, 20-HETE, 11-keto-TXB₂ and PGE₁ in the plasma of
5 patients with AD. Among these, LTE₄ (a member of the slow-reacting substance of
6 anaphylaxis and a pathway marker of pro-inflammatory cysteinyl-LT biosynthesis), 11-keto-
7 TXB₂ (a prothrombotic marker) and 20-HETE (a potent renal vasoconstrictor) have
8 pathophysiological significance in these patients. On the other hand, 9(10)-
9 epoxyoctadecenoic (9(10)-EpOME) and 12(13)-epoxyoctadecenoic (12(13)-EpOME) acids,
10 which are generated by neutrophils during oxidative burst and are markers of bactericidal
11 activity, were remarkably suppressed in patients with AD (**Figure 3C**). In patients with
12 ACLF, the lipid mediators whose levels were significantly increased were the same as in
13 patients with AD, but the profile was enriched in two additional lipid mediators (PGF_{2 α} , a
14 COX-derived vasoconstrictor, and 8-HDoHE, a product of DHA autoxidation) (**Figure 3D**).
15 Of note, the fold changes achieved by some lipid mediators such as 8-HETE, which was
16 increased more than 16-fold, or 9(10)-EpOME and 12(13)-EpOME, which were reduced by
17 8-fold, indicate that PUFA metabolism is severely disrupted in patients with cirrhosis.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 **Unbiased identification of a lipid mediator signature specific of AD cirrhosis**

40
41 To reduce the dimension of our dataset, we next explored whether any combination or
42 combinations of lipid mediators could serve as a fingerprint of patients with AD cirrhosis. To
43 address this question, we performed an unbiased PCA analysis on the 59 lipid mediators
44 collected at baseline in the entire study cohort. As shown in **Figure 3E**, the PCA analysis
45 yielded a clear distinction between patients and HS. After adjusting for gender and age, we
46 refined from 59 to 16 the number of lipid mediators separating the different stages of the
47 disease. The plasma levels of these 16 lipid mediators can be visualized in a heatmap, which
48 reveals that, with the exception of 9(10)-EpOME and 12(13)-EpOME, changes in lipid
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

mediators were highly heterogeneous in our cohort of patients (**Figure 3F**). Of interest, one out of these 16 lipid mediators, the DHA product 4-HDoHE, distinguished patients with AD from HS, whereas 11 lipid mediators discriminated HS from patients at any stage (either AD or ACLF) (**Table 1**). These 11 lipid mediators were 9(10)-EpOME, 12(13)-EpOME and EKODE from LA; 8-HETE, 20-HETE, 11,12-DiHETrE, 14,15-DiHETrE and 11-keto-TXB₂ from AA; 8-HETrE and PGE₁ from DGLA and 13-HOTrE γ from γ -LA. Box plots for 9(10)-EpOME and 12(13)-EpOME are shown in **Figure 4A**. Although 9(10)-EpOME, 12(13)-EpOME are biologically relevant and their levels presented the largest reductions in the volcano plots, these two lipid mediators were not associated with any clinical outcome (**Supplementary Figure 3**). Lipid mediators that were not significantly associated and did not discriminate the different stages of the disease are listed in **Supplementary Table 10**.

Plasma levels of LTE₄ discriminate disease severity

Among the 16 lipid mediators identified in the PCA analysis, two of them (PGF_{2 α} derived from AA [**Figure 4B**] and 8-HDoHE derived from DHA) distinguished patients with ACLF from HS (**Table 1**). Importantly, LTE₄ and 12-hydroxyheptadecatrienoic (12-HHT) derived from AA shaped a minimal plasma fingerprint that discriminated patients with ACLF from patients with AD (**Table 1**). Between these two lipid mediators, LTE₄ appeared to have a robust discriminative power and its levels gradually increased in parallel with the severity of the disease, being significantly higher in AD cirrhosis compared to HS and in ACLF compared to AD (**Figure 4C**). In addition, LTE₄ levels were higher in patients with ACLF grade 3 than in those with ACLF grade 1 and ACLF grade 2 (**Figure 4D**), suggesting that in terms of this lipid mediator, ACLF-1 and ACLF-2 are indistinguishable. Similar LTE₄ levels in plasma were observed when patients were categorized according to the presence or absence of bacterial infections, portal hypertension, ascites and esophageal varices (**Figure**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

4E-G). The association of lipid mediators with bacterial and fungal infections, development of bacterial infection during hospitalization, portal hypertension, ascites and esophageal varices is detailed in **Supplementary Tables 11 and 12**. The dynamics of LTE₄ was also investigated in a subset of 191 patients (96 AD and 95 ACLF at enrollment) who underwent follow-up for a maximum of 28 days. Twenty-one percent of patients improved the status from ACLF to no ACLF, 10.5% became worse (from AD to ACLF), 19 patients presenting ACLF at inclusion increased the degree of ACLF and 7 reduced the ACLF degree but still having ACLF (**Supplementary Table 13A-B**). Paired sample tests between baseline and follow-up measurements showed that plasma levels of LTE₄ paralleled the course of the disease (significant reduction in patients who improved the clinical status from ACLF to AD and significant increase in those who worsened the condition from AD to ACLF) (**Figure 4H**). However, sensitivity analysis in AD patients secondary developing ACLF revealed that LTE₄ has low predictive value (area under the ROC curve=0.304).

A specific lipid mediator profile associates with markers of inflammation and cell death in patients with AD and in those with ACLF

To investigate the association of lipid mediators collected at baseline with markers of inflammation (cytokines and chemokines) and cell death (cK18 and K18), we constructed a correlation matrix plot including the whole group of patients with cirrhosis. As shown in **Figure 5**, lipid mediators had in general a positive correlation among them, except LXA₅, which is an anti-inflammatory and pro-resolution lipid mediator that was inversely correlated. In general, lipid mediators had a weak correlation with cytokines/chemokines, except LTE₄ and LXA₅. LTE₄, which composes the ACLF fingerprint, was the only lipid mediator with distinct positive correlation with inflammatory cytokines/chemokines, in particular with IL-1RA and IL-6, and specially with IL-8. LTE₄ also positively correlated with markers of cell

1 death in AD and ACLF patients (**Figure 5** and **Supplementary Figure 4**). This correlation
2 was stronger with K18 than with cK18, suggesting that LTE₄ can be associated with the non-
3 apoptotic form. On the other hand, LXA₅ was inversely correlated to LTE₄, showed a
4 negative correlation with IL-8 and did not associate with cK18 and K18. Interestingly, PGE₂,
5 which has previously been associated with immunosuppression in cirrhosis (16), was
6 positively correlated with IL-8 ($\rho=0.526$, $P<0.001$), although this lipid mediator was not
7 associated with infection or any other clinical variable (**Supplementary Figure 5** and
8 **Supplementary Tables 11 and 12**).
9
10
11
12
13
14
15
16
17
18
19
20
21

22 **A specific lipid mediator signature associates with organ failures and short-term** 23 **mortality in patients with ACLF**

24 We finally investigated the association of the 59 lipid mediators collected at baseline with the
25 most frequent organ failures (i.e. circulatory, brain, coagulation, liver, kidney and respiratory)
26 in patients with AD and ACLF. The heatmap in **Figure 6A** shows that LXA₅ was the lipid
27 mediator with the strongest association, in particular with liver failure. Other lipid mediators
28 associated with liver failure were autooxidation products such as 9-KODE, 8-HETrE, 8-
29 HDoHE, 4-HDoHE, 11,12-DiHETrE and EKODE. Of interest, LXA₅ together with EKODE
30 constituted a minimal fingerprint of liver and coagulation failures, while PGF_{2 α} was
31 significantly associated with circulatory failure. None of the lipid mediators associated with
32 brain, kidney or respiratory failures. **Figures 6B and C** show the presence of increased
33 EKODE in the context of reduced LXA₅, which were the lipid mediators associated with at
34 least two different organ failures. Moreover, increased EKODE and LTE₄ together with
35 reduced LXA₅ significantly associated with 28-day mortality (**Figure 6D** and
36 **Supplementary Table 12**).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Discussion

1
2 The current study investigated the profile of lipid mediators in plasma from patients of the
3 CANONIC study (1). By LC-MS/MS we screened 100 lipid mediators derived from PUFAs
4
5 in plasma from 246 patients with AD cirrhosis of whom 119 had ACLF. Our major findings
6
7 were the following: 1) Patients with AD, and to a greater extent patients with ACLF, showed
8
9 increased ratio between AA (omega-6-PUFA that serves as substrate precursor for
10
11 inflammatory and vasoconstrictor lipid mediators) and EPA (omega-3-PUFA that serves as
12
13 substrate precursor for anti-inflammatory and pro-resolving lipid mediators), which is a
14
15 surrogate marker of systemic inflammation (14). 2) ACLF was associated with higher
16
17 circulating levels of LTs, PGs, epoxy-keto fatty acids and TX families, in parallel with
18
19 reductions in LXs and epoxy fatty acids. 3) LTE_4 was one of the top differentially regulated
20
21 lipid mediators and gradually increased from HS to AD and ACLF, as well as in ACLF-3 as
22
23 compared with ACLF-1 and -2. In addition, LTE_4 levels followed the clinical course of the
24
25 disease (levels increased when worsening and decreased when improving). Moreover, LTE_4
26
27 positively correlated with markers of cell death (K18) and inflammatory cytokines (IL-8). 4)
28
29 LXA_5 , which was invariably reduced in patients, was the only lipid mediator that inversely
30
31 correlated with IL-8. 5) LTE_4 was part of a minimal plasma fingerprint for ACLF, whereas
32
33 LXA_5 , and EKODE, discriminated organ failures. 6) Finally, increased LTE_4 and EKODE
34
35 together with decreased LXA_5 was associated with higher 28-day mortality. Collectively,
36
37 these findings capture a specific lipid mediator profile in patients with AD cirrhosis, adding
38
39 value to recent studies within the frame of CANONIC, describing a characteristic
40
41 metabolomic fingerprint in these patients (4).
42
43
44
45
46
47
48
49
50
51
52
53
54
55

56 There are findings in our study that deserve particular attention. For example, LTE_4 was the
57
58 lipid mediator enzymatically generated from AA with the largest fold change in ACLF versus
59
60
61
62
63
64
65

1 HS. LTE₄ is formed upon activation of 5-LOX, which converts AA into 5-HpHETE, an
2 intermediate in the generation of LTA₄ (**Supplementary Figure 1A**). LTA₄ is further
3 converted into LTB₄ by LTA₄ hydrolase or into LTC₄ by LTC₄ synthase. LTC₄ is further
4 metabolized to LTD₄ and LTE₄ (generically termed as cysteinyl-LTs), which are potent
5 vasoconstrictors that were previously known as slow-reacting substances of anaphylaxis (17).

6
7 Cysteinyl-LTs are primarily generated by neutrophils, macrophages, eosinophils and mast
8 cells at sites of infection and/or inflammation and their release is enhanced by activation of
9 Toll-like receptors (18). Cysteinyl-LTs participate in a variety of diseases including arthritis,
10 inflammatory bowel disease, atherosclerosis and especially asthma and allergy, conditions in
11 which blockage of their receptors is used as therapy (19). In these conditions, cysteinyl-LTs
12 directly interact with cytokines (i.e. IL-6, IL-10 and TNF α) and chemokines (i.e. IL-8,
13 eotaxin and MIP-1 α) (20). In addition to inflammation, cysteinyl-LTs might also be related to
14 cell death, since in our study LTE₄ levels were strongly correlated with K18, which is a
15 marker of total cell death. Interestingly, LTE₄ negatively correlated with the ratio between
16 cK18 (apoptosis) and K18 (apoptosis and necrosis), indicating that this lipid mediator is
17 related to non-apoptotic cell death, which potentially is more immunogenic (13). On the other
18 hand, cysteinyl-LTs induce hyperreactivity of the arterial vascular tissue to vasoactive
19 compounds (such as angiotensin II and norepinephrine), suggesting that these lipid mediators
20 may have pathological significance in the development of organ failures in ACLF (21).
21 Indeed, elevated urinary LTE₄ levels were reported in patients with hepatorenal syndrome
22 and might contribute to the development of kidney dysfunction in patients with cirrhosis (22).
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51
52
53 Another LOX-derived lipid mediator that could be relevant for the understanding of the
54 pathophysiology of the ACLF syndrome is LXA₅, for which plasma levels were significantly
55 reduced in patients with ACLF but not in those with AD. LXA₅ is an EPA (omega-3-PUFA)-
56
57
58
59
60
61
62
63
64
65

1 derived lipid mediator that belongs to the family of specialized pro-resolving mediators
2 promoting the timely resolution of inflammation (10). Biosynthesis of LXA₅ from
3 endogenous sources of EPA is initiated by 15-LOX and mainly occurs in cells bearing 15-
4 LOX activity, such as those of the immune system. Since circulating LXA₅ levels were
5 suppressed in patients with ACLF without changes in 15-LOX, this impairment was likely
6 related to limited access to EPA. Indeed, EPA abundance was significantly reduced in plasma
7 from patients with AD cirrhosis, who also presented an unbalanced AA/EPA ratio. In
8 agreement with this, we identified unbalanced formation between pro-inflammatory omega-
9 6-derived (i.e. LTE₄) and anti-inflammatory omega-3-derived (i.e. LXA₅) lipid mediators in
10 patients with AD and ACLF. These two lipid mediators showed opposite relationships
11 (positive for LTE₄ and negative for LXA₅) with IL-8, suggesting that this imbalance might be
12 a contributory factor for unresolved systemic inflammation in these patients. It is worth
13 mentioning that Schwarzkopf et al. (23) reported no changes in the plasma levels of omega-6
14 and -3 PUFA in patients with cirrhosis with and without ACLF. However, these authors did
15 not include a group of healthy subjects and the comparisons were made between patients with
16 AD and ACLF with respect to a group of patients with compensated cirrhosis. Moreover,
17 these authors determined the levels of free PUFA whereas in our study we determined not
18 only free PUFA but also the total PUFA content (all PUFA from triglycerides, phospholipids
19 and cholesterol esters after saponification), which more accurately represents the actual
20 PUFA pool in plasma.

21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51 Our study also identified profound alterations in the circulating levels of lipid mediators
52 derived from LA. Among these, 9(10)-EpOME and 12(13)-EpOME, which are produced by
53 the activity of CYP450 in leukocytes during oxidative burst (24), were invariably reduced in
54 patients with AD and in those with ACLF. Since a defect in leukocyte oxidative burst is a

1 hallmark of ACLF (25), these LA-derived lipid mediators could serve as circulating
2 biomarkers of decreased bactericidal activity in these patients. However, no significant
3 differences in the levels of these lipid mediators were seen between patients with infections
4 and those without. In contrast, nonenzymatic autooxidation products of LA were found
5
6
7
8
9
10 invariably increased in patients with AD and in those with ACLF. One of these products was
11
12 EKODE, which modulates aldosterone, corticosterone and dehydroepiandrosterone secretion
13
14 by human adrenal cells (26), suggesting that it might drive adrenal dysfunction in patients
15
16 with cirrhosis.
17

18
19
20
21
22 Finally, COX-derived PGs, which are widely distributed and formed at sites of inflammation,
23 deserve some comments. For example, PGE₂, which was related to immunosuppression in
24 cirrhosis (16), was not significantly associated with the presence of infections at the time of
25 inclusion or with the risk of developing infections during hospitalization. In contrast, PGF_{2α},
26 which is involved in contraction of bronchial, vascular smooth muscle, renin secretion and
27 blood pressure regulation (27), was associated with circulatory failure in patients with ACLF.
28
29
30
31
32
33
34
35
36 On the other hand, 12-HHT, which is biosynthesized by TXA₂ synthase in an equimolar ratio
37
38 to TXA₂, was part of the minimal plasma fingerprint discriminating patients with ACLF from
39
40 those with AD. In the past, 12-HHT was considered a mere byproduct of the biosynthesis of
41
42 the potent vasoconstrictor TXA₂, although recent studies indicate that this lipid mediator
43
44 induces chemotaxis of immune cells by binding to LTB₄ receptor 2 (28).
45
46
47
48
49
50

51 In summary, the current study provides a comprehensive analysis of the plasma levels of 100
52
53 PUFA-derived lipid mediators in a well-clinically defined cohort (i.e. CANONIC) of patients
54
55 with AD cirrhosis with and without ACLF. By using an agnostic approach to data analysis,
56
57 we identified 11 lipid mediators that distinguished healthy from cirrhotic patients at any stage
58
59
60
61
62
63
64
65

1 (either AD or ACLF), 2 lipid mediators (LTE₄ and 12-HHT) that discriminated patients with
2 ACLF from those with AD and 2 other lipid mediators that shaped a minimal plasma
3 fingerprint of liver and coagulation failures (LXA₅ and EKODE). Moreover, LTE₄
4 distinguished ACLF grade 3 from ACLF grades 1 and 2 and its plasma levels followed the
5 clinical course of the disease and together with LXA₅ and EKODE associated with short-term
6 mortality. Overall, our study provides useful insights on the role of bioactive lipid mediators
7 in the initiation and progression of systemic inflammation and organ failures in patients with
8 AD cirrhosis.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Acknowledgements

We are indebted to the IDIBAPS Biobank, integrated in the Spanish National Biobank Network, for samples and data procurement.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- 1
2
3 1. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic
4
5 liver failure is a distinct syndrome that develops in patients with acute
6
7 decompensation of cirrhosis. *Gastroenterology* 2013;144:1426-1437.
8
- 9
10 2. Gustot T, Fernandez J, Garcia E, Morando F, Caraceni P, Alessandria C, et al.
11
12 Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis.
13
14 *Hepatology* 2015;62:243-252.
15
- 16
17 3. Clària J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic
18
19 inflammation in decompensated cirrhosis: Characterization and role in acute-on-
20
21 chronic liver failure. *Hepatology* 2016;64:1249-1264.
22
- 23
24 4. Moreau R, Clària J, Aguilar F, Fenaille F, Lozano J, Junot C, et al. Blood
25
26 metabolomics uncovers inflammation-associated mitochondrial dysfunction as a
27
28 potential mechanism underlying ACLF. *J Hepatol*, doi: [10.1016/j.jhep.2019.11.009](https://doi.org/10.1016/j.jhep.2019.11.009).
29
30
- 31
32 5. Buczynski MW, Dumlao DS, Dennis EA. An integrated omics analysis of eicosanoid
33
34 biology. Thematic Review Series: Proteomics. *J Lipid Res*. 2009;50:1015-1038.
35
- 36
37 6. Stables MJ, Gilroy DW. Old and new generation lipid mediators in acute
38
39 inflammation and resolution. *Prog Lipid Res* 2011;50:35-51.
40
- 41
42 7. Rius B, Clària J. Principles, mechanisms of action and future prospects of anti-
43
44 inflammatory drugs. In: NSAIDS and Aspirin: Recent Advances and Implications for
45
46 Clinical Management; Lanas A editor, Springer, Heidelberg, Germany, 2016.
47
- 48
49 8. Fajardo VA, Mikhaeil JS, Leveille CF, Saint C, LeBlanc PJ. Cardiolipin content,
50
51 linoleic acid composition, and tafazzin expression in response to skeletal muscle
52
53 overload and unload stimuli. *Sci Rep* 2017;7:2060.
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
9. Kolmert J, Fauland A, Fuchs D, Säfholm J, Gómez C, Adner M, et al. Lipid Mediator Quantification in Isolated Human and Guinea Pig Airways: An Expanded Approach for Respiratory Research. *Anal Chem.* 2018;90:10239-10248.
10. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 2014;510:92-101.
11. Rund KM, Heylmann D, Seiwert N, Wecklein S, Oger C, Galano JM, et al. Formation of trans-epoxy fatty acids correlates with formation of isoprostanes and could serve as biomarker of oxidative stress. *Prostaglandins Other Lipid Mediat* 2019;106334.
12. Trebicka J, Amoros A, Pitarch C, Titos E, Alcaraz-Quiles J, Schierwagen R, et al. Addressing Profiles of Systemic Inflammation Across the Different Clinical Phenotypes of Acutely Decompensated Cirrhosis. *Front Immunol.* 2019;10:476.
13. Macdonald S, Andreola F, Bachtiger P, Amoros A, Pavesi M, Mookerjee R, et al. Cell death markers in patients with cirrhosis and acute decompensation. *Hepatology* 2018;67:989-1002.
14. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* 2002;56:365-79.
15. Oettl K, Birner-Gruenberger R, Spindelboeck W, Stueger HP, Dorn L, Stadlbauer V, et al. Oxidative albumin damage in chronic liver failure: relation to albumin binding capacity, liver dysfunction and survival. *J Hepatol.* 2013;59:978-83.
16. O'Brien AJ, Fullerton JN, Massey KA, Auld G, Sewell G, James S, et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. *Nat Med.* 2014;20:518-23.
17. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294:1871-1875.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
18. Lindner SC, Köhl U, Maier TJ, Steinhilber D, Sorg BL. TLR2 ligands augment cPLA2alpha activity and lead to enhanced leukotriene release in human monocytes. *J Leukoc Biol* 2009;86:389-399.
 19. Peters-Golden M, Gleason MM, Togias A. Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. *Clin Exp Allergy* 2006;36:689-703.
 20. Sadik CD, Luster AD. Lipid-cytokine-chemokine cascades orchestrate leukocyte recruitment in inflammation. *J Leukoc Biol* 2012;91:207-215.
 21. Huber M, Kästner S, Schölmerich J, Gerok W, Keppler D. Analysis of cysteinyl leukotrienes in human urine: enhanced excretion in patients with liver cirrhosis and hepatorenal syndrome. *Eur J Clin Invest* 1989;19:53-60.
 22. Moore KP, Taylor GW, Maltby NH, Siegers D, Fuller RW, Dollery CT, et al. Increased production of cysteinyl leukotrienes in hepatorenal syndrome. *J Hepatol* 1990;11:263-271.
 23. Schwarzkopf KM, Queck A, Thomas D, Angioni C, Cai C, Freygang Y, et al. Omega-3 and -6 fatty acid plasma levels are not associated with liver cirrhosis-associated systemic inflammation. *PLoS One* 2019;14:e0211537
 24. Hayakawa M, Sugiyama S, Takamura T, Yokoo K, Iwata M, Suzuki K, et al. Neutrophils biosynthesize leukotoxin, 9, 10-epoxy-12-octadecenoate. *Biochem Biophys Res Commun* 1986;137:424-430.
 25. Korf H, du Plessis J, van Pelt J, De Groot S, Cassiman D, Verbeke L, et al. Inhibition of glutamine synthetase in monocytes from patients with acute-on-chronic liver failure resuscitates their antibacterial and inflammatory capacity. *Gut* 2019;68:1872-1883.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
26. Goodfriend TL, Ball DL, Egan BM, Campbell WB, Nithipatikom K. Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. *Hypertension*. 2004;43:358-363.
27. Watanabe K. Prostaglandin F synthase. *Prostaglandins Other Lipid Mediat* 2002;68-69:401-407.
28. Okuno T, Iizuka Y, Okazaki H, Yokomizo T, Taguchi R, Shimizu T. 12(S)-Hydroxyheptadeca-5Z, 8E, 10E-trienoic acid is a natural ligand for leukotriene B4 receptor 2. *J Exp Med*. 2008;205:759-766.

Figure legends

1
2
3 **Figure 1. Unbalanced omega-6/omega-3 ratio in AD cirrhosis.** (A) Schematic diagram and
4 chemical structures of omega-6 (LA and AA) and omega-3 (EPA and DHA) PUFA families.
5
6 (B) Plasma levels of free and total PUFA in HS (n=38) and AD (n=34) and ACLF (n=25)
7 patients. (C) AA/EPA ratio of free and total PUFA content. (D) Changes in gene expression
8 for delta-5 (*FADS1*), delta-6 (*FADS2*) and delta-9 (*SCD1*) desaturases and ELOVL fatty acid
9 elongase 5 (*ELOVL5*) and 6 (*ELOVL6*) in leukocytes from HS (n=14) and AD (n=14) and
10 ACLF (n=14) patients. Changes in *SCD1* and *ELOVL6* expression are shown at the bottom.
11
12 Results are expressed as median and IQR.
13
14
15
16
17
18
19
20
21
22
23

24 **Figure 2. Altered biosynthesis of lipid mediators in leukocytes of patients with AD**
25 **cirrhosis.** (A) Total amount (upper panel) and box plots of individual values (lower panel) of
26 lipid mediators from omega-6 and omega-3 PUFA families generated by CYP, LOX, COX
27 and non-enzymatic (NE) pathways in plasma from HS (n=18) and AD (n=127) and ACLF
28 (n=119) patients. (B) Percent expression of genes of the CYP, LOX and COX pathways in
29 leukocytes from HS (n=14) and AD (n=14) and ACLF (n=14) patients. (C) Gene expression
30 of individual CYP, LOX and COX enzymes in leukocytes from HS and AD and ACLF
31 patients. Results are expressed as median and IQR.
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 **Figure 3. Identification of an AD cirrhosis lipid mediator signature.** (A) Cleveland dot
47 plot showing a ranked log₂ transformation of fold changes of plasma levels of lipid mediators
48 categorized by chemical families (LTs, epoxy-keto-fatty acids (FA), AA and DHA epoxides,
49 PGs, TX, AA/EPA/DHA diols, oxo-FA, ALA epoxides, monohydroxy-FA, FA triols, LXs,
50 LA diols and LA epoxides). Blue and red dots represent the fold changes between AD
51 (n=127) and ACLF (n=119) patients with respect to HS (n=18), respectively. (B) Cleveland
52
53
54
55
56
57
58
59
60
61
62
63
64
65

dot plot of the analyzed 59 lipid mediators ranked by fold change of AD and ACLF patients versus HS. Color coding on the left represents each biosynthetic precursor ((LA, γ -linolenic acid (GLA), dihomo- γ -linolenic acid (DGLA), oleic acid (OA), EPA, AA, DHA and ALA)). (C) Volcano plot representing the levels of lipid mediators up- or down-regulated in AD patients with respect to HS. Lipid mediators with significant changes are presented in red (significant increase) or in blue (significant decrease). (D) Volcano plot representing changes in ACLF patients with respect to HS. (E) 3D-PCA of lipid mediators in plasma from HS and AD and ACLF patients. (F) Heatmap of the 16 lipids associated with patient status at enrolment (unsupervised analysis). Rows represent individual lipid mediators and columns represent HS, AD and ACLF individuals.

Figure 4. LTE₄ discriminates disease severity. (A) Plasma levels of 9(10)-EpOME, 12(13)-EpOME in the HS (n=18), AD (n=127) and ACLF (n=119) groups at enrollment. (B, C) PGF₂ α and LTE₄ levels in the study groups. (D) LTE₄ in ACLF patients according to severity (grade-1, n=57; grade-2, n=44 and grade-3, n=18). (E) LTE₄ according to the absence (n=219) or presence (n=22) of bacterial infection. (F) LTE₄ according to the absence (n=68) or presence (n=177) of ascites. (G) LTE₄ according to the absence (n=133) or presence (n=133) of esophageal varices. (H) Differences in LTE₄ levels between baseline and follow-up measurements in plasma samples from patients with AD and ACLF according to the course of the disease: steady (n=105), improvement (n=47) or worsening (n=39). Mann-Whitney test was used. Bonferroni correction was applied to correct for multiple-testing. An adjusted P-value \leq 0.05 was considered statistically significant.

Figure 5. Association of lipid mediators with markers of inflammation and cell death. Correlation matrix plot between lipid mediators and cytokines/chemokines/cell death markers

in patients with AD (n=127) and ACLF (n=119). Shades of blue indicate increasing positive correlation coefficient; shades of red indicate increasing negative correlation coefficient.

Correlations of LTE₄ with cK18 and K18, LTE₄ and LXA₅ with IL-8 and LTE₄ with LXA₅.

Figure 6. Association of lipid mediators with organ failures and short-term mortality.

(A) Heatmap representation of the 59 lipid mediators analyzed in the study and their association with organ failures in patients with AD (n=127) and ACLF (n=119). Grey color corresponds to low association (high p-values) and red color to high association (low p-values). (B) EKODE and LXA₅ levels in patients with (n=55) and without (n=191) liver failure. (C) EKODE and LXA₅ in patients with (n=44) and without (n=202) coagulation failure. (D) Association analysis of EKODE, LXA₅ and LTE₄ levels with 28-day mortality (survivors [n=206], non-survivors [n=40]). Mann-Whitney test was used. Bonferroni correction was applied to correct for multiple-testing. An adjusted p-value≤0.05 was considered statistically significant.

Table 1. Association analysis of lipid mediators with the status of the patients at enrollment.

Lipid mediator	Kruskal-Wallis		HS - AD		HS - ACLF		AD - ACLF	
	<i>p</i> -val	Adj <i>p</i> -val	<i>p</i> -val	Adj <i>p</i> -val	<i>p</i> -val	Adj <i>p</i> -val	<i>p</i> -val	Adj <i>p</i> -val
Significant changes between HS vs. AD								
4-HDoHE	<0.01	0.02	<0.01	0.04	0.03	1.00	<0.01	0.18
Significant changes between HS vs. AD and ACLF								
12(13)-EpOME	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.26	1
9(10)-EpOME	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.55
EKODE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	1.00
8-HETE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.05	1.00
20-HETE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.15	1.00
8-HETrE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.54
11,12-DiHETrE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.44	1.00
14,15-DiHETrE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.16	1.00
13-HOTrE γ	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.76	1.00
11-keto-TXB ₂	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.10	1
PGE ₁	<0.01	0.03	<0.01	<0.01	<0.01	0.01	0.61	1
Significant changes between HS vs. ACLF								
PGF _{2α}	<0.01	<0.01	<0.01	0.11	<0.01	0.01	<0.01	0.24
8-HDoHE	<0.01	<0.01	<0.01	0.09	<0.01	<0.01	<0.01	0.09
Significant changes between AD vs. ACLF								
12-HHT	<0.01	<0.01	0.09	1.00	0.65	1.00	<0.01	<0.01
LTE ₄	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01

p-value and adjusted *p*-value after Bonferroni correction for multiple testing are shown for each test (Kruskal-Wallis or pairwise Wilcoxon test). All lipids are statistically significant according to Kruskal-Wallis test ($p < 0.05$).

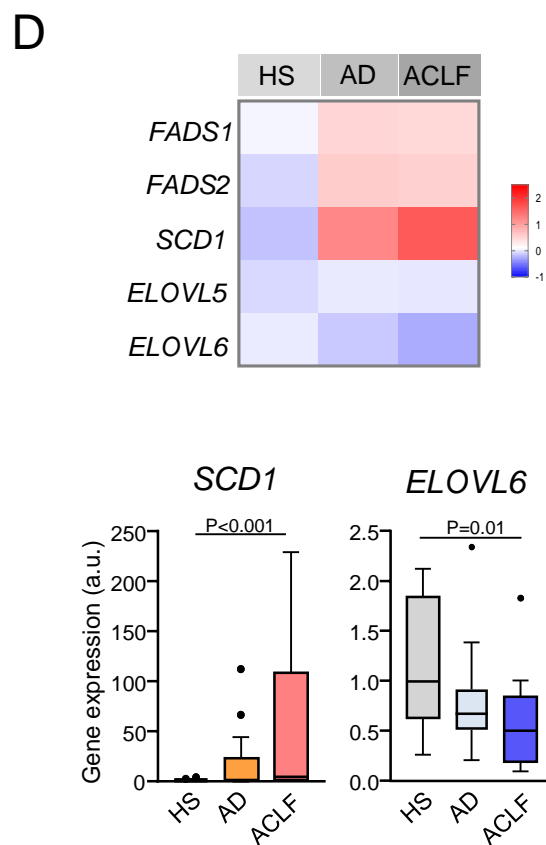
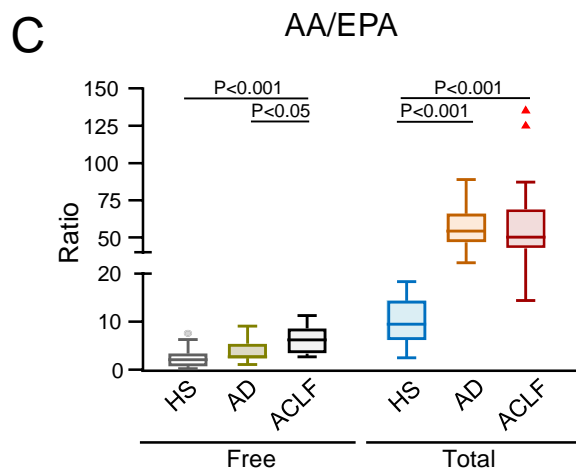
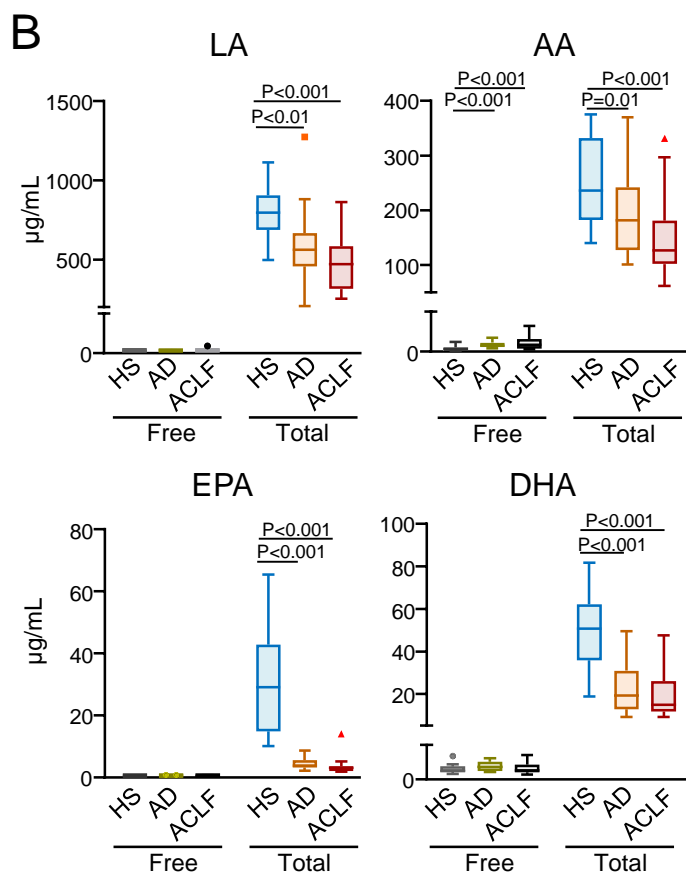
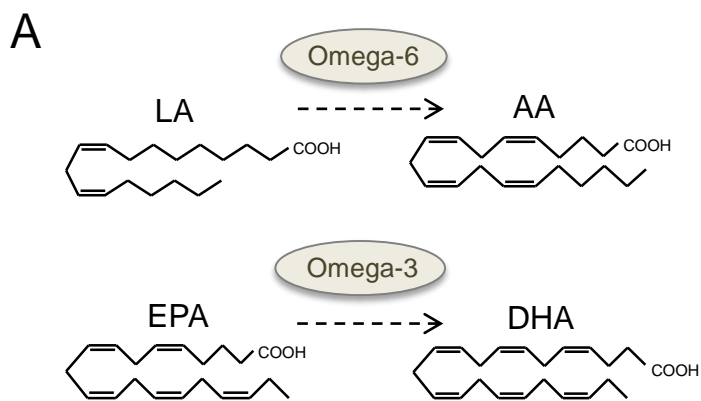
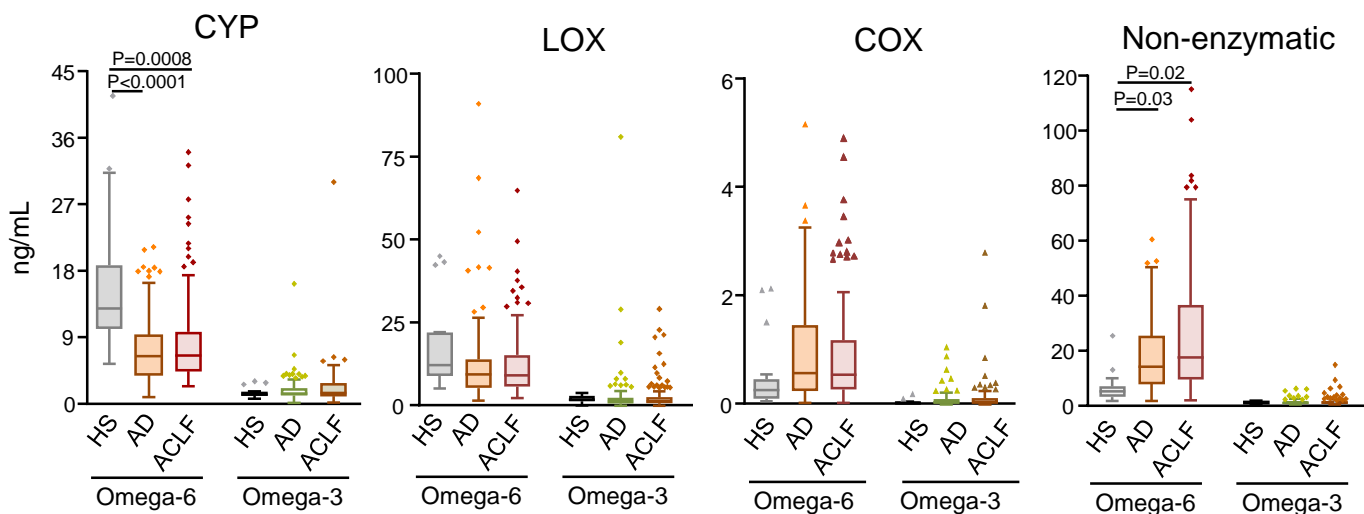
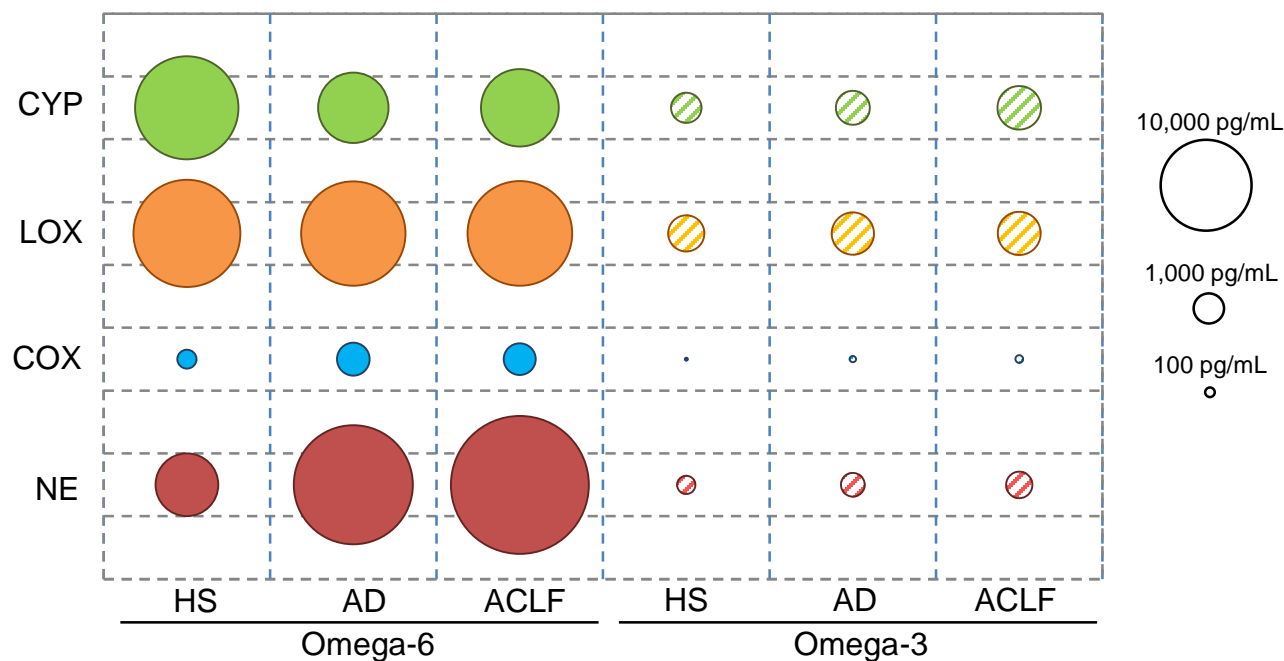


Figure 2

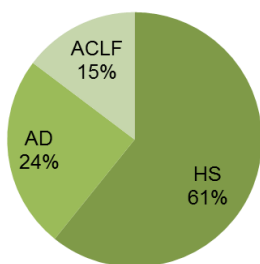
Figure 2

A

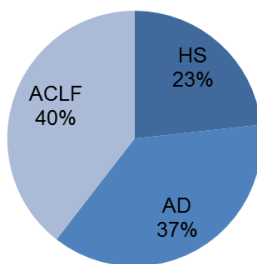


B

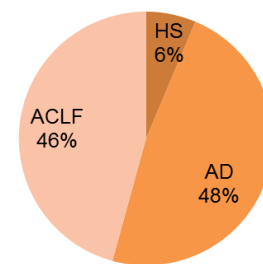
CYP



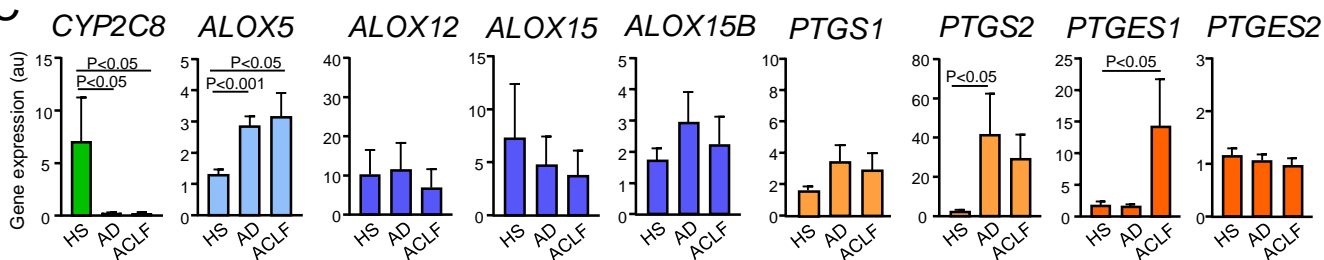
LOX

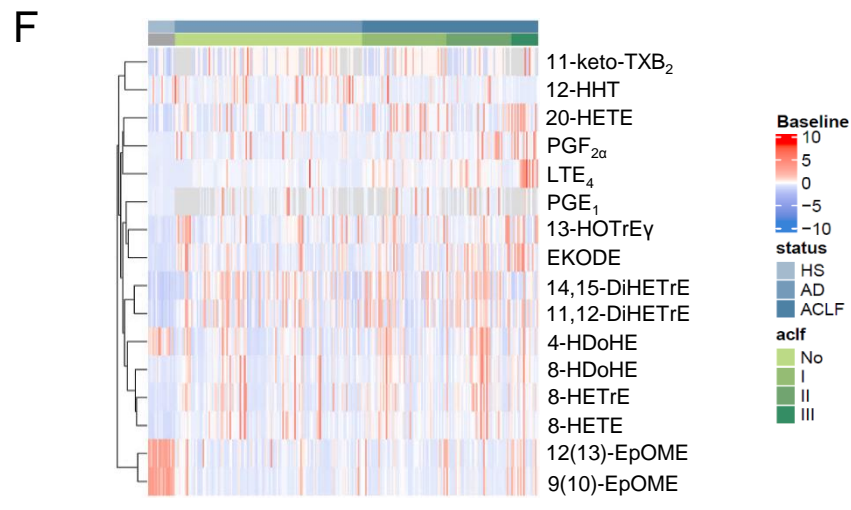
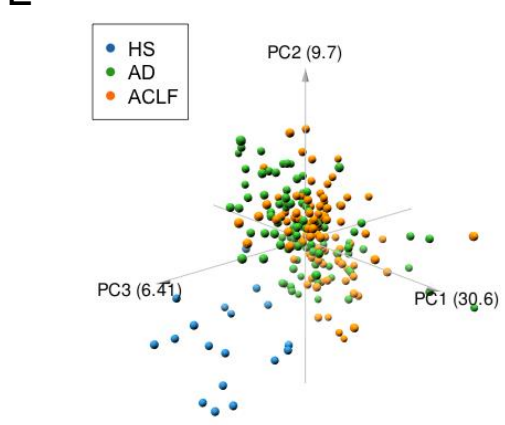
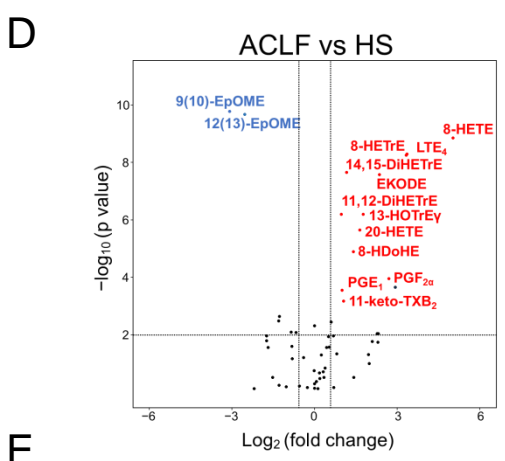
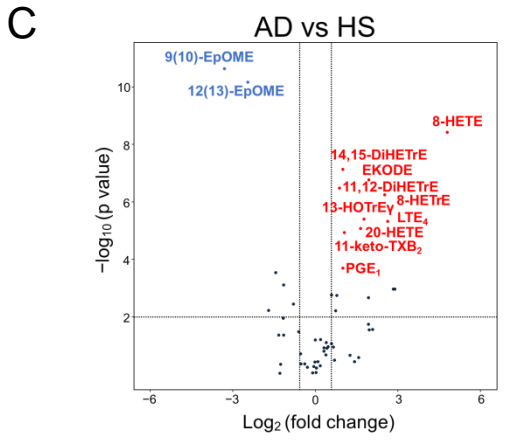
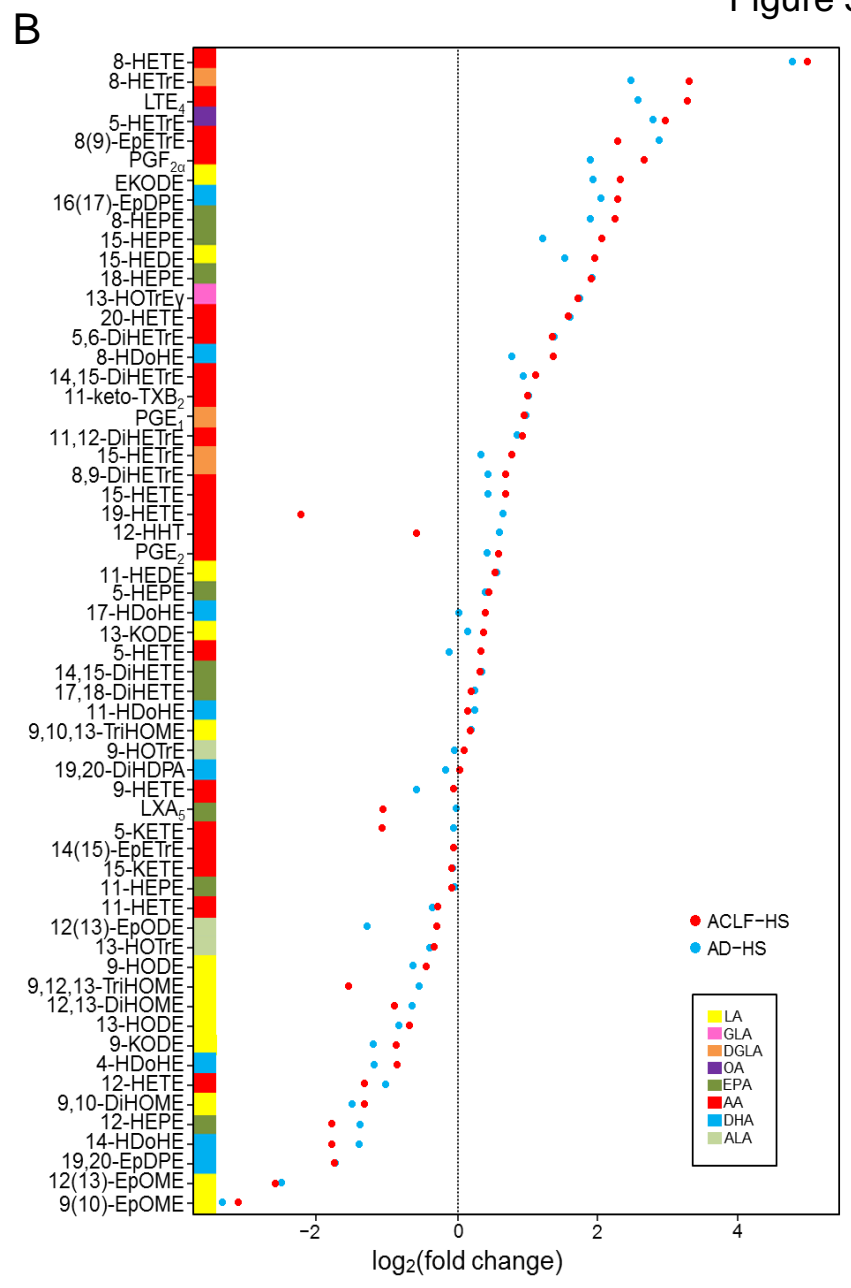
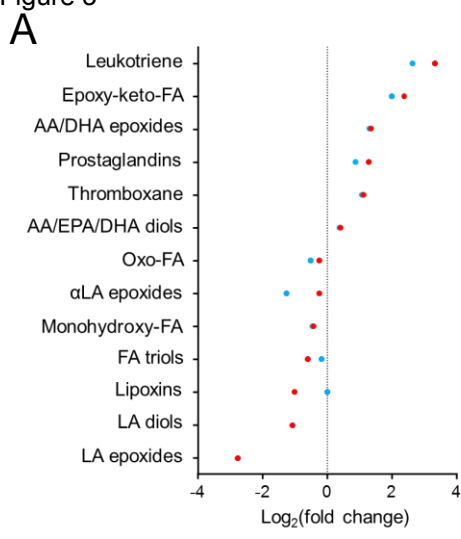


COX



C





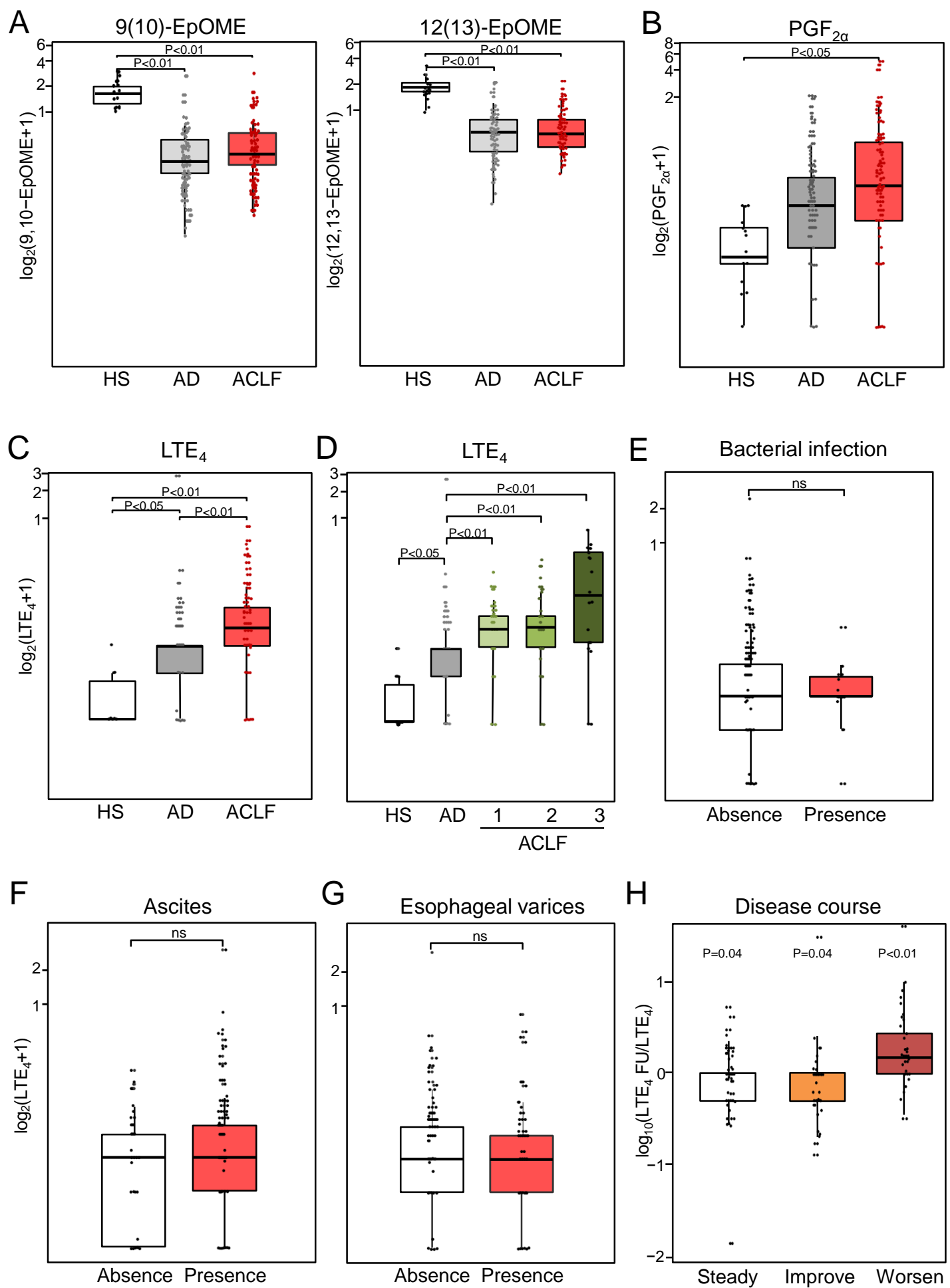


Figure 5

Figure 5

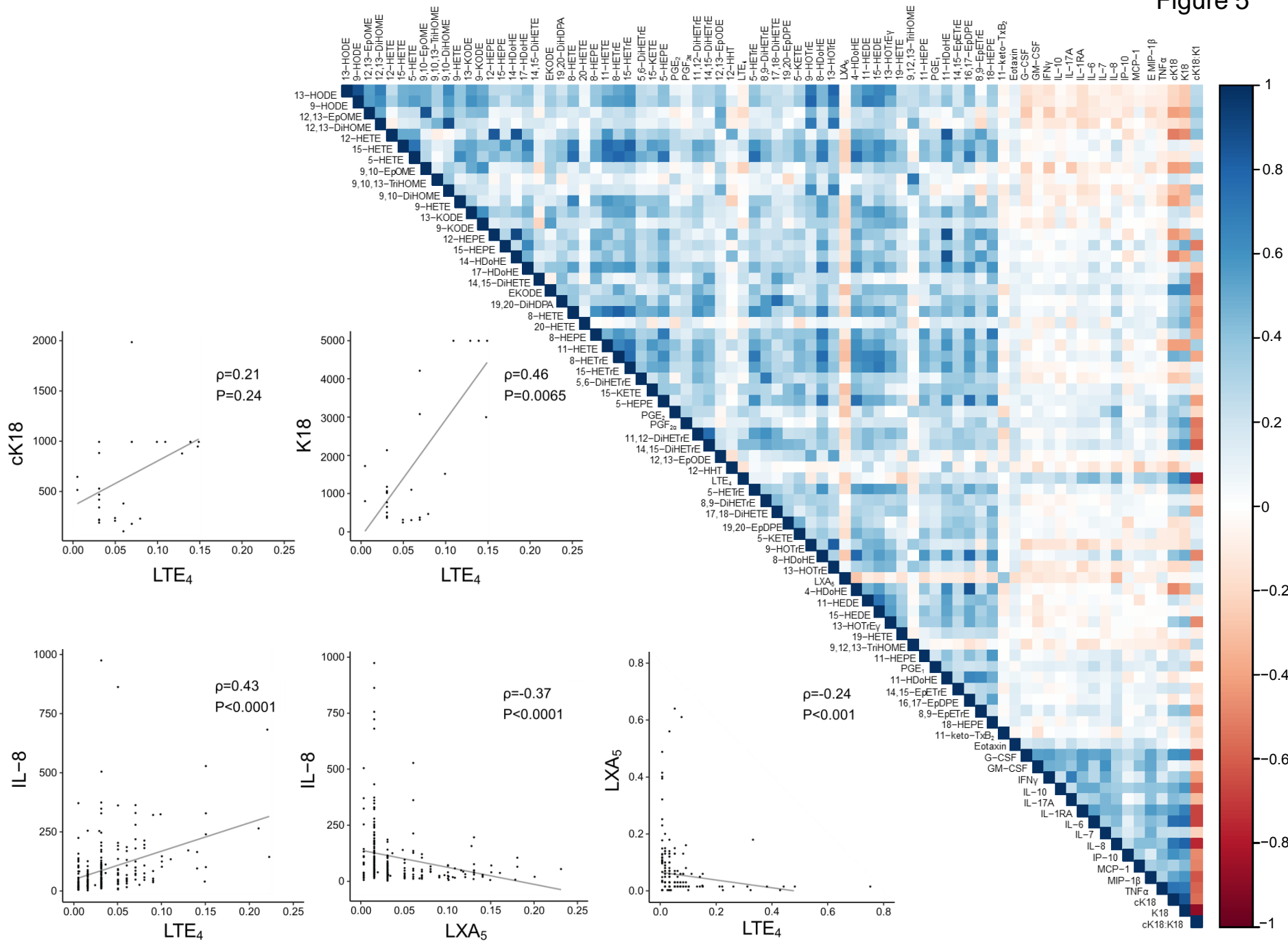
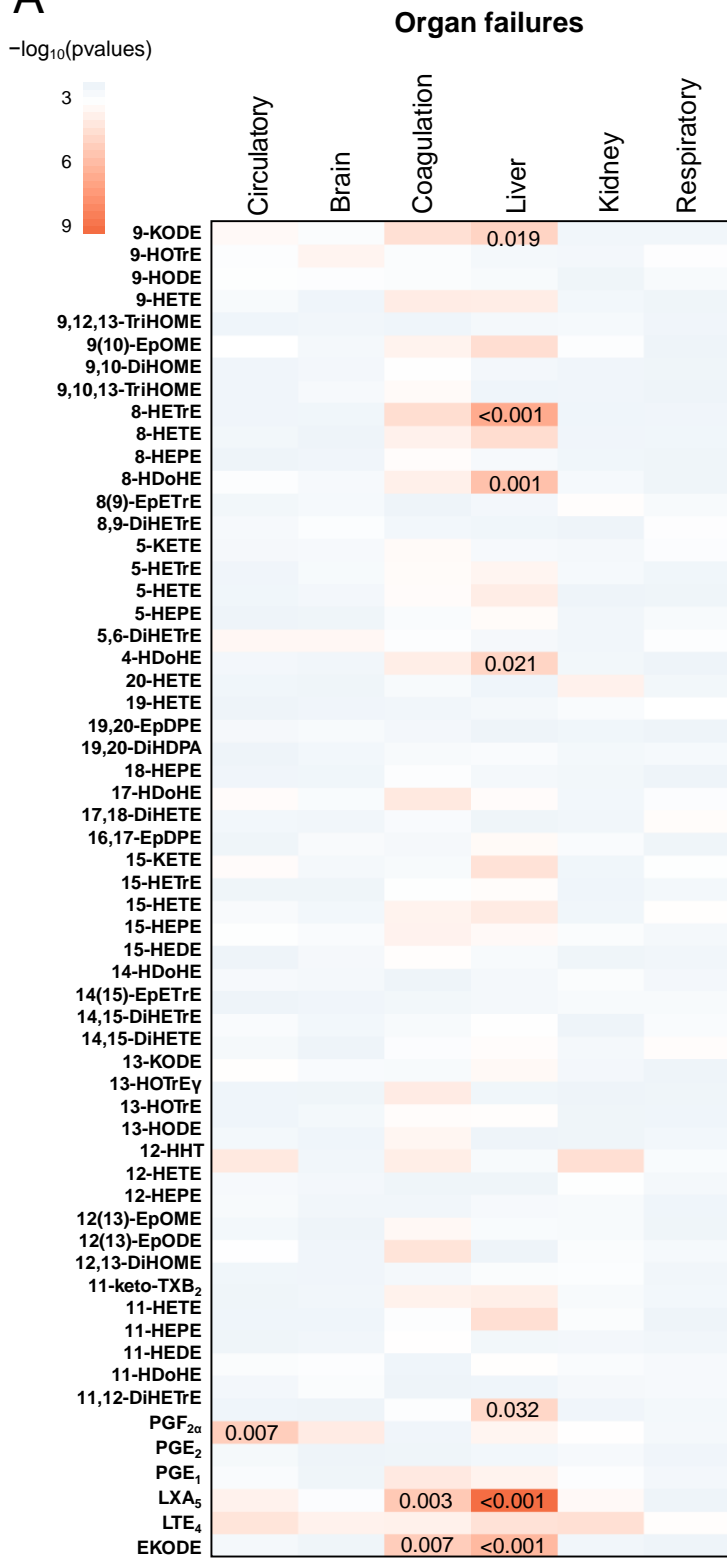
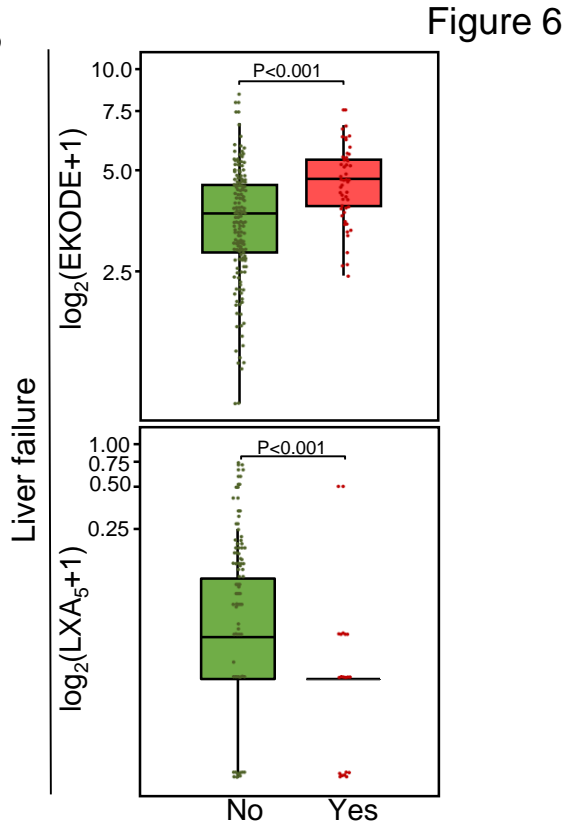


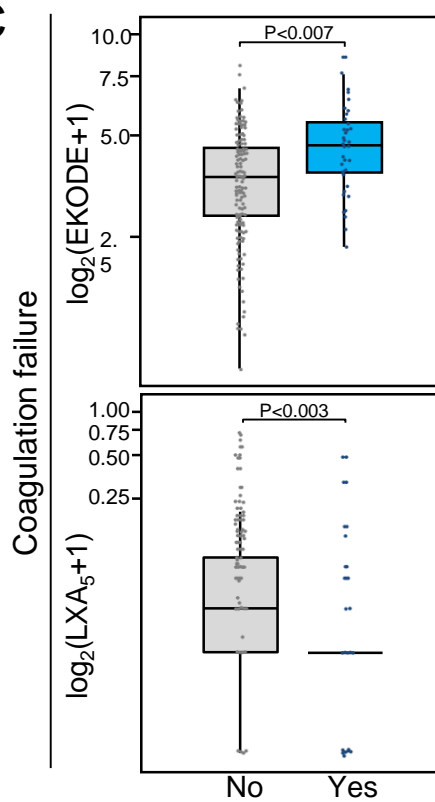
Figure 6



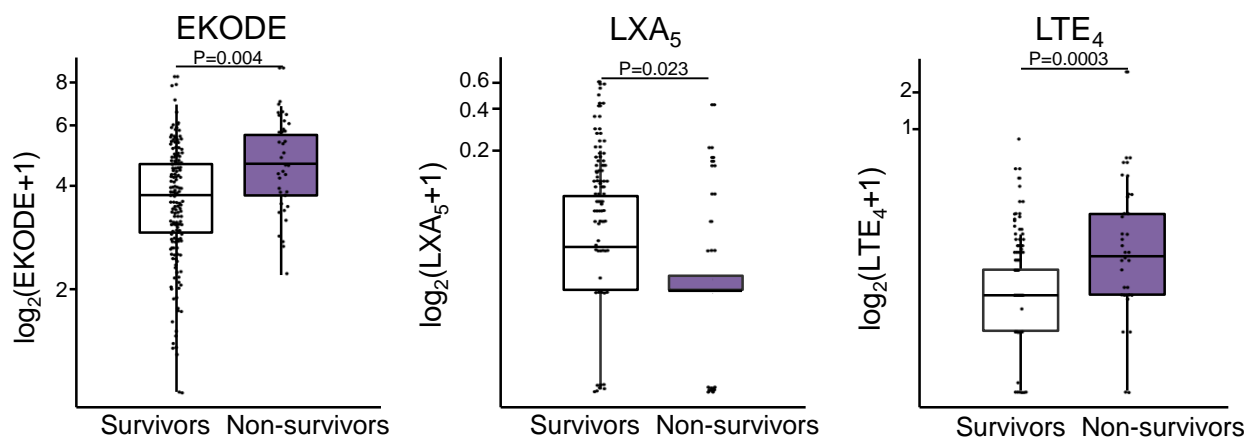
B

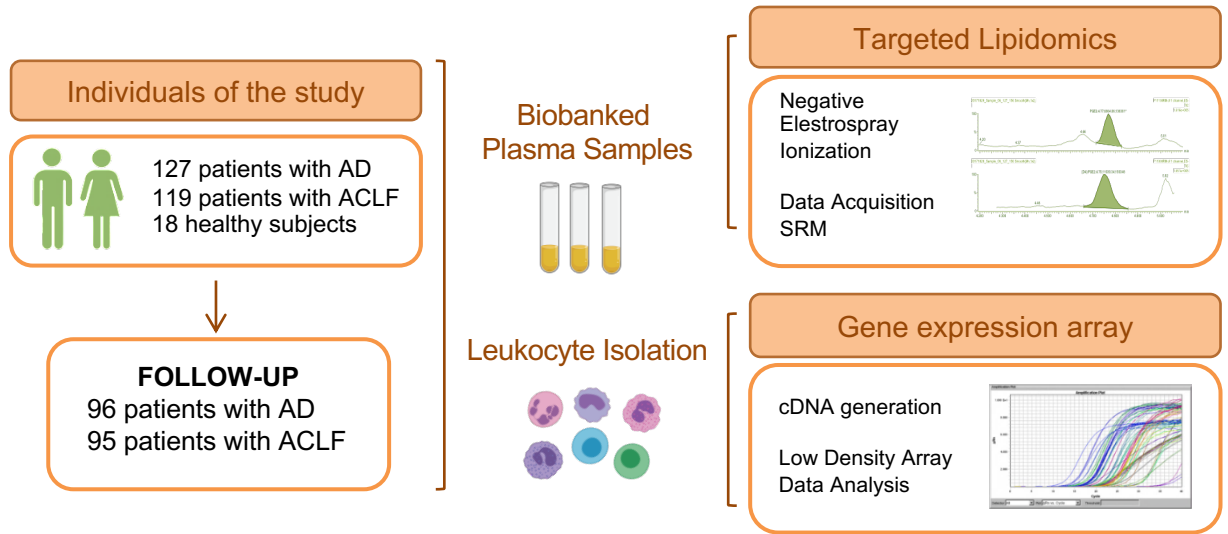


C

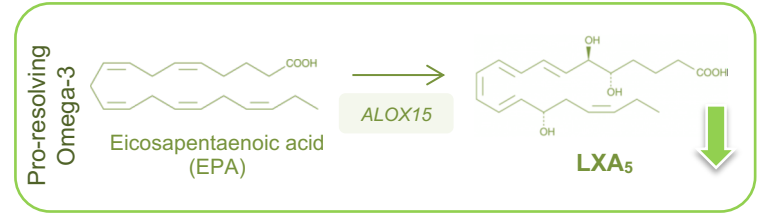
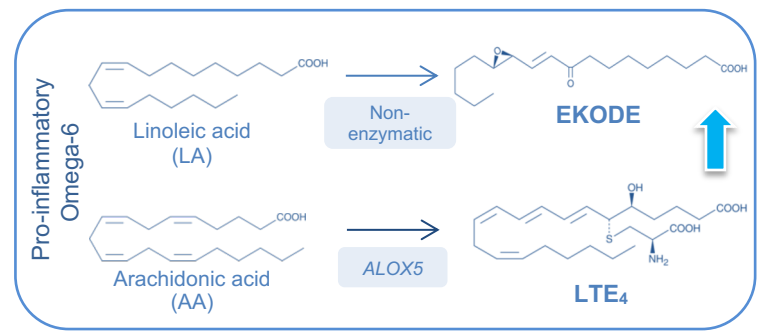
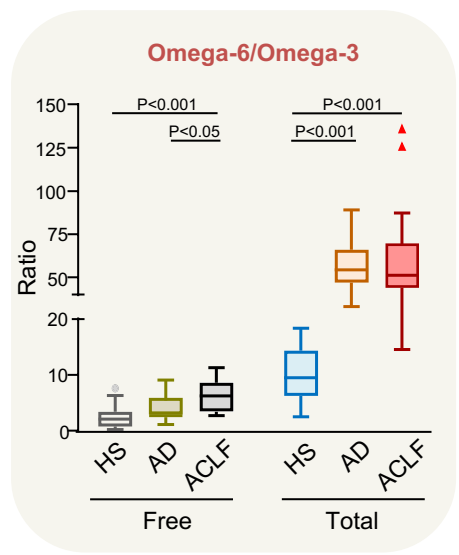


D





Identification of a blood lipid mediator fingerprint in AD cirrhosis



Highlights

- Lipidomics was performed to assess the profile of lipid mediators in plasma from patients with acutely decompensated cirrhosis with and without ACLF.
- Measurements were prospectively repeated during a 28-day follow-up period.
- Fifty-nine lipid mediators were detected in plasma from cirrhotic patients, of which, 16 were significantly associated with the disease status.
- Among these, leukotriene E₄ derived from arachidonic acid was part of a minimal plasma fingerprint that discriminated disease severity and evolution.
- This lipid mediator positively correlated with markers of inflammation and non-apoptotic cell death.