CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: http://orca.cf.ac.uk/121574/

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Maurice, James B., Garvey, Lucy, Tsochatzis, Emmanuel A., Wiltshire, Matthew, Cooke, Graham, Guppy, Naomi, McDonald, Julie, Marchesi, Julian, Nelson, Mark, Kelleher, Peter, Goldin, Robert, Thursz, Mark and Lemoine, Maud 2019. Monocyte-macrophage activation is associated with nonalcoholic fatty liver disease and liver fibrosis in HIV monoinfection independently of the gut microbiome and bacterial translocation. AIDS 33 (5) , pp. 805-814. 10.1097/QAD.0000000000002133 file

Publishers page: http://dx.doi.org/10.1097/QAD.0000000000002133 <http://dx.doi.org/10.1097/QAD.0000000000002133>

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.

information services
gwasanaethau gwybodaeth

1 **Monocyte-Macrophage activation is associated with NAFLD and liver fibrosis in HIV mono-**

2 **infection independently of the gut microbiome and bacterial translocation.**

3 **Running Title: Non-alcoholic fatty liver disease in HIV.**

4 James B MAURICE[1], Lucy GARVEY[2], Emmanuel A. TSOCHATZIS[3], Matthew WILTSHIRE[1],

5 Graham COOKE[4], Naomi GUPPY[5], Julie MCDONALD[1], Julian MARCHESI[1], Mark NELSON[6],

6 Peter KELLEHER[7], Robert GOLDIN[8], Mark THURSZ[1] and Maud LEMOINE[1].

7 Affiliations

8 1 Hepatology Unit, Department of Surgery and Cancer, Imperial College London, London UK

9 2 Department of Infectious Disease, Imperial College Healthcare NHS Trust, London UK

10 3 UCL Institute for Liver and Digestive Health, Royal Free Hospital and University College

11 London, UK

12 4 Division of Infectious Disease, Department of Medicine, Imperial College London, London

13 UK

14 5 University College London Advanced Diagnostics, London, UK

15 6 Department of Infectious Disease, Chelsea & Westminster Hospital NHS Trust, London UK

16 7 Centre for Immunology and Vaccinology, Department of Medicine, Imperial College

17 London UK

18 8 Histopathology Unit, Department of Medicine, Imperial College London, UK

19 **Corresponding Author**

20 Corresponding author

21 Dr James Maurice: james.maurice@imperial.ac.uk. The Liver Unit, 10th Floor QEQM, St

22 Mary's Hospital, South Wharf Road, London W21NY

23 **Author Statement:**

24 Guarantor of the article: Dr Maud Lemoine

25 JBM: Designed the study, collected data, analysed data, wrote the manuscript.

26 LG, GC, PK, MN, MT: Designed the study and critically reviewed the manuscript.

27 ET, MW, NG, JAKM, JM, RG: collected and analysed data, critically reviewed the manuscript.

28 ML: Designed the study, analysed data, wrote the manuscript.

29 **Word Count:** 3716

30 **Structured Summary (Word Count: 250)**

31 **Background:** Non-alcoholic fatty liver disease (NAFLD) is common among people living with

32 HIV. There is limited data available on the pathophysiology of NAFLD and the development of

33 fibrosis in this population.

34 **Objectives:** to investigate the association of bacterial translocation, adipose tissue

35 dysfunction, monocyte activation and gut dysbiosis in patients with HIV mono-infection and

36 NAFLD.

37 **Methods:** Cases with biopsy-proven NAFLD and HIV mono-infection were age and sex-

38 matched to HIV+ and HIV- controls. Markers of bacterial translocation (lipopolysaccharide-

39 binding protein (LBP), bacterial DNA and lipopolysaccharide (LPS)), adipose tissue dysfunction

40 (leptin, adiponectin) and monocyte activation (sCD14 and sCD163) were measured by ELISA.

Hepatic patterns of macrophage activation were explored with immunohistochemistry. 16s rRNA sequencing was performed with stool.

Results: Thirty-three cases were included (≥F2 fibrosis n=16), matched to HIV+ (n=29) and HIV- (n=17) controls. Cases with NAFLD were more obese (BMI 31.0±4.4 kg/m^2 vs 24.1 ±2.8 kg/m^2 p<0.001) and had significantly increased levels of sCD14, sCD163 and higher leptin to adiponectin ratio versus HIV+ controls. Cases with ≥F2 verses <F2 fibrosis had increased sCD14 (1.4 ±0.4 vs 1.1 ±0.3 μg/ml, p=0.023) and sCD163 (1.0 ±0.3 vs 0.8 ±0.3 μg/ml, p=0.060) which correlated with waist circumference (sCD14 p=0.022, sCD163 p=0.011). Immunohistochemistry showed increased hepatic portal macrophage clusters in patients with fibrosis. No markers of bacterial translocation or changes to the microbiome were associated with NAFLD or fibrosis.

Conclusion: NAFLD fibrosis stage in HIV mono-infected patients is associated with monocyte activation in the context of obesity, which may be independent of bacterial translocation and gut microbiome.

Key Words: NAFLD; NASH; fibrosis; HIV; translocation; monocyte.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease worldwide with an estimated prevalence of 25%.[1] NAFLD encompasses a spectrum of disease pathology, ranging from non-alcoholic fatty liver (NAFL) i.e. steatosis without hepatic injury, to non-alcoholic steatohepatitis (NASH), a more severe entity defined by liver steatosis with lobular inflammation and hepatocytes ballooning, and fibrosis.[2] Liver fibrosis is the most important stage of disease progression in NAFLD, as it is the key predictor of increased

63 liver-related mortality.[3] Although only a minority of patients with NAFLD will develop
64 cirrhosis, such is the scale of the problem that NASH is projected to become the leading
65 indication for liver transplant in the next 5-10 years in developed countries.[4] [5]

66 NAFLD, NASH and fibrosis in HIV mono-infected subjects have only been investigated with a
67 limited number of liver biopsy-based analyses.[6][7][8][9] A recent systematic review by our
68 group found a prevalence of NAFLD of 35% in populations mainly investigated with imaging
69 for abnormal liver function tests, and about 20% of patients who had a liver biopsy had
70 significant fibrosis (>=F2).[10]

71 Obesity and the metabolic syndrome are strongly associated with NAFLD and progression to
72 NASH and fibrosis in populations both without[11] [12] and with HIV. [10][13][14][15] In the
73 non- HIV population this may in part be mediated by a complex interaction of adipose tissue
74 dysfunction, bacterial translocation and changes to the structure of the gut
75 microbiome[16][17][18]. However, this has been poorly investigated in patients with HIV. The
76 loss of gut-associated lymphoid tissue (GALT) following HIV infection, bacterial translocation
77 and systemic immune activation has been an important paradigm in our understanding of HIV
78 disease progression,[19] and even in patients established on effective antiretroviral therapy
79 (ART), restoration of the GALT is slower than the peripheral CD4 cell count. Therefore an
80 incomplete resolution of the gut mucosal barrier may contribute to persistent immune
81 activation in these patients,[20] in turn leading to chronic hepatic inflammation and the
82 development of NASH. Furthermore, research on the gut microbiome has demonstrated
83 changes associated with HIV infection that may further modulate the host immune
84 response.[21] Therefore there may be a synergy between HIV and NAFLD driving liver
85 inflammation and fibrosis.

Our study aimed to explore the role of bacterial translocation, adipose tissue dysfunction, immune activation and gut dysbiosis in the development of NAFLD, NASH and fibrosis in HIV mono-infected patients treated with ART.

Methods

Study Population

Patients were prospectively recruited in clinics specialising in HIV and liver disease at three main HIV centres in London, UK (Imperial College Healthcare NHS Trust, Chelsea & Westminster NHS Trust, Royal Free NHS Trust). Controls were prospectively recruited from the same institutions.

Cases were defined as patients with controlled HIV-1 mono-infection i.e. undetectable viral load (<50 cp/ml) and CD4 cell count> 200/mm^3) on ART and liver biopsy proven NAFLD.

Liver biopsy was performed in cases of persistent ALT≥80 IU/l and/or transient elastography (Fibroscan®) ≥7.1kPa according to the treating physician's discretion. Fibroscans were performed after an overnight fast according to standard protocol as previously described, reporting data on both liver stiffness and controlled attenuation parameter (CAP).[22] Exclusion criteria were: patients with alcohol excess within the last 6 months defined as >21units/week for men and >14units/ week for women; CD4 cell count <200/mm3 and/or active AIDS- defining illness; other known causes of chronic liver disease (positive HBs antigen or HCV antibody, autoimmune disease, biliary disease, haemochromatosis or Wilson's disease); current use of steatogenic medication such as methotrexate or long- term steroids.

HIV positive age and sex- matched controls were defined as non-obese subjects with normal liver function tests on at least two occasions over the last 12 months prior the start of the

108 study, alcohol intake less than 21 units per week and no history of liver disease. HIV negative
109 controls were age, sex and body mass index (BMI)- matched to HIV positive controls.

110 Metabolic syndrome was defined by established international guidelines.[23]

111 **Histopathology and Immunohistochemistry**

112 Liver biopsies were formalin- fixed and paraffin- embedded. Sections were stained with
113 trichrome and Haematoxylin & Eosin (H&E) and reported by liver histopathologists blinded to
114 the study data. NASH was defined as the presence of steatosis with ballooning and lobular
115 inflammation, and cases were graded according to the NASH Clinical Research Network (CRN)
116 scoring system.[2] Clinically significant liver fibrosis was defined as at least F2 by the Brunt
117 criteria (pericellular and periportal fibrosis).[24]

118 Liver biopsy slides were stained with antibodies for CD14 and CD163 and reviewed at x100
119 magnification. Clusters were defined as ≥3 positively stained macrophages in a single group.
120 Clusters were identified as portal or lobular, manually counted and divided by the aggregate
121 length of the biopsy cores.[25]

122 **Laboratory Assays**

123 Overnight fasted blood samples were drawn in clinic. Serological markers of bacterial
124 translocation (lipopolysaccharide- binding protein (LBP) (RND Systems, Abingdon, UK) and
125 lipopolysaccharide (LPS) (Cusabio, Wuhan, China)) and monocyte activation (soluble CD14
126 (sCD14) and soluble CD163 (sCD163), RND Systems, Abingdon, UK)), inflammatory cytokines
127 (Interleukin-6 (IL-6) (Life Technologies, Paisley, UK)), tumour necrosis factor alpha receptor 2
128 (TNFαR2) (RND Systems, Abingdon, UK)) and adipokines (adiponectin and leptin (Life
129 Technologies, Carlsbad, USA)) were measured by ELISA following the manufacturer's

instructions. Leptin to adiponectin ratio was used as a marker of adipose tissue dysfunction and insulin resistance.[26][27]

DNA was extracted from whole blood using QIAamp DNA Blood Midi kit (Qiagen Ltd, Manchester, UK) and bacterial DNA quantified by qPCR as previously reported[28] (supplementary methods).

Stool microbial DNA Extraction and 16s rRNA Sequencing

Stool samples were collected at the same time as blood samples or within the following 2 weeks. Faecal DNA extraction was performed as previously described.[29]

Sequencing was performed on an Illumina Miseq instrument (Illumina Inc., Saffron Walden, UK) using the MiSeq Reagent Kit v3 (Illumina) and paired-end 300bp chemistry. The 16s rRNA sequencing data generated on MiSeq was processed on Mothur v.1.39.5 using the MiSeq SOP Pipeline.[30] Further detail is described in the supplementary methods.

Statistical Analysis

Quantitative variables were presented as mean ±SD or median (95% CI) in cases of parametric and non-parametric distribution respectively. Two- group comparisons of continuous data were performed using independent sample t-tests for parametric data and Mann-Whitney U for non-parametric data, and chi-square for proportions. Multiple- group comparisons were conducted using ANOVA or Kruskall-Wallis tests. Associations between laboratory data and liver disease classification were explored using logistic regression and bivariate analyses conducted using biologically relevant variables. Associations between laboratory variables and obesity were explored using linear regression. P values <0.05 were considered significant.

152 Statistical analyses were conducted using GraphPad Prism and IBM SPSS Statistics Software
153 version 23. Microbiome analysis was conducted on Statistical Analysis of Metagenomic Profiles
154 (STAMP) and the R statistical package (Supplementary methods).

155 **Results**

156 **Characteristics of cases and controls**

157 Thirty-three cases, 29 HIV-positive and 17 HIV-negative controls were included in the study.
158 The characteristics are described in table 1 and supplementary table 1.

159 The mean age of cases was 46 ±12.2 years, BMI 31.0±4.4 kg/m^2 and waist circumference
160 104.1±11.4 cm. Twenty-two (66.7%) patients had metabolic syndrome and 5 (15.2%) patients
161 were on treatment for type 2 diabetes. HIV+ and HIV- healthy controls were 48.3 ±11.0 years
162 and 48.0 (36.5-53.5) years respectively, and slim (BMI 24.1 ±2.8 kg/m^2 and 25.2 ±3.5 kg/m^2;
163 waist circumference 85.7 ±8.0 cm and 86.0 ±7.1 cm).

164 Nine (27%) patients had non-alcoholic fatty liver (NAFL), and 24 (73%) patients had NASH;17
165 (51.5%) had none or mild liver fibrosis (F0-1), 3 (9.1%) had significant fibrosis (F2) and 13
166 (39.4%) advanced fibrosis (F3), including 4 patients with historical liver biopsies but with no
167 significant weight change since biopsy. No patients had cirrhosis (Supplementary Table 2).
168 The median time between biopsy and peripheral blood sample collection was 1 month (IQR
169 0-5).

170 **NAFLD and liver fibrosis are not associated with markers of bacterial translocation**

171 There was no difference either in levels of LBP (5.9 ±2.0 vs 5.3 ±1.7 µg/ml, p=0.330), bacterial
172 DNA (0.01 ±0.01 vs 0.01 ±0.00 pg/ml, p=0.566) or LPS (30.2 (0.0-63.1) pg/ml vs 11.3 (0.0-49.7)
173 pg/ml, p=0.269) between NAFLD cases compared to HIV+ controls. There was no difference

174 in any of these markers between HIV+ and HIV- controls (Supplementary Figure 1). These
175 markers of translocation also did not distinguish NASH from NAFL (Supplementary Table 3) or
176 significant fibrosis (Supplementary Figure 1).

177 **NAFLD and liver fibrosis are associated with monocyte activation and adipose tissue**
178 **dysfunction**

179 NAFLD cases had significantly higher levels of sCD14 (1.3 ±0.4 vs 1.1 ±0.4 μg/ml, p=0.031),
180 sCD163 (0.9 ±0.3 vs 0.7 ±0.2 μg/ml, p=0.002) and leptin (11.8 (3.8-20.2) vs 3.5 (2.1-5.5) ng/ml,
181 p<0.0001), lower levels of adiponectin (1.1 (0.5-2.4) vs 2.5 (1.1-4.6) μg/ml, p=0.005), and
182 higher leptin to adiponectin ratio (9.5 (2.5-27.6) vs 1.6 (0.6-4.6), p<0.0001) compared to HIV+
183 controls. IL-6 (7.2 ±2.0 vs 7.1 ±1.8 pg/ml, p=0.821) and TNFαR2 (1.1 ±0.5 vs 1.1 ±0.6 ng/ml,
184 p=0.687) levels did not differ between HIV+ NAFLD cases and HIV+ controls. There was no
185 difference in any of the markers between HIV+ and HIV- controls (Figure 1 and Supplementary
186 Table 3).

187 Cases with F2-F3 fibrosis had significantly higher levels of sCD14 (1.4 ±0.4 vs 1.1 ±0.3 μg/ml,
188 p=0.023, ANOVA p=0.008) compared to cases with F0-F1 fibrosis (Figure 1), whereas there
189 was no difference in sCD14 levels between cases with F0-F1 fibrosis compared to HIV+
190 controls (1.1 ±0.3 v 1.1 ±0.4 μg/ml, p=0.521). There was a trend to increased sCD163 (1.0 ±0.3
191 vs 0.8 ±0.3 μg/ml, p=0.060) and leptin to adiponectin ratio (12.2 (7.5-37.3) vs 5.3 (1.8-21.7),
192 p=0.063) from cases with F0-F1 fibrosis as compared to cases with F2-F3 fibrosis, but a
193 significant increase in these markers by fibrosis stage compared to controls (ANOVA p=0.001
194 and p<0.0001 respectively, Figure 1). There was a significant increase in levels of IL-6 in cases
195 with F2-F3 compared to cases with F0-F1 fibrosis (8.0 ±2.4 vs 6.4 ±1.0 pg/ml, p=0.022), but

196 there was no statistical difference in TNFαR2 levels between both groups (1.2 ±0.6 vs 1.0 ±0.4
197 ng/ml, p=0.341).

198 **Systemic markers of monocyte activation and adipose tissue dysfunction correlate with**
199 **central obesity**

200 We next explored the impact of obesity and metabolic disorders on NAFLD and liver fibrosis.
201 Cases had higher BMI (31.0 ±4.5 vs. 24.1 ±2.8 kg/m2, p<0.001), waist circumference (104.1
202 ±11.4 vs. 85.7 ±8.0 cm, p<0.001), more type 2 diabetes (15% vs. 0% p=0.037), hypertension
203 (61% vs. 28%, p=0.012) and metabolic syndrome (67% vs. 10%, p<0.001) compared to HIV+
204 controls. We correlated markers associated with NAFLD and fibrosis with waist
205 circumference, a surrogate marker for visceral adiposity. Soluble CD14 (r=0.297, p=0.022),
206 sCD163 (r=0.413, p=0.001) and leptin to adiponectin ratio (r=0.487, p<0.0001) all positively
207 correlated with waist circumference (Supplementary Figure 2). Similar results were observed
208 with BMI, although sCD14 did not reach significance (sCD14 r=0.190, p=0.093; sCD163
209 r=0.371, p=0.001; leptin to adiponectin ratio r=0.534 p=<0.0001).

210 Bivariate logistic regression models were used to assess for an association of these markers
211 with liver fibrosis independent of obesity in all HIV+ subjects (Table 2). Interestingly, sCD14
212 and sCD163 remained significantly associated with fibrosis when adjusted for BMI (OR 1.003
213 (1.001-1.005) p=0.016 and OR 1.003 (1.001-1.006) p=0.016) and waist circumference (OR
214 1.002 (1.000-1.005) p=0.049 and OR 1.003 (1.000-1.006) p=0.034), although the effect was
215 blunted, whereas the association with leptin to adiponectin ratio was lost. Age and duration
216 of ART did not affect the associations of these markers with fibrosis. This suggests that obesity
217 contributes to but is not the sole factor in the increased monocyte activation associated with
218 fibrosis.

219 **Liver fibrosis is associated with macrophage clustering in the portal tracts**

220 To investigate the relationship between peripheral monocyte activation and intra-hepatic
221 macrophage activity in HIV-NASH with fibrosis, we performed immunohistochemistry on the
222 liver tissue (n=28; NASH n=21; ≥F2 fibrosis n=14). Clusters of macrophages in the lobules were
223 observed in patients with and without fibrosis. However, there were significantly more
224 CD163- stained portal clusters in ≥F2 versus <F2 fibrosis (0.13 (0.00-0.22) vs 0.0 (0.00-0.04)
225 clusters/mm, p=0.014), which was not observed with CD14 (0.01 (0.00-0.09)vs 0.00 (0.00-
226 0.02) clusters/mm, p=0.122) (Figure 2), although the overall staining with CD14 was weaker
227 than with CD163. There was a significant correlation between both sCD163 with CD163-
228 stained portal clusters (r=0.504, p=0.010), and sCD14 with CD14-stained portal clusters
229 (r=0.431, p=0.029). Neither portal clusters of sCD14 or sCD163 stained macrophages
230 distinguished NASH from NAFL (CD14 0.00 (0.00-0.06) vs 0.00 (0.00-0.04) clusters/mm,
231 p=0.492; CD163 0.04 (0.00-0.16) vs 0.00 (0.00-0.06) clusters/mm, p=0.101).

232 **Gut microbiota**

233 **NAFLD is not associated with a distinct gut microbial profile**

234 Fifty-seven stool samples (cases n=27/33, HIV+ controls n=20/29, HIV-controls n=10/17) were
235 analysed using 16s rRNA sequencing. The characteristics of this subpopulation are shown in
236 Supplementary Table 4. Analysis of the 16s rRNA gene sequencing showed no difference in
237 the relative abundance of bacteria at all levels of the taxanomic classification between HIV+
238 patients with NAFLD and HIV+ controls. Community structures did not differ between groups
239 on the non- metric multidimensional scaling (NMDS) plot (PERMANOVA p=0.809,
240 Supplementary Figure 3A). Similarly, there was no distinct microbiota associated with NASH

241 or significant fibrosis (PERMANOVA p=0.858 and p=0.093, Supplementary Figure 3B and

242 Figure 3A).

243 **HIV infection is associated with a *Prevotella*- enriched enterotype**

244 Given the lack of associations observed within all the HIV positive patients when stratified by
245 NAFLD, NASH or fibrosis, these patients were grouped and compared to HIV negative controls.
246 Interestingly, there were marked differences observed in the microbiome of subjects when
247 stratified by HIV serostatus. NMDS plot demonstrated distinct clustering of microbial
248 communities according to HIV serostatus (PERMANOVA p=0.001, Figure 3B), which remained
249 when only HIV+ controls were compared to HIV- controls, confirming this was not a function
250 of increased BMI or metabolic co-morbidities (data not shown). Significant changes between
251 the groups emerged at the class level, with significantly higher abundance of *Negativicutes*
252 (Mean difference (MD) 7.2% 95%CI 4.9-9.5, corrected p=0.002, Figure 3C). The most striking
253 feature was an enrichment of *Prevotellaceae* (MD 28.0% (19.7-35.6), corrected p=0.011) and
254 *Prevotella* (MD 25.7% (17.6-33.1), corrected p=0.013) at the family and genus level
255 respectively. This was associated with an expected depletion in *Bacteroidaceae* (MD -22.9%
256 (-15.1- -30.1), corrected p=0.022) and *Bacteroides* (MD -22.9% (-15.4- -30.3), corrected
257 p=0.026) compared to HIV- subjects, who are known to compete in the same environmental
258 niche (Figure 3C and supplementary Figure 4).

259

260 **Discussion**

261 We first explored bacterial translocation according to the biopsy-confirmed severity of liver
262 disease, which has not previously been documented in this population, and found that neither

263 LBP,16s rDNA or LPS were associated with NAFLD and liver fibrosis stage. This was in contrast
264 to a strong association with increased levels of sCD14, which in other studies has been used
265 as a surrogate marker of bacterial translocation as CD14 is a co-receptor for LPS and is cleaved
266 from the cell surface of circulating monocytes following activation by LPS.[31] However,
267 sCD14 is not specific to LPS and may be released following monocyte stimulation by multiple
268 ligands and as such also represents a non-specific marker of monocyte activation.[31] Given
269 the lack of association with three other markers of bacterial translocation (LBP, bacterial DNA
270 and LPS), monocyte activation more likely explains the increased circulating levels of sCD14
271 in our patients, which is consistent with the increase in sCD163 levels in cases with NAFLD and
272 fibrosis.

273 There is an extensive literature supporting a role for bacterial translocation in NAFLD,
274 although this is predominantly in animal models.[32] Clinical studies have also demonstrated
275 associations between NAFLD and markers of increased gut permeability, but the results are
276 more inconsistent.[33][34][35] This may be a function of methodological limitations, with LPS
277 in particular lacking robust and reproducible assays.[36] However, it may also be that the
278 absolute levels of systemic bacterial products are much less than in patients with more
279 advanced liver disease (e.g. decompensated cirrhosis)[37][28] and beyond the limit of
280 detection, especially when sampled peripherally rather than in portal blood. Moreover, the
281 similar results between the HIV+ and HIV- control groups suggests there may in fact be
282 restoration of the gut barrier in patients treated with effective ART.[20]

283 Biomarkers of monocyte activation in NAFLD have been investigated in both HIV and general
284 populations. A study from the Multicentre AIDS Cohort Study (HIV+ n=329, NAFLD n=44)
285 found an association between sCD14 and sCD163 with NAFLD which was lost following

286 adjustment for study site, age, race and *PNPLA3* genotype. However, cases were defined by
287 liver steatosis on CT scan rather than biopsy, without stratification by NASH or fibrosis stage,
288 so a detailed analysis of these markers in progressive disease could not be performed.[38]
289 Another study in HIV mono-infected patients with or without metabolic syndrome (n=405)
290 used Fibroscan to stratify by liver fibrosis, and found higher levels of circulating sCD14 and
291 sCD163 in patients with metabolic syndrome, with sCD163 levels significantly associated with
292 fibrosis stage independent of metabolic syndrome. Since clinical features of obesity were also
293 associated with fibrosis stage, the authors concluded adipose tissue dysfunction was
294 important but not the sole factor in monocyte activation and hepatic fibrogenesis. [13] In the
295 non-HIV population, a study combining an Australian (n=157) and Italian (n=174) cohort of
296 biopsy-confirmed NAFLD demonstrated a significant association between serum sCD163
297 levels and fibrosis stage, obesity and insulin resistance, which remained independently
298 associated with liver fibrosis after adjustment for metabolic parameters.[25] Overall, these
299 studies have consistently shown that biomarkers of monocyte activation, especially sCD163,
300 are strongly linked to but not entirely explained by the metabolic complications of obesity,
301 and appear to be key players in the development of NAFLD and fibrosis, regardless of HIV
302 infection. This is consistent with our data: sCD14, sCD163 and leptin to adiponectin ratio (a
303 marker of adipose tissue dysfunction and insulin resistance)[26] increased with fibrosis stage
304 and significantly correlated with waist circumference, but the association between sCD14 and
305 sCD163 with fibrosis remained after adjustment for waist circumference.

306 To investigate the link between peripheral markers of monocyte activation and intra-hepatic
307 macrophages we performed immunohistochemistry in the liver tissue. CD163 - stained portal
308 tract clusters of activated macrophages increased in patients with significant liver fibrosis
309 (≥F2). This pattern was not so clearly seen with CD14, although the staining was weaker

310 throughout the biopsy suggesting it may be a less sensitive marker. Previous studies in non-
311 HIV patients have found clustering of CD163-stained macrophages in NASH compared to NAFL
312 patients, although they did not distinguish portal from lobular clusters, or look specifically at
313 fibrosis.[25][39]

314 Here, the immunohistochemistry data, which significantly correlated with peripheral
315 markers, further supports the notion that monocyte-macrophage activation is associated with
316 progressive fibrosis stage, and the marked differences in peripheral markers between cases
317 and controls is not solely a reflection of obesity rates in the groups. However, the
318 demographic data clearly also highlights how obesity is an important contributor. This is
319 consistent with experimental studies mechanistically linking central obesity to NASH in a
320 disease model where inflamed, insulin resistant adipose tissue enriched with activated
321 macrophages secretes leptin and other pro-inflammatory cytokines into the systemic
322 circulation, in turn stimulating hepatic immune cell infiltration and fibrogenesis.[16] However,
323 additional triggers independent of obesity such as hepatocyte injury from lipotoxicity and
324 oxidative stress may also contribute to local monocyte activation.[40] Therefore, targeting
325 monocyte recruitment is an emerging therapeutic option in NASH clinical trials; a phase 3
326 trial is underway evaluating Cenicriviroc, a CCR2/CCR5 antagonist targeting chemokine
327 signalling important for monocyte infiltration and activation (NCT 03028740),[41] and
328 similarly an early proof-of-concept trial is investigating the potential benefit of Maraviroc, a
329 CCR5 receptor antagonist and licensed antiretroviral, in HIV-associated NASH
330 (ISRCRN15410818).[42]

331 The role of the gut microbiome in NAFLD pathogenesis is an area of significant research
332 interest, and its role in mediating complex metabolic and inflammatory pathways influencing

333 the development of NASH has been elegantly demonstrated in many pre-clinical models,
334 opening new avenues for possible therapeutic targets.[17] However, human studies have
335 often produced inconsistent results.[32] Our study has not observed an association between
336 markers of bacterial translocation or the microbiota with NAFLD, NASH or fibrosis, contrasting
337 with previous studies in the non-HIV population of patients with NAFLD.[43] This may reflect
338 our small sample size, but the fact that associations of specific bacterial populations with
339 NAFLD are rarely repeated in subsequent studies[44] demonstrates the difficulty in exploring
340 a highly complex system in a disease that is slow to evolve.

341 One striking finding was the significant difference in gut microbial communities between
342 cases with HIV and age and sex- matched healthy controls. This was driven principally by an
343 enrichment in the genus *Prevotella* (family *Prevotellaceae*), mirrored by a converse depletion
344 of its competitor *Bacteroides*. Interestingly, *Prevotella* enrichment has been a relatively
345 consistent finding in previous studies investigating the impact of the microbiome in people
346 living with HIV, although this may be a function of lifestyle factors, particularly sexual
347 practices, rather than HIV infection per se.[45] The reasons for this are incompletely
348 understood but may be linked to local environmental perturbations associated with
349 microtrauma and tissue healing.[46] Further mechanistic work is required to investigate a
350 possible role for *Prevotella* in mucosal healing, and whether this affects an individual's
351 susceptibility to acquiring HIV infection.

352 Our study has some limitations. First, the small sample size. The gold standard for diagnosing
353 NASH and fibrosis remains liver biopsy, an invasive procedure and currently only indicated in
354 patients who meet specific criteria following assessment with non-invasive markers. This
355 limits the sample size, restricts analyses to an enriched group with few cases of mild liver

356 disease, and some smaller associations with specific biomarkers may have been missed by
357 lack of statistical power. This may explain why none of the biomarkers could distinguish NASH
358 from NAFL, and negative results in the microbiota analysis. However, there is currently no
359 validated diagnostic marker of NASH, and non-invasive markers have not been well validated
360 in the HIV population, therefore a small study with well-characterised liver histology might be
361 superior to larger studies based on non-invasive markers when investigating mechanisms of
362 NAFLD. Second, some of the results may have been a function of the control group selection,
363 whose BMI was much lower than the cases. However, our bivariate analysis demonstrated an
364 association of monocyte markers independent of BMI and waist circumference. Finally, we
365 were unable to collect Fibroscan values in HIV+ and HIV- controls. However, all had exclusion
366 of acute or chronic liver disease and normal liver function tests and biochemistry.

367 In conclusion, monocyte activation associated with central obesity seems to be a key player
368 in the development of NAFLD and significant liver fibrosis in HIV mono-infected patients
369 independent of dysbiosis and gut translocation.

370 **Acknowledgements:** We would like to thank St Stephens AIDS Trust for funding this work,
371 and Mike Liu and Fouzia Sadiq for their technical support in the laboratory.

372

373 **References**

374 1 Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global
375 epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of
376 prevalence, incidence, and outcomes. *Hepatology* 2016; **64**:73–84.

377 2 Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, *et al.*
378 Design and validation of a histological scoring system for nonalcoholic fatty liver
379 disease. *Hepatology* 2005; **41**:1313–1321.

380 3 Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, *et al.* Fibrosis stage
381 but not NASH predicts mortality and time to development of severe liver disease in

382 biopsy-proven NAFLD. *J Hepatol* 2017; **67**:1265–1273.

383 4 Flemming JA, Kim WR, Brosgart CL, Terrault NA. Reduction in liver transplant wait-
384 listing in the era of direct-acting antiviral therapy. *Hepatology* 2017; **65**:804–812.

385 5 Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA.
386 Frequency and Outcomes of Liver Transplantation for Nonalcoholic Steatohepatitis in
387 the United States. *Gastroenterology* 2011; **141**:1249–1253.

388 6 Morse CG, McLaughlin M, Matthews L, Proschan M, Thomas F, Gharib AM, *et al.*
389 Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With
390 Elevated Aminotransferase Levels on Antiretroviral Therapy. *Clin Infect Dis* 2015;
391 **60**:1569–1578.

392 7 Vodkin I, Valasek M a, Bettencourt R, Cachay E, Loomba R. Clinical, biochemical and
393 histological differences between HIV-associated NAFLD and primary NAFLD: a case-
394 control study. *Aliment Pharmacol Ther* 2014; :1–11.

395 8 Ingiliz P, Valantin MA, Duvivier C, Medja F, Dominguez S, Charlotte F, *et al.* Liver
396 damage underlying unexplained transaminase elevation in human immunodeficiency
397 virus-1 mono-infected patients on antiretroviral therapy. *Hepatology* 2009; **49**:436–
398 442.

399 9 Benmassaoud A, Ghali P, Cox J, Wong P, Szabo J, Deschenes M, *et al.* Screening for
400 nonalcoholic steatohepatitis by using cytokeratin 18 and transient elastography in HIV
401 mono-infection. *PLoS One* 2018; **13**:1–13.

402 10 Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk
403 factors of nonalcoholic fatty liver disease in HIV-monoinfection. *AIDS* 2017; **31**:1621–
404 1632.

405 11 Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis Progression in
406 Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A Systematic Review and
407 Meta-analysis of Paired-Biopsy Studies. *Clin Gastroenterol Hepatol* 2015; **13**:643–654.

408 12 Parikh ND, Marrero WJ, Wang J, Steuer J, Tapper EB, Konerman M, *et al.* Projected
409 increase in obesity and non-alcoholic steatohepatitis-related liver transplantation
410 waitlist additions in the United States. *Hepatology* Published Online First: 17 August
411 2017. doi:10.1002/hep.29473

412 13 Lemoine M, Lacombe K, Bastard JP, Sébire M, Fonquernie L, Valin N, *et al.* Metabolic
413 syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected
414 patients. *Aids* 2017; **44**:1.

415 14 C. D, A. P. Central obesity and non-alcoholic fatty liver disease in people living with
416 HIV: A pragmatic approach. *HIV Med* 2018; **19**:S74.

417 15 G. G, N. S, C. S, G. O, R. D, G. L, *et al.* Nonalcoholic fatty liver disease in HIV-infected
418 patients referred to a metabolic clinic: Prevalence, characteristics, and predictors.
419 Clin. Infect. Dis. 2008; **47**:250–257.

420 16 Bijnen M, Josefs T, Cuijpers I, Maalsen CJ, van de Gaar J, Vroomen M, *et al.* Adipose
421 tissue macrophages induce hepatic neutrophil recruitment and macrophage

422 accumulation in mice. *Gut* 2017; :gutjnl-2016-313654.

423 17 Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J*
424 *Hepatol* 2018; **68**:280–295.

425 18 Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic
426 fatty liver disease (NAFLD). *Metabolism* 2016; **65**:1038–1048.

427 19 Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, *et al.* Microbial
428 translocation is a cause of systemic immune activation in chronic HIV infection. *Nat*
429 *Med* 2007; **12**:1365–1371.

430 20 Mudd JC, Brenchley JM. Gut Mucosal Barrier Dysfunction, Microbial Dysbiosis, and
431 Their Role in HIV-1 Disease Progression. *J Infect Dis* 2016; **214**:S58–S66.

432 21 Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, *et al.*
433 Dysbiosis of the gut microbiota is associated with HIV disease progression and
434 tryptophan catabolism. *Sci Transl Med* 2013; **5**:193ra91.

435 22 Cassinotto C, Boursier J, de L??dinghen V, Lebigot J, Lapuyade B, Cales P, *et al.* Liver
436 stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear
437 imaging, FibroScan, and ARFI with liver biopsy. *Hepatology* 2016; **63**:1817–1827.

438 23 Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, *et al.*
439 Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International
440 Diabetes Federation Task Force on Epidemiology and Prevention; National Heart,
441 Lung, and Blood Institute; American Heart Association; World Heart Federation;
442 International . *Circulation* 2009; **120**:1640–1645.

443 24 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR.
444 Nonalcoholic steatohepatitis: A proposal for grading and staging the histological
445 lesions. *Am J Gastroenterol* 1999; **94**:2467–2474.

446 25 Kazankov K, Barrera F, Møller HJ, Rosso C, Bugianesi E, David E, *et al.* The macrophage
447 activation marker sCD163 is associated with morphological disease stages in patients
448 with non-alcoholic fatty liver disease. *Liver Int* 2016; **36**:1549–1557.

449 26 Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, *et al.* Correlation of
450 the leptin: Adiponectin ratio with measures of insulin resistance in non-diabetic
451 individuals. *Diabetologia* 2009; **52**:2345–2349.

452 27 Tsochatzis E, Papatheodoridis G V, Archimandritis AJ. The Evolving Role of Leptin and
453 Adiponectin in Chronic Liver Diseases. *Am J Gastroenterol* 2006; **101**:2629–2640.

454 28 Vergis N, Atkinson SR, Knapp S, Maurice J, Allison M, Austin A, *et al.* In Patients With
455 Severe Alcoholic Hepatitis, Prednisolone Increases Susceptibility to Infection and
456 Infection-Related Mortality, and Is Associated With High Circulating Levels of Bacterial
457 DNA. *Gastroenterology* 2017; **152**:1068–1077.e4.

458 29 Mullish BH, Pechlivanis A, Barker GF, Thursz MR, Marchesi JR, McDonald JAK.
459 Functional microbiomics: Evaluation of gut microbiota-bile acid metabolism
460 interactions in health and disease. *Methods* Published Online First: 26 April 2018.
461 doi:10.1016/j.ymeth.2018.04.028

462 30 Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-
463 index sequencing strategy and curation pipeline for analyzing amplicon sequence
464 data on the miseq illumina sequencing platform. *Appl Environ Microbiol* 2013;
465 **79**:5112–5120.

466 31 Shive CL, Jiang W, Anthony DD, Lederman MM. Soluble CD14 is a nonspecific marker
467 of monocyte activation. doi:10.1097/QAD.0000000000000735

468 32 Brandl K, Schnabl B. Intestinal microbiota and nonalcoholic steatohepatitis. *Curr Opin*
469 *Gastroenterol* 2017; **33**:128–133.

470 33 Pang J, Xu W, Zhang X, Wong GLH, Chan AWH, Chan HY, *et al.* Significant positive
471 association of endotoxemia with histological severity in 237 patients with non-
472 alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017; **46**:175–182.

473 34 Du Plessis J, Korf H, Van Pelt J, Windmolders P, Vander Elst I, Verrijken A, *et al.* Pro-
474 inflammatory cytokines but not endotoxin-related parameters associate with disease
475 severity in patients with NAFLD. *PLoS One* 2016; **11**:1–15.

476 35 Farhadi A, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny MM, *et al.*
477 Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-
478 alcoholic steatohepatitis. *Liver Int* 2008; **28**:1026–33.

479 36 Gnauck A, Lentle RG, Kruger MC. Chasing a ghost? – Issues with the determination of
480 circulating levels of endotoxin in human blood. *Crit Rev Clin Lab Sci* 2016; **53**:197–215.

481 37 Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J*
482 *Hepatol* 2014; **60**:197–209.

483 38 Price JC, Wang R, Seaberg EC, Budoff MJ, Kingsley LA, Palella FJ, *et al.* The Association
484 of Inflammatory Markers With Nonalcoholic Fatty Liver Disease Differs by Human
485 Immunodeficiency Virus Serostatus. *Open Forum Infect Dis* 2017; **4**:1–9.

486 39 Kazankov K, Tordjman J, Møller HJ, Vilstrup H, Poitou C, Bedossa P, *et al.* Macrophage
487 activation marker soluble CD163 and non-alcoholic fatty liver disease in morbidly
488 obese patients undergoing bariatric surgery. *J Gastroenterol Hepatol* 2015; **30**:1293–
489 300.

490 40 Peterson KR, Cottam MA, Kennedy AJ, Hasty AH. Macrophage-Targeted Therapeutics
491 for Metabolic Disease. *Trends Pharmacol Sci* 2018; **39**:536–546.

492 41 AURORA: Phase 3 Study for the Efficacy and Safety of CVC for the Treatment of Liver
493 Fibrosis in Adults With NASH - Full Text View - ClinicalTrials.gov.
494 https://clinicaltrials.gov/ct2/show/NCT03028740 (accessed 25 Sep2018).

495 42 Clinical Trials register - Search for 2017-003172-32.
496 https://www.clinicaltrialsregister.eu/ctr-search/search?query=2017-003172-32
497 (accessed 30 May2018).

498 43 Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, *et al.* The
499 severity of NAFLD is associated with gut dysbiosis and shift in the metabolic function
500 of the gut microbiota. *Hepatology* 2015; :1–32.

501 44 Brenner DA, Paik Y-H, Schnabl B. Role of Gut Microbiota in Liver Disease. *J Clin*
502 *Gastroenterol* 2015; **49**:S25–S27.

503 45 Noguera-Julian M, Rocafort M, Guillén Y, Rivera J, Casadellà M, Nowak P, *et al.* Gut
504 Microbiota Linked to Sexual Preference and HIV Infection. *EBioMedicine* 2016; **5**:135–
505 146.

506 46 Kelley CF, Kraft CS, De Man TJB, Duphare C, Lee HW, Yang J, *et al.* The rectal mucosa
507 and condomless receptive anal intercourse in HIV-negative MSM: Implications for HIV
508 transmission and prevention. *Mucosal Immunol* 2017; **10**:996–1007.

509

510

511

512

513

514

515

516

517

518

519

520

521 **Table 1**

Parameters	Cases n=33	HIV+ Controls n=29	HIV- Controls n=17	P value*
Age (years)	46.4 (12.4)	48.3 (11.0)	48.0 (36.5-53.5)	0.523
Male Gender (%)	32 (97.0)	29 (100)	15 (88.2)	0.525
Ethnicity				
Caucasian (%)	27 (81.8)	22 (75.9)	10 (58.8)	0.756
Black (%)	2 (6.1)	3 (10.3)	0 (0.0)	0.658
Asian (%)	3 (9.1)	2 (6.9)	5 (29.4)	1.000
Other (%)	1 (3.0)	2 (6.9)	2 (11.8)	0.595
Transmission Risk Factor		29 (100)	0 (0.0)	0.241
MSM (%)	30 (90.9)	0 (0)	17 (0.0)	0.494
Heterosexual (%)	2 (6.1)	0 (0)	N/A	1.00
Vertical Transmission (%)	1 (3.0)			
BMI (kg/m2)	31.0 (4.5)	24.1 (2.8)	25.2 (3.5)	**<0.001***
Waist circumference (cm)	104.1 (11.4)	85.7 (8.0)	86.0 (7.1)	**<0.001***
Type 2 Diabetes (%) [1]	5 (15.2)	0 (0)	0 (0)	**0.037***
Hypertension (%) [2]	22 (66.7)	9 (31.0)	0 (0)	**0.010***
High serum Triglycerides (%) [3]	22 (66.7)	8 (27.6)	0 (0)	**0.003***
Low serum HDL (%) [4]	22 (66.7)	9 (31.0)	0 (0)	**0.010***
Metabolic Syndrome [5]	22 (66.7)	3 (10.3)	0 (0)	**<0.001***
Time since HIV Diagnosis (years)	9.0 (5.0-15.0)	12.0 (5.5-18.5)	N/A	0.385
CD4 cell count Nadir	262.1 (168.4)	292.5 (225.7)	N/A	0.536
Duration ART (years)	7.6 (6.5)	10.2 (8.0)	N/A	0.221
Cumulative duration of ART Class (years)				
NRTI	8.2 (6.5)	20.3 (8.0)	N/A	0.290

NNRTI	4.6 (4.6)	6.4 (6.9)	N/A	0.233
PI	0.0 (0.0-3.8)	0.0 (0.0-2.3)	N/A	0.584
II	0.0 (0.0-1.2)	0.0 (0.0-0.0)	N/A	**0.027***
ALT (IU/L)	104.7 (62.1)	28.3 (8.3)	-	**<0.001***
AST (IU/L)	56.9 (42.0-59.5)	27.8 (24.0-31.0)	-	**0.002***
ALP (IU/ml)	91.1 (25.1)	76.4 (16.3)	-	**0.011***
Cholesterol (mmol/L)	5.0 (0.9)	4.8 (1.4)	-	0.617
LDL (mmol/L)	3.1 (1.1)	3.0 (1.1)	-	0.795
HDL (mmol/L)	1.3 (1.2)	1.3 (0.4)	-	0.873
Triglycerides (mmol/L)	2.2 (1.1)	1.5 (0.8)	-	**0.011***
Glucose (mmol/L)	5.4 (1.2)	5.0 (0.8)	-	0.253
CD4 (cells/mm^3)	815.5 (309.2)	765.7 (235.1)	-	0.506
CD8 (cells/mm^3)	1048.8 (417.3)	830.8 (317.0)	-	**0.046***
Liver Stiffness (kPa)	8.7 (3.7)	-	-	-
CAP (dB/min)	308.8 (36.2)	-	-	-

522

523 **Table 1:** Demographic data of cases compared to age- and sex- matched HIV+ and HIV-
524 controls. Categorical variables are expressed as raw numbers and percentages, continuous
525 variables are reported as mean (SD) or median (IQR). [1] Active treatment with anti-diabetic
526 medications; [2] Systolic BP ≥130mmHg, diastolic BP ≥85mmHg or active treatment anti-
527 hypertensive medication; [3] Serum triglycerides >1.7mmol/L or active treatment with a fibrate;
528 [4] Serum HDL < 1.0 or active treatment with a statin. [5]As per international guidelines.[23]
529 *Cases vs HIV+ controls, P value<0.05. MSM: men who have sex with men; BMI: body mass
530 index; HDL: high density lipoprotein; LDL: low density lipoprotein; ART: antiretroviral therapy;
531 NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse

532 transcriptase inhibitors; PI: protease inhibitors; II: integrase inhibitors; ALT: alanine

533 aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; CAP:

534 controlled attenuation parameter.

535

536

537

538

539

540

541

542

543

544

545

546

547

548

Biomarker	Unadjusted OR	P Value	Model	Adjust NAFLD
sCD14	1.003 (1.001-1.005)	***0.006***	+Age	1.003 (1.005)
			+Waist Circumference	1.002 (1.005)
			+BMI	1.003 (1.005)
			+ Duration of ART	1.003 (1.005)
sCD163	1.004 (1.001-1.006)	***0.003***	+Age	1.003 (1.006
			+Waist Circumference	1.003 (1.006)
			+BMI	1.003 (1.006)
			+ Duration of ART	1.003 (1.006)
Leptin:Adiponectin	1.059 (1.016-1.104)	**0.007**	+Age	1.057 (1.101)
			+Waist Circumference	1.034 (1.081)
			+BMI	1.033 (1.081)
			+ Duration of ART	1.061 (1.108)

Table 2: Odds ratios for sCD14, sCD163 (per 1ng/ml increase) and leptin to adiponectin ratio as biomarkers for significant fibrosis in all subjects with HIV (n=62), adjusted for either age, waist circumference, BMI or duration of antiretroviral therapy (ART). Italics indicate p<0.05.

Figure Legends

Figure 1: Markers of monocyte activation and adipose tissue function in cases and controls. A-E: NAFLD; F-J: sub-categorised by fibrosis stage. sCD14: soluble CD14; sCD163: soluble CD163.

Figure 2: Liver immunohistochemistry**.** A-B: Sample liver sections (Magnification x100) without (A)

and with (B, arrow) portal clusters of CD163-stained macrophages; C-D: Portal clusters/mm liver tissue with CD163 (C) and CD14 (D) staining; E-F: Correlation between liver portal macrophage clusters and peripheral markers of monocyte activation.

Figure 3: Gut microbial communities in liver fibrosis and HIV infection. Non-metric dimensional scaling (NMDS) plot comparing microbial community structures between A). HIV positive cases with NAFLD and ≥F2 Fibrosis vs NAFLD and <F2 Fibrosis vs HIV+ controls. PERMANOVA p=0.093; B). HIV+ (all) vs HIV- subjects. PERMANOVA p=0.001. C). Extended error bar plots comparing the mean difference of significantly altered proportions at Class, Order, Family and Genus taxonomic classification between HIV+ subjects vs HIV- subjects (White's non-parametric t-test with Benjamini-Hochberg FDR correction).