Metabolomics approach in the investigation of depression biomarkers in pharmacologically induced immune-related depression

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Abstract

Background

The aim of this study was to identify previously unrecognised biological pathways and biomarkers that might expand the inflammatory hypothesis of depression.

Methods

Broad metabolomics analyses in plasma samples from 31 chronic hepatitis C-infected patients with and without immune-related depression were carried out using the Absolute IDQ p180 kit—a targeted metabolomics approach of combined direct flow injection and liquid chromatography that measures acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids, and sugars.

Results

The measurements showed that the average concentration of the branched-chain amino acid isoleucine was significantly lower in depressive HCV patients in comparison to non-depressive HCV patients [depression group: Median 51.35 (43.4–60.2 μmol/L) vs. Median 62.10 (38.4–81.7 μmol/L); U = -2.958; p = 0.002]. All other amino acids, acylcarnitines, biogenic amines, glycerophospholipids, sphingolipids, and sugars, liver enzymes and thyroid levels showed no statistically significant differences.

Conclusions

The results of the present study suggest that the branched-chain amino acid isoleucine might play a role in the pathophysiology of immune-related major depression, which expands existing knowledge about inflammatory hypothesis of depression.
Introduction

According to the WHO (2017), worldwide more than 300 million people of all ages suffer from depression, and globally, depression is the leading cause of disability. Thus, depressive illness is one of the major contributors to the overall global disease burden and is one of the priority conditions covered by the WHO’s Mental Health Gap Action Programme (mhGAP) [1]. Depressive patients often suffer from pronounced emotional, somatic, and functional impairments [2,3].

Several pathomechanistic theories for major depression have been proposed. According to the classic monoaminergic hypothesis, depression is the result of a dysfunction of the noradrenaline and 5-hydroxytryptamine (5-HT) mediated neurotransmission. Another well-known concept of major depression is the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. However, both these concepts are not sufficient to explain comprehensively the pathophysiological processes leading to mood imbalances and major depression. Newer models focus on the alterations of neuroplasticity and neurotrophins or the involvement of inflammatory processes [4]. The inflammatory hypothesis of depression proposes that inflammatory cytokines, such as interferon (IFN)-α, IFN-γ, and tumor necrosis factor-α (TNF-α), increase the activity of the enzyme indoleamine 2,3-dioxygenase (IDO), leading to an activation of the catabolism of the serotonin precursor tryptophan to kynurenine [5–9]. The resulting larger amount of kynurenine crosses the blood-brain barrier and is broken down into its neurotoxic metabolites, 3-hydroxykynurenine (3-HK) and quinolinic acid. Quinolinic acid has strong agonistic effects on the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is a glutamate receptor that can induce neuronal damage upon overstimulation [6–9]. Considering the pathophysiological heterogeneity of major depression, the most effective therapy might differ between various forms of the disease. The correct identification of underlying biochemical causes might be a prerequisite for efficient treatment strategies in different forms of major depression [10, 11]. Here we aimed to identify new biomarkers for immune-related depression using the model of pharmacologically induced immune-related depression (PIRD). PIRD is a well-established condition that is often used to study the inflammatory hypothesis of depression in humans.

A prototype of PIRD is the IFN-α induced depression in hepatitis C (HCV) patients. Even in the era of directly acting antivirals (DAAs) [12], IFN-α induced depression still remains an important model to study inflammatory pathways in depression and other mental disease. In the present study we aimed to identify new biomarkers for immune-related depression in IFN-α treated depressive HCV patients using targeted metabolomics. This approach allows the simultaneous analysis of multiple small molecular compounds including acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids, and sugars. The results provide a comprehensive overview of an individual’s metabolic status and may help to explore new biochemical markers for PIRD.

Methods

Study design

We investigated 31 hepatitis C patients treated with IFN-α who had participated in a previous research project on immune-related depression [5, 6, 13, 14]. Detailed information about the primary study has been published before [5]. All patients were treated in the outpatient clinic of the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Austria. At baseline and after one month of IFN-α therapy, patients were assessed in a clinical psychiatric interview performed by an experienced consultation-
liaison psychiatrist (A.B.) for the presence of depressive symptoms. In addition, at baseline and after one month of IFN-α therapy all patients were evaluated with the psychometric observer-rated scale Hamilton Depression Rating Scale (HAMD-17, Hamilton, 1967 [15]). One month after the initial IFN-α therapy the diagnosis of major depression required the diagnosis of major depression in a clinical psychiatric interview performed by an experienced consultation-liaison psychiatrist (A.B.) and additionally the exceeding of the cut-offs of the HAMD-17 [15] and the self-reporting Beck Depression Inventory (BDI, Beck et al., 1961 [16]). After the making of the clinical diagnosis in the clinical psychiatric interview the consultation-liaison psychiatrist (A.B.) had also access to the scores of the HAMD-17 and BDI.

Fasting plasma samples were collected between 9 and 10am one month after the commencement of IFN-α therapy. All samples were centrifuged within 0.5 hours after blood collection and stored until batched analysis. These samples were used for metabolomics analysis. The study was approved by the Institutional Review Board of the University of Medicine of Graz, Austria, and all patients gave their written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

Participants

Participants were recruited through the outpatient clinic of the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Austria. A detailed description of the study cohort has been published before [5, 6, 13]. All patients had chronic hepatitis C infection and were treated with standard pegylated (peg) IFN-α therapy. Exclusion criteria from enrolment were as follows: (1) chronic liver diseases other than HCV, (2) other somatic co-morbidities (i.e. diabetes, cardiovascular disease, cancer), and/or (3) a former diagnosis of any neurological disease.

Metabolomics analyses

Plasma samples were analysed with the Absolute IDQ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) [17] on a SCIEX4000 triple quadrupole mass spectrometer (MS/MS; SCIEX) coupled to an Agilent 1100 liquid chromatography (LC) system equipped with a Zorbax Eclipse C18 150x4.6 mm, 5 μm column (Agilent, Palo Alto, USA).

The Absolute IDQ p180 kit measures 186 analytes: acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids and sugars, using a combined approach of liquid chromatography tandem mass spectrometry (LC-MS/MS) and direct flow injection mass spectrometry (DFI-MS/MS). DFI-MS/MS bypasses the LC system by injecting samples directly into the MS/MS [17].

Prior to analysis samples were prepared as follows: 10 μl of the internal standard solution supplied with the kit and 10 μl of plasma were transferred to each well of a 96-well filterspot extraction plate. After drying the plate under a gentle stream of nitrogen, samples were derivatized with phenyl-isothiocyanate (PITC). Subsequently the plate was dried again and samples were eluted with 5 mM ammonium acetate in methanol. 100 μl of the extracts were diluted with 200 μl of 40% methanol in water for LC-MS/MS analysis. In addition, 10μl of extract were mixed with 190μl of kit running solvent (Biocrates Life Sciences AG) for DFI-MS/MS.

Analytes with results under the limit of detection (LOD) and analytes with insufficient validation for human plasma have been excluded. Analytes with levels between the lower limit of quantification (LOQ) and the limit of detection (LOD) are marked in Table 1 by a bullet. In accordance with the BIOCRATES guidelines of the Absolute IDQ p180 kit (BIOCRATES Life Sciences, 2010) [17], analytes with a semi-quantitative determination are mentioned in a separate column of Table 1.
Table 1. Depression questionnaires, thyroid levels, liver enzymes and metabolomic analyses (Absolute IDQ p180kit) one month after the initial IFN-α treatment.

|                        | Patients without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values | Semi-quantitative | Mann-Whitney-U Test | Benjamini Hochberg Critical Value |
|------------------------|-------------------------------------|-----------------------------------|------------------------|--------------------|---------------------|----------------------------------|
|                        | Median | Range  | Mean  | SD     | Median | Range  | Mean  | SD     | Values | U      | p (exact) |                          |
| Depression Questionnaires |        |        |       |        |        |        |       |        |        |        |          |                          |
| BDI 1                  | 5.0    | 8.0    | 3.91  | 2.28   | 17.0   | 31.0   | 19.2  | 9.11   | 12.00  | 0      | -4.468  | 0.00**               | 0.002               |
| HAMD-17 2              | 4.0    | 10.0   | 4.14  | 3.15   | 14.5   | 11.0   | 15.0  | 3.62   | 10.50  | 0      | -4.449  | 0.00**               | 0.004               |
| Thyroid Levels         |        |        |       |        |        |        |       |        |        |        |          |                          |
| Thyroid-stimulating hormone [mU/L] | 1.54 | 3.01   | 1.60  | 0.75   | 1.12   | 2.21   | 1.19  | 0.75   | -0.42  | 42     | -1.162  | 0.265               | 0.049               |
| Free thyroxine [pmol/L] | 13.5   | 7.4    | 13.19 | 2.19   | 12.2   | 5.5    | 13.54 | 2.31   | -1.30  | 59.5   | -0.032  | 0.975               | 0.234               |
| Free triiodothyronine [pmol/L] | 4.4  | 2.8    | 4.61  | 0.73   | 4.7    | 5.7    | 4.99  | 1.76   | 0.30   | 55.5   | 0.291   | 0.776               | 0.194               |
| Liver Enzymes          |        |        |       |        |        |        |       |        |        |        |          |                          |
| Bilirubin [mg/dL]      | 0.71   | 2.81   | 0.82  | 0.61   | 0.79   | 2.9    | 1.56  | 1.21   | 0.08   | 53     | -1.879  | 0.063               | 0.011               |
| Alkaline phosphatase [U/L] | 66.0 | 70.0   | 68.80 | 15.72  | 69.0   | 45.0   | 66.0  | 14.89  | 3.00   | 87     | 0.142   | 0.908               | 0.219               |
| Gamma-glutamyltransferase [U/L] | 32.0 | 115.0  | 43.67 | 31.02  | 37.0   | 156.0  | 57.89 | 48.71  | 5.00   | 70.5   | 1.087   | 0.234               | 0.054               |
| Aspartate transaminase [U/L] | 39.0 | 67.0   | 42.52 | 16.95  | 38.0   | 57.0   | 38.56 | 17.13  | -1.00  | 77.5   | -0.771  | 0.449               | 0.103               |
| Alanine transaminase [U/L] | 38.0 | 92.0   | 45.05 | 25.2   | 28.0   | 68.0   | 40.67 | 24.34  | -10.00 | 80     | 0.657   | 0.533               | 0.124               |
| Cholinesterase [U/L]   | 8422.0 | 8531.0 | 8103.0 | 2351.4 | 10276.5 | 4433.0 | 9518.0 | 2085.7 | 1854.5 | 12     | -1.131  | 0.304               | 0.061               |
| Metabolomic Analyses—Absolute IDQ p180kit Acylcarnitines |        |        |       |        |        |        |       |        |        |        |          |                          |
| C0 (Carnitine) [µmol/L] | 37.01  | 33.63  | 37.58 | 9.18   | 34.11  | 32.29  | 4.05  | 2.90   | -2.90  | 84     | -0.887  | 0.393               | 0.094               |
| C2 (Acetylcarnitine) [µmol/L] | 5.11 | 8.22   | 5.55  | 2.10   | 5.37   | 4.98   | 5.42  | 1.59   | 0.36   | 102    | -0.127  | 0.917               | 0.221               |
| C3 (Propionylcarnitine) [µmol/L] | 0.23 | 0.26   | 0.22  | 0.05   | 0.17   | 0.1    | 0.18  | 0.03   | -0.06  |        |          |                          |
| C4 (Butyrylcarnitine) [µmol/L] | 0.15 | 0.16   | 0.15  | 0.04   | 0.12   | 0.19   | 0.14  | 0.06   | -0.03  |        |          |                          |
| C5 (Valerylcarnitine) [µmol/L] | 0.13 | 0.36   | 0.19  | 0.11   | 0.1    | 0.35   | 0.17  | 0.12   | -0.03  |        |          |                          |
| C9 (Nonaylcarnitine) [µmol/L] | 0.05 | 0.06   | 0.05  | 0.01   | 0.04   | 0.04   | 0.05  | 0.01   | -0.01  |        |          |                          |
| C14-1 (Tetradecenoylcarnitine) [µmol/L] | 0.13 | 0.17   | 0.13  | 0.04   | 0.16   | 0.13   | 0.15  | 0.05   | 0.03   | 84     | -1.31   | 0.201               | 0.031               |
| C16 (Hexadecanoylcarnitine) [µmol/L] | 0.10 | 0.1    | 0.11  | 0.03   | 0.13   | 0.14   | 0.13  | 0.05   | 0.03   | 102    | -0.127  | 0.917               | 0.221               |
| C18 (Octadecanoylcarnitine) [µmol/L] | 0.04 | 0.08   | 0.05  | 0.02   | 0.04   | 0.05   | 0.05  | 0.02   | 0.00   |        |          |                          |
| C18:1 (Octadecenoylcarnitine) [µmol/L] | 0.13 | 0.18   | 0.14  | 0.05   | 0.15   | 0.22   | 0.16  | 0.07   | 0.02   | 89     | 0.676   | 0.519               | 0.119               |
| C18:2 (Octadecadienoylcarnitine) [µmol/L] | 0.06 | 0.08   | 0.06  | 0.02   | 0.06   | 0.1    | 0.06  | 0.03   | 0.00   | 98     | -0.296  | 0.787               | 0.196               |
| Amino Acids            |        |        |       |        |        |        |       |        |        |        |          |                          |
| Alanine [µmol/L]       | 290.0  | 293.0  | 312.24 | 83.0   | 288.0  | 164.0  | 293.6 | 51.14  | -2.00  | 94.5   | -0.444  | 0.663               | 0.158               |
| Arginine [µmol/L]      | 81.0   | 56.4   | 84.04  | 16.0   | 76.7   | 63.8   | 74.84 | 22.07  | -4.30  | 76.5   | 1.205   | 0.233               | 0.038               |

(Continued)
| Table 1. (Continued) |
|-----------------------|
| **Patients without Depression (n = 21)** | **Patients with Depression (n = 10)** | **Diff. of Median values** | **Semi-quantitative** | **Mann-Whitney-U Test** | **Benjamini Hochberg Critical Value** |
| **Median** | **Range** | **Mean** | **SD** | **Median** | **Range** | **Mean** | **SD** | **Mann-Whitney-U** | **p (exact)** | **0.05** | **0.10** |
| 39.1 | 33.8 | 39.09 | 8.03 | 32.8 | 25.0 | 34.76 | 7.83 | -6.30 | 77 | -1.183 | 0.25 | 0.045 |
| 463.0 | 450.0 | 442.29 | 120.59 | 437.5 | 330.0 | 428.3 | 100.38 | -25.50 | 101 | -0.169 | 0.884 | 0.212 |
| 283.0 | 439.0 | 330.52 | 131.13 | 283.0 | 354.0 | 321.2 | 112.36 | 0.00 | 101 | -0.169 | 0.884 | 0.214 |
| 207.0 | 167.0 | 214.33 | 44.14 | 189.5 | 141.0 | 208.3 | 48.61 | -17.50 | 88.5 | -0.697 | 0.492 | 0.110 |
| 62.1 | 43.3 | 62.0 | 11.30 | 51.35 | 16.8 | 51.07 | 5.48 | -10.75 | 35 | -2.958 | 0.002** | 0.005 |
| 111.0 | 101.2 | 116.31 | 21.85 | 99.8 | 43.9 | 98.48 | 14.85 | -11.20 | 51 | -2.283 | 0.022 | 0.007 |
| 17.0 | 94.0 | 171.76 | 21.91 | 161.0 | 115.0 | 167.4 | 35.92 | -9.00 | 88 | -0.719 | 0.348 | 0.074 |
| 18.9 | 32.17 | 19.41 | 6.37 | 16.45 | 13.4 | 17.32 | 4.66 | -2.45 | 82 | -0.972 | 0.348 | 0.074 |
| 61.8 | 85.6 | 65.09 | 19.66 | 60.5 | 54.8 | 63.79 | 17.75 | -1.30 | 97 | -0.338 | 0.755 | 0.185 |
| 70.20 | 75.7 | 73.75 | 18.24 | 67.65 | 27.0 | 65.81 | 8.23 | -2.55 | 81 | -1.014 | 0.327 | 0.068 |
| 210.0 | 271.0 | 214.1 | 69.42 | 174.5 | 151.0 | 175.8 | 41.60 | -35.50 | 69 | -1.521 | 0.135 | 0.022 |
| 106.0 | 60.4 | 104.06 | 16.21 | 109.0 | 64.2 | 109.81 | 21.97 | 3.00 | 90 | -0.634 | 0.546 | 0.126 |
| 125.0 | 106.6 | 134.77 | 31.13 | 135.5 | 153.2 | 132.94 | 45.17 | -10.50 | 94.5 | -0.444 | 0.663 | 0.160 |
| 55.2 | 29.7 | 52.9 | 8.84 | 51.2 | 21.2 | 50.84 | 8.23 | -2.55 | 80 | -1.057 | 0.306 | 0.063 |
| 76.2 | 111.8 | 77.63 | 25.03 | 67.95 | 39.8 | 75.01 | 15.33 | -8.25 | 98 | -0.296 | 0.787 | 0.198 |
| 227.0 | 129.0 | 232.29 | 37.46 | 199.0 | 69.0 | 202.8 | 25.03 | -28.00 | 55.5 | -2.092 | 0.035 | 0.009 |
| **Biogenic Amines** | **Creatinine [μmol/L]** | 75.6 | 40.10 | 74.74 | 12.52 | 71.8 | 49.9 | 71.15 | 15.93 | -3.80 | 91.5 | 0.571 | 0.574 | 0.138 |
| **Putrescine [μmol/L]** | 0.13 | 0.13 | 0.14 | 0.04 | 0.16 | 0.2 | 0.18 | 0.07 | -0.08 | 0.3 | -0.063 | 0.546 | 0.126 |
| **Sarcosine [μmol/L]** | 7.92 | 6.41 | 8.03 | 1.69 | 6.8 | 6.87 | 7.85 | 2.60 | -1.12 | 84 | -0.887 | 0.393 | 0.095 |
| **Taurine [μmol/L]** | 46.4 | 49.40 | 52.83 | 14.92 | 44.4 | 38.5 | 47.76 | 12.60 | -2.00 | 81 | 1.014 | 0.327 | 0.070 |

| **Glycerophospholipids** |
|--------------------------|
| **lysoPhosphatidylcholine acyl C14:0 [μmol/L]** | 2.67 | 2.69 | 2.98 | 0.72 | 2.87 | 1.23 | 3.10 | 0.51 | -0.20 | X | 79 | -1.099 | 0.287 | 0.056 |
| **lysoPhosphatidylcholine acyl C16:0 [μmol/L]** | 97.86 | 98.54 | 102.3 | 29.09 | 92.62 | 110.44 | 109.37 | 38.86 | -5.24 | X | 104 | -0.042 | 0.983 | 0.236 |
| **lysoPhosphatidylcholine acyl C16:1 [μmol/L]** | 2.15 | 1.91 | 2.27 | 0.59 | 2.4 | 1.76 | 2.5 | 0.57 | 0.25 | X | 75 | -1.268 | 0.217 | 0.034 |
| **lysoPhosphatidylcholine acyl C17:0 [μmol/L]** | 1.95 | 3.12 | 2.14 | 0.83 | 2.15 | 2.89 | 2.38 | 0.94 | 0.20 | X | 92 | -0.549 | 0.603 | 0.146 |
| **lysoPhosphatidylcholine acyl C18:0 [μmol/L]** | 39.84 | 46.59 | 40.45 | 13.14 | 33.32 | 40.6 | 41.89 | 15.13 | -6.52 | X | 103 | -0.085 | 0.95 | 0.228 |
| **lysoPhosphatidylcholine acyl C18:1 [μmol/L]** | 21.99 | 25.31 | 21.79 | 6.96 | 19.14 | 19.7 | 22.4 | 7.94 | -2.85 | X | 104 | -0.042 | 0.983 | 0.237 |
| **lysoPhosphatidylcholine acyl C18:2 [μmol/L]** | 27.81 | 34.41 | 29.99 | 10.23 | 25.75 | 35.51 | 29.45 | 12.73 | -2.06 | X | 94 | -0.465 | 0.663 | 0.162 |
| **lysoPhosphatidylcholine acyl C20:3 [μmol/L]** | 2.65 | 2.36 | 2.52 | 0.76 | 1.87 | 2.05 | 2.21 | 0.75 | -0.78 | X | 80 | -1.056 | 0.306 | 0.065 |

(Continued)
| Patient without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values | Mann-Whitney-U Test | Benjamini Hochberg Critical Value |
|-------------------------------------|----------------------------------|------------------------|----------------------|----------------------------------|
| **Median** | **Range** | **Mean** | **SD** | **Median** | **Range** | **Mean** | **SD** | **P** | **U (exact)** |
| **lysoPhosphatidylcholine acyl C20:4 [μmol/L]** | 5.32 (4.01-6.47) | 8.26 | 4.16 | 10.15 (9.04-11.42) | 6.47 | 4.96 | 2.86 | 0.36 | X | 0.128 |
| **lysoPhosphatidylcholine acyl C24:0 [μmol/L]** | 0.44 (0.29-0.64) | 0.64 | 0.16 | 0.64 (0.45-0.86) | 0.45 | 0.49 | 0.27 | 0.11 | X | 0.230 |
| **lysoPhosphatidylcholine acyl C26:0 [μmol/L]** | 0.91 (0.74-1.38) | 1.26 | 0.36 | 1.38 (1.08-1.74) | 1.08 | 0.84 | 0.49 | 0.27 | X | 0.205 |
| **lysoPhosphatidylcholine acyl C26:1 [μmol/L]** | 0.29 (0.23-0.36) | 0.36 | 0.11 | 0.36 (0.25-0.49) | 0.25 | 0.26 | 0.09 | 0.04 | X | 0.205 |
| **lysoPhosphatidylcholine acyl C28:0 [μmol/L]** | 1.09 (0.84-1.47) | 1.38 | 0.36 | 1.47 (1.10-1.86) | 1.10 | 0.88 | 0.53 | 0.27 | X | 0.232 |
| **lysoPhosphatidylcholine acyl C28:1 [μmol/L]** | 0.72 (0.56-0.94) | 0.94 | 0.31 | 0.94 (0.69-1.23) | 0.69 | 0.69 | 0.26 | 0.12 | X | 0.176 |
| **Phosphatidylcholine diacyl C24:0 [μmol/L]** | 0.28 (0.23-0.32) | 0.32 | 0.13 | 0.32 (0.24-0.45) | 0.24 | 0.25 | 0.08 | 0.04 | X | 0.103 |
| **Phosphatidylcholine diacyl C26:0 [μmol/L]** | 0.70 (0.52-0.94) | 0.94 | 0.31 | 0.94 (0.69-1.23) | 0.69 | 0.69 | 0.26 | 0.12 | X | 0.176 |
| **Phosphatidylcholine diacyl C28:0 [μmol/L]** | 1.0 (0.75-1.26) | 1.26 | 0.36 | 1.26 (0.99-1.58) | 0.99 | 0.97 | 0.26 | 0.12 | X | 0.176 |
| **Phosphatidylcholine diacyl C30:0 [μmol/L]** | 3.09 (2.60-3.74) | 4.76 | 1.38 | 4.76 (4.19-5.32) | 4.19 | 3.45 | 1.26 | 0.87 | X | 0.176 |
| **Phosphatidylcholine diacyl C32:0 [μmol/L]** | 16.16 (14.20-18.32) | 18.32 | 2.34 | 18.32 (16.47-20.46) | 16.47 | 13.16 | 5.32 | 1.98 | X | 0.176 |
| **Phosphatidylcholine diacyl C34:0 [μmol/L]** | 35.15 (32.50-37.82) | 37.82 | 3.51 | 37.82 (35.94-40.00) | 35.94 | 33.79 | 5.88 | 2.04 | X | 0.176 |
| **Phosphatidylcholine diacyl C36:0 [μmol/L]** | 198.21 (176.56-220.87) | 220.87 | 19.15 | 220.87 (198.44-248.29) | 198.44 | 194.73 | 11.87 | 3.93 | X | 0.176 |
|                      | Patients without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values | Semi-quantitative | Mann-Whitney-U Test | Benjamini Hochberg Critical Value |
|----------------------|--------------------------------------|---------------------------------|------------------------|--------------------|---------------------|---------------------------------|
|                      | Median | Range | Mean | SD    | Median | Range | Mean | SD    |                     |                                   |                                  |
| Phosphatidylcholine diacyl C36:3 [μmol/L] | 112.24 | 153.13 | 121.2 | 34.07 | 110.55 | 52.38 | 111.03 | 15.11 | -1.69 | X | 89 | -0.676 | 0.519 | 0.121 |
| Phosphatidylcholine diacyl C36:4 [μmol/L] | 132.58 | 127.76 | 139.69 | 34.46 | 128.23 | 76.59 | 129.80 | 20.36 | -4.35 | X | 96 | -0.38 | 0.724 | 0.178 |
| Phosphatidylcholine diacyl C36:5 [μmol/L] | 13.9 | 13.41 | 15.48 | 4.19 | 14.63 | 7.64 | 15.0 | 2.20 | 0.73 | X | 99 | -0.254 | 0.819 | 0.207 |
| Phosphatidylcholine diacyl C36:6 [μmol/L] | 1.07 | 1.48 | 1.12 | 0.32 | 1.07 | 0.46 | 1.08 | 0.14 | 0.00 | X | 104 | -0.042 | 0.983 | 0.243 |
| Phosphatidylcholine diacyl C38:0 [μmol/L] | 3.36 | 3.33 | 3.5 | 0.78 | 3.49 | 2.21 | 3.76 | 0.82 | 0.13 | X | 85 | -0.845 | 0.416 | 0.099 |
| Phosphatidylcholine diacyl C38:1 [μmol/L] | 2.83 | 4.81 | 2.80 | 1.16 | 2.7 | 2.72 | 2.75 | 0.86 | -0.13 | X | 104 | -0.042 | 0.983 | 0.245 |
| Phosphatidylcholine diacyl C38:2 [μmol/L] | 33.88 | 36.64 | 37.41 | 10.35 | 33.22 | 17.02 | 34.26 | 5.42 | -0.66 | X | 96 | -0.38 | 0.724 | 0.180 |
| Phosphatidylcholine diacyl C38:3 [μmol/L] | 67.46 | 74.59 | 69.81 | 18.52 | 63.86 | 34.29 | 65.36 | 10.08 | -3.60 | X | 98 | -0.296 | 0.787 | 0.200 |
| Phosphatidylcholine diacyl C38:4 [μmol/L] | 27.73 | 27.18 | 29.22 | 6.93 | 27.99 | 5.56 | 28.04 | 1.74 | 0.26 | X | 98 | -0.296 | 0.787 | 0.201 |
| Phosphatidylcholine diacyl C38:5 [μmol/L] | 35.71 | 32.84 | 35.4 | 9.18 | 38.18 | 31.49 | 38.9 | 8.88 | 2.47 | X | 90 | -0.634 | 0.546 | 0.129 |
| Phosphatidylcholine diacyl C38:6 [μmol/L] | 0.92 | 1.04 | 0.91 | 0.25 | 0.77 | 0.61 | 0.84 | 0.2 | -0.15 | X | 83 | -0.93 | 0.37 | 0.083 |
| Phosphatidylcholine diacyl C40:1 [μmol/L] | 1.7 | 1.6 | 1.57 | 0.41 | 1.41 | 1.28 | 1.48 | 0.36 | -0.29 | X | 87 | -0.761 | 0.466 | 0.104 |
| Phosphatidylcholine diacyl C40:2 [μmol/L] | 1.69 | 1.47 | 1.59 | 0.41 | 1.43 | 1.13 | 1.5 | 0.33 | -0.26 | X | 96 | -0.38 | 0.724 | 0.182 |
| Phosphatidylcholine diacyl C40:3 [μmol/L] | 3.32 | 2.22 | 3.29 | 0.66 | 3.08 | 1.15 | 3.14 | 0.31 | -0.24 | X | 91 | -0.592 | 0.574 | 0.140 |
| Phosphatidylcholine diacyl C40:4 [μmol/L] | 6.5 | 4.53 | 6.4 | 1.39 | 6.37 | 2.71 | 6.33 | 0.84 | -0.13 | X | 95 | -0.423 | 0.693 | 0.169 |
| Phosphatidylcholine diacyl C40:5 [μmol/L] | 13.79 | 12.95 | 14.2 | 3.94 | 14.64 | 9.31 | 15.65 | 3.08 | 0.85 | X | 83 | -0.93 | 0.37 | 0.085 |
| Phosphatidylcholine diacyl C40:6 [μmol/L] | 0.83 | 0.62 | 0.81 | 0.17 | 0.73 | 0.52 | 0.76 | 0.18 | -0.10 | X | 84 | -0.887 | 0.393 | 0.097 |
| Phosphatidylcholine diacyl C42:0 [μmol/L] | 0.52 | 0.49 | 0.51 | 0.13 | 0.45 | 0.36 | 0.47 | 0.12 | -0.07 | X | 87 | -0.761 | 0.466 | 0.106 |
| Phosphatidylcholine diacyl C42:1 [μmol/L] | 0.58 | 0.55 | 0.58 | 0.15 | 0.58 | 0.34 | 0.56 | 0.12 | 0.00 | X | 95 | -0.423 | 0.693 | 0.171 |
| Phosphatidylcholine diacyl C42:2 [μmol/L] | 0.59 | 0.64 | 0.61 | 0.15 | 0.63 | 0.16 | 0.6 | 0.07 | 0.04 | X | 92 | -0.549 | 0.603 | 0.147 |
| Phosphatidylcholine diacyl C42:5 [μmol/L] | 0.58 | 0.57 | 0.6 | 0.14 | 0.64 | 0.19 | 0.62 | 0.07 | 0.06 | X | 73 | -1.352 | 0.186 | 0.027 |
Table 1. (Continued)

|                      | Patients without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values \(^4\) | Semi-quantitative Mann-Whitney-U Test | Benjamini Hochberg Critical Value \(^5\) |
|----------------------|-------------------------------------|----------------------------------|-------------------------------|---------------------------------------|----------------------------------|
|                      | Median | Range | Mean | SD   | Median | Range | Mean | SD | Mann-Whitney-U | U | p (exact) |                      |                      |
| Phosphatidylcholine diacyl C42:6 [μmol/L] | 0.57   | 0.66  | 0.57 | 0.13 | 0.6    | 0.16  | 0.57 | 0.06 | 0.03          | X | -0.507    | 0.633 | 0.151          |
| Phosphatidylcholine acyl-alkyl C30:0 [μmol/L] | 0.48   | 0.45  | 0.49 | 0.13 | 0.54   | 0.27  | 0.52 | 0.09 | 0.06          | X | -0.592    | 0.574 | 0.142          |
| Phosphatidylcholine acyl-alkyl C30:2 [μmol/L] | 0.26   | 0.37  | 0.25 | 0.09 | 0.23   | 0.26  | 0.24 | 0.08 | -0.03         | X | -0.549    | 0.603 | 0.149          |
| Phosphatidylcholine acyl-alkyl C32:1 [μmol/L] | 3.32   | 2.69  | 3.27 | 0.75 | 3.44   | 2.09  | 3.46 | 0.59 | 0.12          | X | -0.676    | 0.519 | 0.122          |
| Phosphatidylcholine acyl-alkyl C32:2 [μmol/L] | 0.85   | 0.7   | 0.86 | 0.19 | 0.85   | 0.69  | 0.89 | 0.2  | 0.00          | X | -0.127    | 0.917 | 0.225          |
| Phosphatidylcholine acyl-alkyl C34:0 [μmol/L] | 1.51   | 1.81  | 1.67 | 0.45 | 1.92   | 0.66  | 1.85 | 0.24 | 0.41          | X | -1.733    | 0.087 | 0.016          |
| Phosphatidylcholine acyl-alkyl C34:1 [μmol/L] | 11.07  | 8.73  | 11.52| 2.19 | 12.75  | 4.44  | 12.16| 1.35 | 1.68          | X | -0.099    | 0.287 | 0.058          |
| Phosphatidylcholine acyl-alkyl C34:2 [μmol/L] | 10.96  | 14.71 | 12.39| 4.06 | 11.74  | 7.74  | 11.05| 2.47 | 0.78          | X | -0.634    | 0.546 | 0.131          |
| Phosphatidylcholine acyl-alkyl C34:3 [μmol/L] | 6.99   | 10.0  | 7.79 | 2.51 | 7.78   | 5.04  | 7.07 | 1.89 | 0.79          | X | -0.634    | 0.546 | 0.133          |
| Phosphatidylcholine acyl-alkyl C36:0 [μmol/L] | 1.13   | 0.95  | 1.07 | 0.25 | 1.08   | 0.38  | 1.09 | 0.15 | -0.05         | X | -0.423    | 0.693 | 0.173          |
| Phosphatidylcholine acyl-alkyl C36:1 [μmol/L] | 13.07  | 14.37 | 12.99| 3.63 | 12.97  | 6.29  | 12.85| 2.21 | -0.10         | X | -0.211    | 0.852 | 0.210          |
| Phosphatidylcholine acyl-alkyl C36:2 [μmol/L] | 13.32  | 16.72 | 14.26| 4.46 | 15.00  | 9.11  | 14.54| 3.02 | 1.68          | X | -0.93     | 0.37  | 0.086          |
| Phosphatidylcholine acyl-alkyl C36:3 [μmol/L] | 6.06   | 7.34  | 6.54 | 2.07 | 6.01   | 3.12  | 5.61 | 1.12 | -0.05         | X | -0.761    | 0.466 | 0.108          |
| Phosphatidylcholine acyl-alkyl C36:4 [μmol/L] | 16.84  | 24.66 | 18.67| 5.82 | 16.67  | 15.00 | 16.77| 5.02 | -0.17         | X | -0.845    | 0.416 | 0.101          |
| Phosphatidylcholine acyl-alkyl C36:5 [μmol/L] | 8.50   | 12.5  | 9.06 | 2.59 | 8.56   | 7.98  | 8.61 | 3.02 | 0.06          | X | -0.042    | 0.983 | 0.246          |
| Phosphatidylcholine acyl-alkyl C38:0 [μmol/L] | 2.79   | 3.25  | 2.94 | 0.76 | 2.93   | 1.59  | 3.18 | 0.59 | 0.14          | X | -1.056    | 0.306 | 0.067          |
| Phosphatidylcholine acyl-alkyl C38:1 [μmol/L] | 4.18   | 6.37  | 4.36 | 1.54 | 4.42   | 3.27  | 4.32 | 1.02 | 0.24          | X | -0.254    | 0.819 | 0.209          |
| Phosphatidylcholine acyl-alkyl C38:2 [μmol/L] | 7.24   | 9.6   | 7.47 | 2.46 | 7.04   | 4.49  | 7.01 | 1.4  | -0.20         | X | -0.93     | 0.37  | 0.088          |
| Phosphatidylcholine acyl-alkyl C38:3 [μmol/L] | 9.22   | 8.41  | 9.51 | 2.43 | 8.91   | 4.01  | 8.72 | 1.19 | -0.31         | X | -0.465    | 0.663 | 0.164          |
| Phosphatidylcholine acyl-alkyl C38:4 [μmol/L] | 11.69  | 9.72  | 11.89| 2.6  | 11.49  | 7.21  | 11.27| 2.26 | -0.20         | X | -0.676    | 0.519 | 0.122          |
| Phosphatidylcholine acyl-alkyl C38:5 [μmol/L] | 13.09  | 14.34 | 13.19| 3.52 | 12.63  | 9.10  | 12.55| 3.29 | -0.46         | X | -0.718    | 0.492 | 0.115          |

(Continued)
|                          | Patients without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values | Mann-Whitney-U Test | Benjamini Hochberg Critical Value |
|--------------------------|-------------------------------------|----------------------------------|------------------------|---------------------|----------------------------------|
|                          | Median | Range | Mean | SD | Median | Range | Mean | SD | Mann-Whitney-U | U | p (exact) |                              |
| Phosphatidylcholine acyl-alkyl C38:6 [μmol/L] | 4.47   | 5.2   | 4.56 | 1.04 | 4.59   | 3.76   | 4.62 | 1.19 | 0.12 | X | 104 | -0.042 | 0.983 | 0.248 |
| Phosphatidylcholine acyl-alkyl C40:1 [μmol/L] | 2.31   | 1.71   | 2.14 | 0.5 | 2.03   | 1.38   | 1.99 | 0.44 | -0.28 | X | 78 | -1.141 | 0.268 | 0.052 |
| Phosphatidylcholine acyl-alkyl C40:2 [μmol/L] | 2.78   | 2.44   | 2.88 | 0.67 | 2.89   | 1.88   | 2.87 | 0.58 | 0.11 | X | 102 | -0.127 | 0.917 | 0.227 |
| Phosphatidylcholine acyl-alkyl C40:3 [μmol/L] | 4.26   | 4.50   | 4.39 | 1.06 | 4.15   | 2.83   | 4.12 | 0.75 | -0.11 | X | 95 | -0.423 | 0.693 | 0.174 |
| Phosphatidylcholine acyl-alkyl C40:4 [μmol/L] | 4.46   | 3.65   | 4.61 | 0.97 | 4.46   | 2.18   | 4.31 | 0.65 | 0.00 | X | 90 | -0.634 | 0.546 | 0.135 |
| Phosphatidylcholine acyl-alkyl C40:5 [μmol/L] | 4.49   | 3.71   | 4.7 | 0.9 | 4.7    | 2.57   | 4.69 | 0.71 | 0.21 | X | 93 | -0.507 | 0.633 | 0.153 |
| Phosphatidylcholine acyl-alkyl C40:6 [μmol/L] | 3.27   | 2.29   | 3.35 | 0.58 | 3.32   | 1.93   | 3.51 | 0.60 | 0.05 | X | 96 | -0.38  | 0.724 | 0.183 |
| Phosphatidylcholine acyl-alkyl C42:1 [μmol/L] | 1.22   | 0.93   | 1.11 | 0.27 | 0.98   | 0.96   | 1.03 | 0.29 | -0.24 | X | 83 | -0.93  | 0.37  | 0.090 |
| Phosphatidylcholine acyl-alkyl C42:2 [μmol/L] | 1.12   | 0.96   | 1.04 | 0.27 | 0.97   | 0.78   | 0.98 | 0.24 | -0.15 | X | 90 | -0.634 | 0.546 | 0.137 |
| Phosphatidylcholine acyl-alkyl C42:3 [μmol/L] | 1.25   | 1.16   | 1.24 | 0.3 | 1.24   | 0.69   | 1.2  | 0.23 | -0.01 | X | 91 | -0.592 | 0.574 | 0.144 |
| Phosphatidylcholine acyl-alkyl C42:4 [μmol/L] | 1.43   | 1.17   | 1.4 | 0.29 | 1.29   | 0.85   | 1.26 | 0.26 | -0.14 | X | 76 | -1.225 | 0.233 | 0.040 |
| Phosphatidylcholine acyl-alkyl C42:5 [μmol/L] | 2.89   | 1.49   | 2.87 | 0.41 | 2.74   | 1.5    | 2.77 | 0.44 | -0.15 | X | 88 | -0.718 | 0.492 | 0.117 |
| Phosphatidylcholine acyl-alkyl C44:3 [μmol/L] | 0.37   | 0.38   | 0.37 | 0.1 | 0.39   | 0.25   | 0.37 | 0.09 | 0.02 | X | 101 | -0.169 | 0.884 | 0.216 |
| Phosphatidylcholine acyl-alkyl C44:4 [μmol/L] | 0.55   | 0.45   | 0.55 | 0.12 | 0.47   | 0.33   | 0.49 | 0.11 | -0.08 | X | 79 | -1.099 | 0.287 | 0.059 |
| Phosphatidylcholine acyl-alkyl C44:5 [μmol/L] | 1.97   | 1.89   | 1.87 | 0.48 | 1.38   | 1.49   | 1.55 | 0.48 | -0.99 | X | 67 | -1.606 | 0.114 | 0.020 |
| Phosphatidylcholine acyl-alkyl C44:6 [μmol/L] | 1.37   | 1.04   | 1.29 | 0.28 | 1.06   | 0.89   | 1.15 | 0.33 | -0.31 | X | 73 | -1.352 | 0.186 | 0.029 |

**Sphingolipids**

|                          | Median | Range | Mean | SD | Median | Range | Mean | SD | Mann-Whitney-U | U | p (exact) |
|--------------------------|--------|-------|------|----|--------|-------|------|----|----------------|---|-----------|
| Hydroxylysingomycine C14:1 [μmol/L] | 6.08   | 6.53   | 6.74 | 1.91 | 7.52   | 6.8    | 7.34 | 1.97 | -1.44 | X | 76 | -1.225 | 0.233 | 0.031 |
| Hydroxylysingomycine C16:1 [μmol/L] | 3.44   | 3.34   | 3.7  | 0.95 | 4.52   | 4.09   | 4.31 | 1.20 | -1.08 | X | 65 | -1.69  | 0.096 | 0.018 |
| Hydroxylysingomycine C22:1 [μmol/L] | 7.25   | 8.01   | 7.88 | 2.07 | 8.72   | 6.72   | 8.65 | 1.86 | 1.47 | X | 72 | -1.395 | 0.173 | 0.025 |
| Hydroxylysingomycine C22:2 [μmol/L] | 7.13   | 7.45   | 7.47 | 1.82 | 7.93   | 7.24   | 8.04 | 1.98 | 0.80 | X | 76 | -1.225 | 0.233 | 0.043 |

(Continued)
Table 1. (Continued)

| Analyte                     | Patients without Depression (n = 21) | Patients with Depression (n = 10) | Diff. of Median values | Semi-quantitative | Mann-Whitney-U Test | Benjamini Hochberg Critical Value |
|-----------------------------|-------------------------------------|----------------------------------|------------------------|-------------------|---------------------|----------------------------------|
|                             | Median | Range | Mean | SD | Median | Range | Mean | SD | U | p (exact) |                                |
| Hydroxylysingomyeline C24:1 | 0.65   | 0.82  | 0.66 | 0.18 | 0.68   | 0.47  | 0.73 | 0.14 | 0.03 | X           | 75 | -1.268 | 0.217 | 0.036 |
| Sphingomyeline C16:0 [μmol/L] | 121.95 | 78.9  | 123.69 | 18.26 | 137.69 | 96.65 | 131.77 | 27.79 | 15.74 | X           | 74 | -1.31  | 0.201 | 0.032 |
| Sphingomyeline C16:1 [μmol/L] | 16.45  | 11.13 | 16.5 | 2.81 | 17.26 | 13.97 | 17.71 | 4.41 | 0.81 | X           | 83 | -0.93  | 0.37  | 0.092 |
| Sphingomyeline C18:0 [μmol/L] | 20.96  | 20.59 | 22.12 | 4.87 | 25.21 | 24.38 | 25.25 | 7.21 | 4.25 | X           | 61 | -1.859 | 0.065 | 0.013 |
| Sphingomyeline C18:1 [μmol/L] | 8.65   | 9.37  | 8.82 | 1.97 | 9.92   | 11.39 | 10.36 | 3.5  | 1.27 | X           | 70 | -1.479 | 0.147 | 0.023 |
| Sphingomyeline C20:2 [μmol/L] | 0.37   | 0.38  | 0.36 | 0.10 | 0.35   | 0.36  | 0.38 | 0.1  | -0.02 | X           | 98 | -0.296 | 0.787 | 0.203 |
| Sphingomyeline C24:0 [μmol/L] | 14.24  | 11.72 | 15.02 | 3.02 | 16.39 | 10.93 | 15.5 | 3.19 | 2.15 | X           | 94 | -0.465 | 0.663 | 0.165 |
| Sphingomyeline C24:1 [μmol/L] | 38.12  | 25.91 | 37.07 | 5.72 | 38.13 | 23.78 | 37.2 | 7.34 | 0.01 | X           | 93 | -0.507 | 0.633 | 0.155 |
| Sphingomyeline C26:1 [μmol/L] | 0.2    | 0.39  | 0.21 | 0.1 | 0.19   | 0.33  | 0.22 | 0.09 | -0.01 | X           | 104 | -0.042 | 0.983 | 0.225 |
| Sugars                      | Hexose | 4400.9 | 2320.2 | 4589.83 | 662.92 | 4394.96 | 2757.6 | 4635.0 | 868.07 | -6.00 | 101 | -0.169 | 0.884 | 0.218 |

1 BDI: Beck Depression Inventory
2 HAMD-17: Hamilton Depression Scale-17
3 Difference of Median values: Median of patients with depression — Median of patients without depression
4 Benjamini Hochberg critical value for multiple comparisons: (i/m)xQ; i = the individual p-value’s rank, m = total number of tests, Q = false discovery rate; 0.25
5 Range is the difference between the largest and the smallest value

● Analytes with levels between the lower limit of quantification (LOQ) and the limit of detection (LOD)

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In addition to the metabolomics analysis we also measured alkaline phosphatase, gamma-glutamyltransferase, aspartate transaminase (ASAT), alanine transaminase (ALAT), cholinesterase and bilirubin with routine methods on a Roche COBAS C411 automated analyser (Roche Diagnostics, Mannheim, Germany). Thyroid-stimulating hormone, free thyroxine and free triiodothyronine concentrations have been analyzed by competitive immunoassay and direct chemiluminescence technology on an ADVIA Centaur XP automated analyser (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.).

**Statistical analyses**

Descriptive statistics are presented as mean ± standard deviation (SD), median, difference of medians and range. Group differences for categorical variables (gender, way of transmission, type of hepatitis C infection, type of interferon) were assessed using the Fisher’s exact test. Continuous variables (results of the depression questionnaires, laboratory values) were analysed by the Mann-Whitney-U test. Adjustment of p-values for multiple testing was performed where indicated using the Benjamini–Hochberg procedure, with a false discovery rate of 0.25 for screening experiments [18, 19]. All statistical analyses were performed with SPSS 22.0 for Windows (IBM-SPSS Statistics).

**Results**

**Sociodemographic, clinical and treatment characteristics**

The 31 participants of the study included 14 (45.2%) females and 17 (54.8%) males with a mean age of 43.5 (±14.4) years and 46.1 (±12.6) years respectively. Table 2 summarizes the sociodemographic, clinical and treatment characteristics such as the subtype of HCV infection, the presumed means of transmission and the type of interferon.

**IFN-α induced depression**

Although the total scores of the depression scale HAMD-17 were elevated marginally in three patients before IFN-α therapy, they indicated only minor degrees of depressed mood in these patients before the start of treatment. One month after the initial IFN-α treatment the psychiatric assessments, based on a clinical psychiatric interview and supported by the HAMD-17 and BDI scales, identified 10 (32.3%) patients with treatment-related depression. These patients had a mean baseline HAMD-17 score of 6.1 (±5.3), which increased to 15.0 (±3.6) one month after the initial IFN-α treatment (Wilcoxon Test: -2.805, p < 0.005). Non-depressive patients had a baseline HAMD-17 score of 2.9 (±3.1), which remained at 3.9 (±2.3) one month after the initial IFN-α treatment (Wilcoxon Test: -3.135, p < 0.002).

Table 2 summarizes the sociodemographic, clinical and treatment characteristics of the depressive and non-depressive patients. Table 1 provides the HAMD-17 and BDI scores.

**Laboratory analyses**

Metabolomic analysis identified the amino acid isoleucine as the only compound that differed significantly between depressive and non-depressive patients (Table 1). Depressive patients had a median isoleucine concentration of 51.35 (43.4–60.2) μmol/L that was significantly lower than in non-depressive subjects 62.1 (38.4–81.7) μmol/L (U = -2.958; p = 0.002). All other amino acids, acylcarnitines, biogenic amines, glycerophospholipids, sphingolipids, sugars, liver enzymes and thyroid levels did not differ between the two groups.
Discussion

Metabolomic assessment of the biochemical signature of IFN-α treated HCV patients identified a decline of the branched chain amino acid isoleucine as a potential surrogate marker for immune-related depression. The absence of significant effects for any of the other molecules that were measured in parallel may highlight the particular role of isoleucine in the pathogenesis of immune-related depression.

The finding of decreased isoleucine levels in patients with immune-related depression is consistent with the results of a previous study by our group showing a significant reduction of the total sum of tryptophan-competing amino acids after the onset of IFN-α induced depression [5]. In addition, Capuron et al. [20] reported a significant decline of large neutral amino acids during the first four weeks of pegIFN-α therapy. Another comparison of somatically healthy depressive patients and mentally healthy controls showed significantly lower concentrations of isoleucine in depressed subjects [21]. Additional support for an involvement of

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Table 2. Sociodemographic-, hepatitis C-, and treatment characteristics.

|                                | All patients (n = 31) | Patients with Depression (n = 10) | Patients without Depression (n = 21) | p    |
|--------------------------------|-----------------------|----------------------------------|-----------------------------------|------|
| **Sociodemographic characteristics** |                       |                                  |                                   |      |
| **Gender**                     |                       |                                  |                                   |      |
| Male                           | 17 (54.8%)            | 6 (60.0%)                        | 13 (61.9%)                        | p exact = 0.441 ^1 |
| Female                         | 14 (45.2%)            | 4 (40.0%)                        | 8 (38.1%)                         |      |
| **Age**                        |                       |                                  |                                   |      |
| Mean (years)                   | 44.9                  | 47.05                            | 43.9                              |      |
| SD                             | ±13.3                 | ±14.7                            | ±12.7                             |      |
| Median                         | 46.0                  | 52.0                             | 46.0                              |      |
| Range ^2                       | 46.0                  | 43.0                             | 45.0                              |      |
| **Hepatitis C infection characters** |                       |                                  |                                   |      |
| **Way of transmission**        |                       |                                  |                                   |      |
| Unknown                        | 13 (41.9%)            | 2 (20.0%)                        | 11 (52.4%)                        | p exact = 0.271 ^1 |
| History of transfusion         | 13 (41.9%)            | 6 (60.0%)                        | 7 (33.3%)                         |      |
| Intravenous drug abuse         | 5 (16.1%)             | 2 (20.0%)                        | 3 (14.3%)                         |      |
| **Type of hepatitis C infection** |                       |                                  |                                   |      |
| HCV Type 1                     | 8 (25.8%)             | 1 (10.0%)                        | 7 (33.3%)                         | p exact = 0.204 ^1 |
| HCV Type 1a                    | 7 (22.6%)             | 4 (40.0%)                        | 3 (14.3%)                         |      |
| HCV Type 1b                    | 12 (38.7%)            | 3 (30.0%)                        | 9 (42.9%)                         |      |
| HCV Type 3                     | 3 (9.7%)              | 1 (10.0%)                        | 2 (9.5%)                          |      |
| HCV Type 4                     | 1 (3.2%)              | 1 (10.0%)                        | 0 (0%)                            |      |
| **Treatment characteristics**  |                       |                                  |                                   |      |
| **Type of interferon**         |                       |                                  |                                   |      |
| Peg Interferon α-2a            | 23 (74.2%)            | 8 (80.0%)                        | 15 (71.4%)                        | p exact = 1.00 ^1 |
| Peg Interferon α-2b            | 8 (25.8%)             | 2 (20.0%)                        | 6 (28.6%)                         |      |

SD = Standard deviation
^1 Fisher’s exact test
^2 Range is the difference between the largest and the smallest value

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isoleucine in depression comes from animal studies. Treatment of mice with paroxetine, a selective serotonin reuptake inhibitor (SSRI), increases the blood concentration of isoleucine, valine and leucine by 50–70% [22]. However, not all previous studies found lower isoleucine concentrations in depressed patients. Compared to 22 non-depressive controls, Woo et al. [23] reported comparable isoleucine concentrations in 68 depressed individuals before and six weeks after commencement of SSRI pharmacotherapy.

There are several pathways through which isoleucine could drive immune-related depression. Aquilani et al. [24] suggested that the oxidative degradation of BCAAs causes alterations of Krebs cycle intermediates, which may impact neurotransmitter synthesis. Lower concentrations of isoleucine may also dysregulate the mammalian target of rapamycin (mTOR) pathway facilitating the occurrence of immune-related depressive episodes. BCAAs, especially leucine, are well-known activators of mTOR [25]. Previous studies have shown that the mTOR pathway is activated in peripheral blood cells of depressed patients after acute sub-anaesthetic administration of ketamine, a NMDA receptor antagonist with well-known anti-depressive properties [26]. Diaz Granados et al. [27] reported that ketamine might even decrease suicidal ideation in treatment-resistant patients with major depression. In contrast, the inhibition of mTOR by rapamycin reverses the antidepressant effects of ketamine [28]. These findings suggest that an activation of mTOR improves depressive symptoms in patients with major depression. In view of these results we hypothesize that lower concentrations of isoleucine might be indicators of lower mTOR activation in depressive HCV patients, resulting in depressive symptomatology.

Lastly, isoleucine might also have an impact on the kynurenine pathway in patients with immune-related depression. A low isoleucine concentration might result in a greater kynurenine uptake from the blood stream into the astrocytes. The uptake of kynurenine by astrocytes occurs via large neutral amino acids transporters (LATs), which also transport BCAAs (e.g. isoleucine) and aromatic amino acids. Therefore it can be speculated that lower isoleucine concentrations result in less competition for LATs facilitating the uptake of kynurenine into the astrocytes and the production of kynurenic acid. Nanomolar increases of kynurenic acid might contribute to cognitive dysfunction, a common feature of depression [29, 30].

As described above, low levels of isoleucine might aggravate depression; however, it needs to be mentioned that high concentrations of isoleucine might have the opposite effect because the serotonin precursor tryptophan as well as isoleucine make use of the same transport system to cross the blood-brain barrier, and competition for the carrier protein might be the result [31]. The clinical impact of this competitive effect of isoleucine is controversially discussed [9], and further studies might address the best isoleucine balance.

**Limitations**

In screening experiments with small sample sizes the risk of Type I errors might be increased. There are also analytical aspects that have to be considered when interpreting the result of this project. For several analytes the sensitivity of the metabolomics method was insufficient to generate quantitative results. Therefore, we may have missed relevant differences for analytes that are present at low concentrations. Furthermore, the high cost of the Biocrates Absolute IDQ p180 kit made a structured assessment of its analytical performance not feasible. Data on imprecision, LoD and LoQ is only available from the pack insert provided by the manufacturer. Therefore it would be desirable to confirm our results with compound specific targeted methods that harbour an optimized analytical performance.

While previous studies have found great support for the inflammatory hypothesis of depression in depressive states with preceding immune activation, the impact of the inflammatory...
hypothesis of depression might be less important in somatically healthy depressive patients without such a previous immune activation [32].

**Conclusions**

The activation of the Kynurenine-Quinolinic acid cascade is the well-established basis of the inflammatory hypothesis of depression. The results of this study now demonstrate that inflammatory processes might have further important consequences such as an isoleucine reduction. Thus, the results of this metabolomic pilot study identified isoleucine as a potential biomarker that is reduced in patients with immune-modulated major depression. This result should be confirmed in a prospective study that uses a targeted assay for BCAAs. Experimental studies should investigate if isoleucine plays a mechanistic role in the pathogenesis of major depression.

**Supporting information**

S1 Dataset.
(XLS)

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