Protocols

Sample Collection and Grouping

All the convalescent patients (both CA and CO groups) were discharged from the hospital after two consecutive negative results from throat swab tests for SARS-CoV-2. All patients were followed up for months after discharging and their plasma samples were collected 60 -100 days after the first onset of the disease symptoms onset. All blood samples were collected with potassium-EDTA blood collection tubes after overnight fasting. We classified the convalescent patients into the CA and CO groups according to the results of the ELISA test of anti-SARS-CoV-2 IgG following the manufacturer's instructions. Briefly, 100 μL of diluted plasma samples (1:100 to 1: 800 dilution) was added to pre-coated plates, and the plates were then incubated at 37°C for 1 h. After washing, 100 μL of horseradish peroxidase (HRP)-conjugated RBD protein of SARS-CoV-2 was added into each well, followed by 30 more min of incubation at 37°C. After washing, the OD value at 450 nm (A450) was determined. The cutoff for negative was calculated by summing 0.090 and the average A450 of negative-control. A sample was determined negative when its A450 was below this cutoff value. The remained plasma samples were then stored at -80°C.

Absolutely Quantitative Metabolomics of Plasma Samples

Metabolomics of plasma samples was performed and quantified according to the previously described method with modification. In brief, the standard solutions (5.0 mg/mL) were made by dissolving the accurately weighed chemicals in the appropriate solutions including water, methanol, sodium hydroxide solution, or hydrochloric acid solution. Then the stock calibration solutions were mixed from the appropriate amounts of individual stock solutions following the instruction of the manufacturer. After thawing at 4°C, 20-μL aliquots of the samples were added to a 96-well plate. Also were added to the plate the calibration solutions of eight various concentrations, quality control (QC) samples (equally mixed samples), as well as solvent blank. Then 120 μL of the standard solution was added to each well. The microporous plate was covered with aluminum foil, placed on a constant-temperature mixer, and vibrated at 10℃, 650rpm for 20min. After

centrifugation at 4000g for 20 min, 30μl supernatant from each well was transferred to a new 96-well plate. The derivative reagents of the Q300 Kit were added to all wells and the plate was covered and incubated at 1200 rpm at 30℃ to carry out derivatization for 60 min. After derivatization, 330 μL of precooled 50% methanol solution was added to each well and mixed with the samples at 1200 rpm at 10℃ for 5 minutes. Then the plate was centrifuged at 4000g, 4°C for 30 minutes. Finally, the supernatant was further transferred to a new 96-well plate and put into the automatic sampler of UPLC-MS analysis.

The Shim-pack UFLC SHIMADZU CBM A ultrahigh-performance liquid chromatography (UHPLC) system (SHIMADZU, Japan) coupled with QTRAP 6500+ triple quadrupole mass spectrometer (Sciex, Washington, USA) was used to analyze the metabolomics. ACQUITY UPLC BEH C18 1.7 μm VanGuard pre-column (2.1mm × 5 mm) and ACQUITY UPLC BEH C18 1.7 μm analytical column (2.1 × 100 mm) (Waters Corporation, Milford, MA, USA) were applied to this system. The mobile phases A and B were 0.1% formic acid solution in water and acetonitrile-IPA mixture (70:30, v/v), respectively. A 5-μL injection of each sample was maintained at 40 ˚C and the flow rate was 0.40 mL/min. The mobile phase gradient was: 0-1 min (5% B), 1-11min (5-78% B), 11-13.5 min (78-95% B), 13.5-14 min (95-100% B), 14-16 min (100% B), 16-16.1 min (100-5% B), and 16.1-18 min (5% B). The mass spectrometer was operated in both positive and negative modes, with capillary voltages of 1.5 and 2.0 kV, respectively. The source and desolvation temperatures were 150°C and 550 °C, respectively. The flow rate of desolvation gas was 1000 L/Hr.

The raw MS data were processed using a website-based platform named Targeted Metabolome Batch Quantification (TMBQ, Metabo-profile, Shanghai, China). Peaks A calibration curve was plotted for each standard based on the results from calibration solutions of various concentrations. The metabolite concentration in each sample was calculated from the equation $y = ax + b$ fitted to the calibration curve. The correctness of all calibration curves and integrated peak areas corresponding to each compound was checked manually.