Unveiling the Metabolic and Coagulation Disruptions in SARS-CoV-2-Associated Acute Macular Neuroretinopathy: A Case-control Study

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April 18, 2024

Abstract

Background: SARS-CoV-2 infection has been associated with the increased incidence of acute macular neuroretinopathy (AMN), an infrequent ocular disorder. However, the precise mechanisms underpinning AMN in the context of SARS-CoV-2 infection (AMN-SARS-CoV-2) remain elusive. Methods: In this case-control study 14 patients diagnosed with AMN-SARS-CoV-2 between 2022/12 and 2023/3 were enrolled in this study. 14 SARS-CoV-2-infected individuals without AMN (SARS-CoV-2-no AMN) as control. 14 AMN-SARS-CoV-2 patients were compared with 14 SARS-CoV-2-no AMN. Metabolomic profiling using Ultra-High-Performance Liquid Chromatography-Online Electrospray Mass Spectrometry (UHPLC-OE-MS) revealed significant alterations in serum metabolites in AMN-SARS-CoV-2 patients. Abnormal blood clotting was observed in AMN-SARS-CoV-2 patients, and its relationship with metabolic disorders was studied. Finally, a predictive model for AMN-SARS-CoV-2 was established. Results: 76 upregulated and 42 downregulated metabolites were discovered in AMN-SARS-CoV-2. Notably, arginine metabolism within the urea cycle showed substantial changes, evidenced by variations in ornithine, citrulline, L-proline, and ADAM levels, correlating with abnormal coagulation markers like platelet crit (PCT), fibrinogen degradation products (FDP), and fibrinogen (Fbg). Additionally, increased arginase 1 (AGR1) activity within the urea cycle and reduced nitric oxide synthase (NOS) activity were observed in AMN-SARS-CoV-2. Combining these urea cycle metabolites with coagulation parameters effectively distinguished AMN-SARS-CoV-2 from SARS-CoV-2-no AMN, with an area under the curve (AUC) value of 0.96. Conclusion: The findings of the present study enhance our comprehension of the underlying metabolic mechanisms associated with AMN-SARS-CoV-2 and offer potential diagnostic markers for this uncommon ocular disorder within the context of SARS-CoV-2 infection.

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ABSTRACT

Background: SARS-CoV-2 infection has been associated with the increased incidence of acute macular neuroretinopathy (AMN), an infrequent ocular disorder. However, the precise mechanisms underpinning AMN in the context of SARS-CoV-2 infection (AMN-SARS-CoV-2) remain elusive.

Methods: In this case-control study 14 patients diagnosed with AMN-SARS-CoV-2 between 2022/12 and 2023/3 were enrolled in this study. 14 SARS-CoV-2-infected individuals without AMN (SARS-CoV-2-no AMN) as control. 14 AMN-SARS-CoV-2 patients were compared with 14 SARS-CoV-2-no AMN. Metabolomic profiling using Ultra-High-Performance Liquid Chromatography-Online Electrospray Mass Spectrometry (UHPLC-OE-MS) revealed significant alterations in serum metabolites in AMN-SARS-CoV-2 patients. Abnormal blood clotting was observed in AMN-SARS-CoV-2 patients, and its relationship with metabolic disorders was studied. Finally, a predictive model for AMN-SARS-CoV-2 was established.

Results: 76 upregulated and 42 downregulated metabolites were discovered in AMN-SARS-CoV-2. Notably, arginine metabolism within the urea cycle showed substantial changes, evidenced by variations in ornithine, citrulline, L-proline, and ADAM levels, correlating with abnormal coagulation markers like platelet crit (PCT), fibrinogen degradation products (FDP), and fibrinogen (Fbg). Additionally, increased arginase 1 (AGR1) activity within the urea cycle and reduced nitric oxide synthase (NOS) activity were observed in AMN-SARS-CoV-2. Combining these urea cycle metabolites with coagulation parameters effectively distinguished AMN-SARS-CoV-2 from SARS-CoV-2-no AMN, with an area under the curve (AUC) value of 0.96.

Conclusion: The findings of the present study enhance our comprehension of the underlying metabolic mechanisms associated with AMN-SARS-CoV-2 and offer potential diagnostic markers for this uncommon ocular disorder within the context of SARS-CoV-2 infection.

Keywords: SARS-CoV-2, Acute macular neuroretinopathy, Serum metabolome, Coagulation disruptions

Introduction

Over the past few years, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed a significant threat to global health systems, with over 700 million confirmed cases and a cumulative death toll exceeding 6 million. Patients afflicted with the coronavirus disease (COVID-19), resulting from SARS-CoV-2 infection, typically present a range of systemic symptoms, including fever, cough, fatigue, dyspnea, myalgia, headaches, diarrhea, anosmia, and ageusia[1]. It is now understood that COVID-19 predisposes to a prothrombotic state. In this regard, the virus directly impacts vascular endothelial cells, conducive to endothelial impairment and initiating a sequence of events activating coagulation pathways and platelets, thus fostering the formation of blood clots[2]. Meanwhile, the virus-induced inflammatory response contributes to a prothrombotic state[3]. Accordingly, COVID-19 has been linked to various thromboembolic complications[4], affecting diverse organ systems, including the lungs, heart, kidneys, and brain.

While ocular symptoms associated with SARS-CoV-2 infection are infrequent, the external eye is typically affected, with follicular or pseudomembranous conjunctivitis, keratoconjunctivitis, and episcleritis being most prevalent[5, 6]. It has been established that the retina, characterized by extensive vasculature, is susceptible to thromboembolic disorders. While less frequent, reports have emerged of posterior segment ocular manifestations among COVID-19 patients, primarily in the form of retinal vascular occlusion [7]. Acute macular neuroretinopathy (AMN), an infrequent condition initially documented by Bos and Deutman in 1975, has been associated with viral infections, intravenous injections, and preeclampsia[8]. Its characteristic features encompass fundus images that typically display dark red petaloid lesions surrounding the fovea, often accompanied by progressive, variable degrees of visual impairment and central visual field defects[9]. Ischemia of the deep capillary plexus (DCP) is reportedly the predominant cause of this condition[10]. An increasing body of evidence suggests that the emergence of the novel coronavirus has been paralleled by a surge in cases of AMN[11, 12]. Over the years, AMN has been primarily documented through case reports, emphasizing the analysis of optical coherence tomography (OCT) characteristics[13, 14].

Metabolism is a fundamental characteristic and essential life process, with metabolic dysregulation playing a pivotal role in the context of COVID-19. In recent years, metabolomics has attracted significant interest in mechanistic research and diagnostics pertaining to COVID-19 and related disorders[15, 16]. Indeed, multiple research teams have investigated the metabolite profiles associated with SARS-CoV-2 infections, revealing alterations in metabolites or metabolic pathways[17], including free fatty acids, kynurenine, sphingolipids, glucose, amino acids, tricarboxylic acid (TCA) cycle, and urea cycle. These alterations have been posited to contribute to changes in organ function and immune responses. However, no studies have hitherto examined the metabolomics of AMN-SARS-CoV-2 interactions. Our study addresses this gap by examining serum samples from 14 patients afflicted by this rare condition. Importantly, we sought to unravel the serum metabolomic profile of AMN-SARS-CoV-2, explore potential correlations between these metabolic shifts and coagulation parameters, identify potential biomarkers, and construct predictive models capable of differentiating AMN-SARS-CoV-2 from SARS-CoV-2 infection in the absence of AMN (SARS-CoV-2-no AMN). Indeed, by unraveling the metabolic alterations associated with AMN within the context of SARS-CoV-2 infection, this study has the potential to provide invaluable insights into the underlying mechanisms governing AMN development.

Materials and methods

Sample:

This cross-sectional study documented novel clinical and metabolomics findings in patients diagnosed with AMN associated with SARS-CoV-2 infection (AMN-SARS-CoV-2). The study was conducted at the Ophthalmology Department of the Second Affiliated Hospital of Chongqing Medical University between November 2022 and March 2023. The study was approved by the Institutional Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University and adhered to relevant guidelines and regulations. Informed written consent was obtained from all patients.

Diagnosis of AMN-SARS-CoV-2 was based on the following clinical criteria: 1) Positive SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) test; 2) acute onset paracentral or central scotoma history; 3) parafoveal grey dark wedge or teardrop-shaped lesions on near-infrared reflectance (NIR) imaging; 4) near-infrared parafoveal hypo-reflective lesions, hyper-reflectivity of the outer plexiform layer (OPL), and attenuation of ellipsoid and interdigitation zones on spectrum domain optical coherence tomography (SD-OCT) B-scans. The exclusion criteria included any other retinal diseases such as age-related macular degeneration or diabetic retinopathy. A total of 16 AMN-SARS-CoV-2 cases were identified, with 14 eventually enrolled. Control group samples were collected from age- and sex-matched SARS-CoV-2-positive patients without AMN (SARS-CoV-2-no AMN). All patients underwent comprehensive ophthalmological examinations, including best corrected visual acuity (BCVA), intraocular pressure, NIR, SD-OCT, OCT angiography (OCTA), and fundus photography. Peripheral blood was also collected from each individual for subsequent experiments.

UHPLC-OE-MS untargeted metabolomics:

Blood samples collected in serum separator tubes were subjected to centrifugation at 4°C and 1500×g for 10 minutes. The resulting serum was aliquoted and stored at -80°C until analysis. A 100 μL sample was combined with 400 μL of an extraction solution (MeOH: ACN = 1:1, including an isotopically-labelled IS mixture) in an EP tube. The mixture underwent vortexing for 30 seconds, followed by sonication for 10 minutes in an ice-water bath. Subsequently, it was incubated at -40°C for 1 hour to facilitate protein precipitation (PPT). After centrifugation at 12000 rpm (RCF = 13800 xg, R = 8.6 cm) for 15 minutes at 4°C, the resulting supernatant was transferred to a fresh glass vial for analysis. Additionally, a quality control (QC) sample was created by combining equal volumes of supernatants from all samples.

A UHPLC system (Vanquish, Thermo Fisher Scientific) coupled with an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo) and a Waters BEH Amide column (2.1 mm × 50 mm, 1.7 μm) was used for LC-MS/MS analyses. The mobile phase comprised a water-based solution (pH = 9.75) containing NH4OAc and NH4OH (A), as well as acetonitrile (B). The autosampler temperature was maintained at 4°C, and 2 μL injections were introduced. The mass spectrometer operated with Xcalibur software in information-dependent acquisition (IDA) mode. ESI source conditions included sheath gas flow rate = 50 Arb, auxiliary gas flow rate = 15 Arb, capillary temperature = 320°C, full MS resolution = 60000, MS/MS resolution = 30000, collision energy = 20/30/40 in NCE mode, and spray voltage at 3 kV (positive) or -3 kV (negative). The raw data obtained from the experiments were converted into mzXML format using ProteoWizard. Subsequently, an in-house program developed in R, incorporating XCMS, was utilized for peak detection, extraction, alignment, and integration processes. Metabolite annotation utilized the MS2 database (BiotreeDB), KEGG, and HMDB, with an annotation threshold set at 0.3.

Clinical laboratory tests:

Blood samples from the patients were used for routine laboratory tests such as the leukocyte count (WBC), red cell count (RBC), haemoglobin (HGB), platelet count (PLT), plateletcrit (PCT), C-reactive protein concentration (CRP), Alanine aminotransferase (AAT), alanine transaminase concentration (ALT), Total bilirubin (TB), blood urea nitrogen (BUN), serum creatinine (Scr), creatinine kinase (CK), lactate dehydrogenase (LDH), prothrombin time (PT), prothrombin time international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), D-dimer, and fibrinogen level (Fbg). The cryopreserved plasma was used for additional tests such as plasma plasmin-α2-plasmin inhibitor complex (PIC), fibrin monomer complex level

(FMC), thrombin antithrombin complex level (TAT), von Willebrand factor activity (VWF:RCo), fibrinogen/fibrin degradation product (FDP), thrombomodulin level (TM), and coagulation factor VIII activity (FVIII).

RNA extraction and RT-qPCR Analysis:

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to assess the expression of the ARG1 gene and NOS1 gene in serum using specific primers. The extraction of total RNA was carried out using the Steady Pure Universal RNA Extraction Kit obtained from Accurate Biotechnology, Hunan Co., Ltd. Reverse transcription was performed using the Evo M-MLVRT Kitt kit from the same manufacturer. Subsequently, qRT-PCR was conducted on the CFX96 Real-Time System by Bio-Rad, utilizing the SYBR Green Supermix provided by Accurate Biotechnology, Hunan Co., Ltd. The $2^{-\Delta\Delta}C_t$ method was employed to calculate the relative gene expression levels, with the housekeeping gene GAPDH serving as the internal control. The designed primers are listed in Table S1.

Statistical analysis:

To compare metabolome profiles among groups, orthogonal partial least squares-discriminant analysis (OPLS-DA) was employed as a visualization tool. Emphasis was placed on identifying differential metabolites based on statistical significance, using Variable Importance in Projection (VIP) values from the OPLS-DA model and P values from the Mann Whitney U test on normalized peak areas. Specifically, metabolites with

VIP values > 1 and P values < 0.05 were considered as differential metabolites. KEGG pathway enrichment analysis of these metabolites utilized the Fisher's exact test and was compared with all identified metabolites. Correlation analysis employed Pearson's correlation coefficient analysis, utilizing the corrplot R package.

Student's t -test analyses were conducted to identify group differences. The potential predictive capability of factors for AMN-SARS-CoV-2 was evaluated using receiver operating characteristic (ROC) curves. The Spearman's rank correlation test was employed to analyze correlations between variables. For all analyses, two-tailed probabilities were used, and significance was indicated by a two-tailed P value < 0.05.

Results

Demographics and clinical ophthalmological examination

Supplementary table 2 and supplementary table 3 summarize the characteristics of the included AMN-SARS-CoV-2 cases. Fourteen AMN-SARS-CoV-2 cases were recruited, exhibiting female predominance (71.43%) and a mean age of 25.21 ± 5.75 years. The time from the onset of fever to visual acuity (VA) defects was 6.29 ± 4.41 days. Among these patients, six (42.86%) exhibited bilateral AMN, and the best-corrected visual acuity (BCVA) in the affected eye was 0.36 ± 0.26. Importantly, the intraocular pressure (IOP) was within the normal range for all patients. The AMN-SARS-CoV-2 and control groups demonstrated comparable age and body mass index (BMI), with no significant differences in age and BMI observed.

Comparison of clinical laboratory and physical findings

As shown in supplementary table 2, there were no statistically significant variations in RBC, WBC, HGB, CRP, AST, ALT, TB, BUN, Scr, CK, and LDH levels between the AMN and control groups. However, significant alterations were observed in blood coagulation function parameters within the AMN group. Parameters including PCT, TAT complex, APTT, and FDP exhibited significant increases, while Fbg demonstrated a notable decrease (supplementary Table 4). These findings collectively underscore the presence of coagulation abnormalities within the AMN group.

Untargeted metabolomic profiling of sera from AMN-SARS-CoV-2 cases

Each serum sample underwent meticulous processing and analysis using UHPLC-OE-MS for untargeted metabolomics, following a standardized protocol. This approach encompassed the examination of both hydrophilic and hydrophobic molecules via positive and negative ionization modes, thereby ensuring a comprehensive assessment of diverse endogenous biochemical classes. The analysis revealed a total of 15,585 metabolite peaks, including 7,182 metabolites in the negative ion mode and 8,403 differential metabolites in the positive ion mode. Quality control (QC) samples exhibited consistency within ± 2 standard deviations, thus validating the precision, reliability, and consistent outcomes of the experimental method employed. OPLS-DA plots visually depicted the distinct clustering of AMN-SARS-CoV-2 and SARS-CoV-2-no AMN groups in both discovery and validation cohorts (Figure 1A). Subsequent analysis led to the identification of 498 metabolites through MS/MS spectra based on the MS2 database (BiotreeDB), KEGG, and HMDB databases. Of these, 118 metabolites were significantly correlated with AMN-SARS-CoV-2 (Figure 1B; $p < 0.05$; VIP > 1). A volcano plot was generated, revealing 76 significantly upregulated metabolites and 42 downregulated metabolites in the AMN-SARS-CoV-2 group compared to the SARS-CoV-2-no AMN group (Figure 1C). Notably, several amino acids exhibited marked changes among these significant metabolites. Specifically, the AMN-SARS-CoV-2 group displayed significant upregulation of L-asparagine, L-serine, L-pyroglutamic acid, glycine, asymmetric-dimethylarginine (ADMA), ornithine, D-ornithine, L-proline, sarcosine, L-threonine, citrulline, D-serine, N-acetyl-L-alanine, N-acetyl-L-methionine, L-lysine, L-beta-aspartyl-L-threonine, prolylhydroxyproline, and N-acetylserine (Figure 1D). Conversely, leucyl-isoleucine, alanyl-leucine, serylalanine, leucyl-phenylalanine, phenylalanylphenylalanine, and phenylalanyl-glycine levels demonstrated significant downregulation (Figure 1E).

Altered main metabolites pathway and dysregulated urea cycle in AMN-SARS-CoV-2:

Pathway analysis conducted via PubChem and KEGG unveiled significantly dysregulated pathways, in-

cluding arginine biosynthesis, arginine and proline metabolism, D-arginine and D-D-ornithine metabolism, glycine, serine, and threonine metabolism, and D-amino acid metabolism (Supplementary figure 1A and 1B). The dysregulated arginine metabolism pathway was pivotal in AMN-SARS-CoV-2. Specifically, the urea cycle, pivotal in arginine metabolism (Figure 2A), demonstrated a significant role in AMN-SARS-CoV-2. Although plasma arginine levels showed no significant variance between the AMN-SARS-CoV-2 and AMN-SARS-CoV-2-no AMN groups in this study, notable increases were observed in ornithine and citrulline levels within the AMN-SARS-CoV-2 group. Both are critical intermediate metabolites in the urea cycle (Figure 2B), indicative of urea cycle dysregulation in AMN-SARS-CoV-2. Furthermore, ornithine's capacity to convert into proline, utilized for synthesizing structural proteins, particularly collagen involved in fibrosis, has been reported[18]. In this study, the AMN-SARS-CoV-2 group demonstrated significantly elevated proline levels compared to the AMN-SARS-CoV-2-no AMN group (Figure 2B). Receiver operating characteristic (ROC) analysis of ornithine, citrulline, and L-proline yielded an area under the curve (AUC) values of 0.7704, 0.7296, and 0.7704, respectively (Figure 2C).

Changes in NOS and ARG1 in AMN-SARS-CoV-2

Growing evidence suggests the roles of NOS and ARG1 in immune response modulation via arginine catabolism and the urea cycle[19, 20]. It has been shown that ARG1 activity is increased in COVID-19 patients and correlated with disease severity[21]. Consistently, our study revealed significantly increased ARG1 mRNA expression in the AMN-SARS-CoV-2 group (Figure 3A). It is well-established that elevated ARG1 competes with NOS for arginine, resulting in decreased NOS activity. Three distinct isoforms of NOS, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), regulate vascular health, with eNOS contributing to healthy blood flow by releasing nitric oxide, which effectively counteracts arterial tone[22]. Consequently, eNOS levels were significantly elevated in the AMN-SARS-CoV-2 group (Figure 3A). UHPLC-LC/MS analysis also identified the upregulation of ADMA (Figure 3B), a known endogenous NOS inhibitor. Increased ADMA could further contribute to NOS suppression. ROC analysis for ARG1, NOS, and ADMA yielded AUC values of 0.8571, 0.6837, and 0.7959, respectively (Figure 3C). Correlation analysis indicated that NOS was negatively correlated with ARG1 (r = -0.428) and ADMA (-0.469). However, NOS displayed no significant correlation with the metabolites ornithine, citrulline, and L-proline.

Correlation between differential metabolites and coagulation function testing parameters

Our findings highlighted significant changes in coagulation parameters in the AMN-SARS-CoV-2 group compared to the AMN-SARS-CoV-2-no AMN group. To further elucidate the link between differential metabolites and coagulation parameters, Pearson's correlation analysis was performed. Significant correlations were observed between differential metabolites (ornithine, citrulline, and L-proline) and coagulation parameters (PCT, Fbg, and FDP). These metabolites exhibited a significant positive correlation with PCT and FDP and a negative correlation with Fbg (Figure 4A-D).

Integration of sera metabolites and coagulation indicators yielded higher predictive performance

To assess the potential of metabolites and coagulation indicators as biomarkers for risk stratification of AMN-SARS-CoV-2, logistic regression was employed. AUC values of 0.8393, 0.7029, and 0.7653 were obtained for PCT, Fbg, and FDP, respectively (Figure 5A). Upon inclusion of the three coagulation indicators in the logistic regression model, an improved AUC value of 0.8878 was observed. The incorporation of ornithine, citrulline, L-proline, and ADMA further enhanced the AUC to 0.9082 (Figure 5B). Finally, when both the coagulation indicators and metabolites were integrated into the logistic regression model, the highest AUC (0.964) was obtained, indicating better accuracy in discriminating between AMN-SARS-CoV-2 and SARS-CoV-2-no AMN cases (Figure 5C).

Discussion

Extrapulmonary manifestations of SARS-CoV-2 infection encompass ocular presentations, with documented instances of retinal involvement. Retinal disorders associated with COVID-19 have been extensively repor-

ted[7, 23]. These ocular manifestations often include vaso-occlusive events characterized by flame-shaped retinal hemorrhages, cotton wool spots, and sectoral pallor. Although AMN is inherently rare, an elevated prevalence of AMN has been observed among individuals afflicted with SARS-CoV-2, and the underlying mechanisms driving this association remain to be elucidated[24, 25]. To our knowledge, this is the first study to offer a comprehensive overview of the metabolic profile of serum in AMN-SARS-CoV-2, thereby unveiling intricate associations between these metabolites and alterations in coagulation markers. Our findings significantly enhance the current understanding of the relationship between SARS-CoV-2 infection and AMN, providing valuable insights to guide future research and clinical applications.

COVID-19 patients frequently exhibit diverse pathological mechanisms associated with microvascular dysfunction and thrombogenesis, encompassing microvascular blood clots and thromboembolism across the pulmonary and other organ systems. Accordingly, changes in coagulation parameters are often observed, including elevated levels of circulating fibrin degradation products and von Willebrand factor, APTT, and thrombocytopenia in hospitalized and critically ill COVID-19 patients, which may hold prognostic relevance[26]. Most importantly, extensive microthrombi can be disseminated throughout organs such as the heart, kidneys, and liver in COVID-19 patients, indicative of multi-organ thrombotic microangiopathy[2, 27]. This prothrombotic state appears to contribute to the vascular complications observed in COVID-19 patients. The underlying mechanisms encompass direct viral-mediated injury to endothelial cells, excessive activation of inflammatory responses, and perturbed activation of the coagulation cascade. In parallel, AMN represents a microvascular disorder typified by microvascular impairment and inflammatory responses, culminating in intravascular clot formation, leading to microvascular occlusion and tissue ischemia[8]. This study detected significant changes in peripheral blood levels of procalcitonin, TAT complex, APTT, and FDP in AMN-SARS-CoV-2 patients compared to those without AMN-SARS-CoV-2. These changes underscore the involvement of coagulation markers in AMN pathogenesis within the context of SARS-CoV-2 infection and indicate the intricate interplay of coagulation processes in the emergence and progression of AMN amidst SARS-CoV-2 infection.

Subsequent application of the LC-MS method uncovered distinct alterations in serum metabolites of AMN-SARS-CoV-2 patients. Interestingly, dysregulations in amino acid metabolism have been documented in COVID-19 patients[15]. The current investigation consistently identified amino acid metabolism perturbations in AMN-SARS-CoV-2. Specifically, 18 amino acid-related metabolites were upregulated, while 6 were downregulated.

Arginine, a semi-essential amino acid, is a critical regulator of immune and vascular cell functions. The dysregulated metabolism of arginine in the context of COVID-19 fosters dysfunctions in immune and endothelial cells, coupled with proliferation and migration of vascular smooth muscle cells, inflammation, vasoconstriction, thrombus formation, arterial thickening, fibrosis, and heightened stiffness[28]. This multifaceted cascade can culminate in dire outcomes, including vascular occlusion, multi-organ failure, and mortality. Within this study, noteworthy modifications in the arginine metabolism pathway were observed in AMN-SARS-CoV-2 cases in comparison to those without AMN-SARS-CoV-2. This metabolic shift involved several differential metabolites, such as ornithine, citrulline, L-proline, and ADAM. Notably, ornithine and citrulline occupy crucial roles as intermediate metabolites within the urea cycle, constituting an integral facet of arginine metabolism. The conversion of arginine can lead to either citrulline and nitric oxide (NO) via NOS or ornithine and urea through the action of the enzyme ARG1[29]. Moreover, prior research has revealed the close connection between dysregulated ornithine cycling, inflammation, and coagulation, which potentially unveils a mechanism contributing to COVID-19 pathogenesis[30]. Correspondingly, the study identified a notable elevation in ornithine levels in AMN-SARS-CoV-2 patients, significantly correlating with coagulation markers such as PCT, FDP, and Fbg. Thus, ornithine potentially assumes a pivotal role within the AMN-SARS-CoV-2 pathogenic framework, especially in relation to coagulation mechanisms. This emphasizes exploring strategies to modulate ornithine metabolism, which could conceivably ameliorate patient conditions and mitigate complications.

Furthermore, it was observed that ARG1 expression is upregulated in COVID-19 patients depending on di-

sease severity, suggesting the potential involvement of the rewired arginine metabolism catalyzed by ARG1 in unfavorable outcomes for COVID-19 patients[31]. This study unveiled significant upregulation of ARG1 in AMN-SARS-CoV-2 cases, accompanied by a considerable downregulation of NOS and a robust negative correlation between ARG1 and NOS expression levels. NOS and ARG1 play a determining role in the function of vascular cells; NOS protects vessels through the basal release of nitric oxide, which effectively counteracts arterial tone, whereas ARG1's association lies with endothelial cell (EC) dysfunction and vascular maladies[32, 33]. Indeed, ARG induction has been implicated in endothelial dysfunction across diverse cardiovascular conditions, spanning systemic and pulmonary arterial hypertension, sickle cell disease, diabetes, atherosclerosis, trauma, obesity, aging, myocardial ischemia-reperfusion injury, and hemorrhagic shock. Experimental models have demonstrated that inhibiting or eliminating ARG1 can reinstate endothelial function by enhancing bioavailability[34]. Additionally, researchers have noted that ARG1 stimulates collagen synthesis in smooth muscle cells (SMCs) by channeling arginine metabolism toward proline, stimulating SMC proliferation, migration, and collagen deposition, thereby fostering vascular fibrosis and stiffness that ultimately underlie vascular occlusion[35]. In this context, the study corroborated elevated levels of both ARG1 and L-proline in the serum of AMN-SARS-CoV-2 patients, suggesting the activation of the ARG1-associated arginine metabolism and collagen synthesis pathways within the AMN-SARS-CoV-2 milieu, suggesting ARG1 represents a promising therapeutic target for AMN-SARS-CoV-2. The reduced NOS activity observed could be attributed to ARG1's upregulation, which competes with NOS for arginine, thus diverting arginine metabolism from NO synthesis. Notably, the study revealed an elevated level of ADMA, an endogenous NOS inhibitor[36], within AMN-SARS-CoV-2 cases, which may further contribute to diminished NOS activity and subsequent reduction in NO production. The cumulative impact of these mechanisms significantly influences vascular health and coagulation processes. Extensive exploration is warranted to unravel the intricate roles and interplay of these mechanisms, which is essential for a comprehensive understanding of the implications of NOS reduction across both physiological and pathological contexts.

To assess the diagnostic relevance of metabolic biomarkers and coagulation parameters within AMN-SARS-CoV-2, a ROC analysis was conducted on pivotal differential metabolites (ornithine, citrulline, L-proline, ADAM) and coagulation abnormality indicators (PCT, Fbg, FDP). The outcomes revealed the individual diagnostic significance of these markers for AMN-SARS-CoV-2, while their combination yielded the most robust diagnostic performance. However, further validation and prospective studies are warranted to establish the efficacy of these biomarkers and their combined utility in diagnosing AMN-SARS-CoV-2.

While this study reveals the potential contributions of metabolism and coagulation markers to the diagnosis and pathogenesis of AMN-SARS-CoV-2, several limitations should be acknowledged. Firstly, the survey excluded individuals not infected by SARS-CoV-2; their inclusion in future investigations could enhance our comprehension of AMN-SARS-CoV-2. Secondly, using serum samples, though fast and convenient, predominantly reflects comprehensive metabolic changes across patients rather than specific metabolic alterations within the ocular domain. Finally, the study's sample size was limited due to the rarity of AMN as an ocular condition. Although the incidence of AMN increases with SARS-CoV-2 infection, the paucity of AMN-SARS-CoV-2 cases emphasizes the potential benefits of augmenting the sample size through multicenter collaborations.

Taken together, the metabolomic insights delineated in this study offer a glimpse into the serum metabolism of AMN-SARS-CoV-2, pinpointing alterations in urea cycle dynamics and coagulation parameters, along with their interplay, as plausible pathological mechanisms underlying AMN-SARS-CoV-2, enhancing our knowledge of the mechanisms driving this condition. This advances our comprehension of the pathogenesis of AMN-SARS-CoV-2, potentially leading to improved early prognostic predictions and opportunities for therapeutic interventions.

Conflict of Interest

All authors do not have conflicts of interest.

Ethical Approval statement

The study was approved by the Institutional Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University and adhered to relevant guidelines and regulations. Informed written consent was obtained from all patients.

Acknowledgments

We thank all of the patients involved in the study and all of the frontline medical staff in the The Second Affiliated Hospital of Chongqing Medical University. The authors also would like to thank the technical support of the SHANGHAI BIOTREE BIOTECH CO., LTD.

Authors' contributions

XX and MZ designed the study and wrote the first draft of the manuscript. ZZ, CL, XW, and SL collected the clinical data and finished the metabolic experiment. ZZ, YL, QX and QC performed the statistical analyses. XX, CL and SL finished the figures and tables. MZ and QC provided data analyses, critical revision and final approval.

Funding Source

This work was supported by This work was supported by the National Natural Science Foundation of China (NSFC) (Grant No. 31871182), Chongqing Natural Science Foundation (Grant No. CSTB2023NSCQ-MSX0115), the Scientifc Research Project of Chongqing Sports Bureau (Grant No. D202124), the Science and Technology Project Afliated with the Science Department of Yuzhong District of Chongqing City (Grant No. 20210134), Kuanren Talents Program of the Second Affiliated Hospital of Chongqing Medical University (Grant No. 202228).

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