Decayed GLUT1/NHE1 RNA expression in whole blood predicts disease severity in patients with COVID-19

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Abstract

Aims We aimed to assess whether expression of whole-blood RNA of sodium proton exchanger 1 (NHE1) and glucose transporter 1 (GLUT1) is associated with COVID-19 infection and outcome in patients presenting to the emergency department with respiratory infections. Furthermore, we investigated NHE1 and GLUT1 expression in the myocardium of deceased COVID-19 patients.

Methods and results Whole-blood quantitative assessment of NHE1 and GLUT1 RNA was performed using quantitative PCR in patients with respiratory infection upon first contact in the emergency department and subsequently stratified by SARS-CoV-2 infection status. Assessment of NHE1 and GLUT1 RNA using PCR was also performed in left ventricular myocardium of deceased COVID-19 patients.

NHE1 expression is up-regulated in whole blood of patients with COVID-19 compared with other respiratory infections at first medical contact in the emergency department (control: 0.0021 ± 0.0002, COVID-19: 0.0031 ± 0.0003, P = 0.01). The ratio of GLUT1 to NHE1 is significantly decreased in the blood of COVID-19 patients who are subsequently intubated and/or die (severe disease) compared with patients with moderate disease (moderate disease: 0.497 ± 0.083 vs. severe disease: 0.294 ± 0.0336, P = 0.036). This ratio is even further decreased in the myocardium of patients who deceased from COVID-19 in comparison with the myocardium of non-infected donors.

Conclusions NHE1 and GLUT1 may be critically involved in the disease progression of SARS-CoV-2 infection. We show here that SARS-CoV-2 infection critically disturbs ion channel expression in the heart. A decreased ratio of GLUT1/NHE1 could potentially serve as a biomarker for disease severity in patients with COVID-19.

Keywords COVID-19; Infection; Heart; GLUT1; NHE1

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Introduction

In the current SARS-CoV-2 pandemic, markers of increased risk for progression to severe disease are urgently needed to allocate limited healthcare resources to patients who need them most. Furthermore, it is important to identify baseline medication that could be harmful (or beneficial) for patients with SARS-CoV-2 infection. The ion transporter sodium proton exchanger 1 (NHE1) is an important regulator of cellular pH in many tissues and is involved in the cellular response to stress, such as inflammation and infection.1,2 Additionally,
the glucose transporter 1 (GLUT1) is a critical glucose transporter in many tissues, especially the heart.3

It has previously been shown that inhibitors of sodium–glucose cotransporter 2 (SGLT2 inhibitors) may exert their beneficial effects directly on the heart via inhibition of NHE1,4,5 as SGLT2 is not present in the heart.6 Additionally, our working group has shown recently in that gliflozins increase myocardial expression of GLUT1, thus increasing glucose uptake into cardiomyocytes7 while also altering myocardial sodium homeostasis.8 Furthermore, expression and activity of NHE1 have been shown to be affected by hypoxia and glucocorticoid levels, among others, via serum and glucocorticoid-inducible kinase 1 (Sgk1).8,9 It has been shown that GLUT1 activity (but not expression) is also stimulated by Sgk1.10 Also, regulation of cellular pH is dependent on both GLUT1 expression and NHE1-expression.11 Interestingly, in the kidney, both low and high sodium levels can alter GLUT1 expression,12 and NHE1-deficient mice show strongly increased cardiac GLUT1 expression.13 As such, it is reasonable that GLUT1 and NHE1 expression could be directly linked. We aimed to assess if SARS-CoV-2 infection altered the expression profile of these important cellular transporters in the blood of patients with COVID-19. Furthermore, as COVID-19 has been shown to affect cardiac function as well, we also investigated NHE1 and GLUT1 expression in the myocardium of deceased COVID-19 patients.

We present data from our ongoing prospective clinical observational study investigating COVID-19 patients at initial presentation to the emergency department (ED). We report on whole-blood quantitative assessment of NHE1 and GLUT1 RNA using quantitative PCR (qPCR).

Methods

Patients were included in the study if they presented with signs of acute respiratory infection to the ED. Pre-specified inclusion criteria were age ≥ 18 years and signs of acute respiratory infection, possibly COVID-19. As all SARS-CoV-2-positive patients reported symptomatic illness, the term COVID-19 is used synonymously for these patients. In this study, we first included all patients with suspected COVID-19 (defined by respiratory tract infection ± reported fever). Patients were then tested for SARS-CoV-2 infection. The patients with negative SARS-CoV-2 test result, who presented themselves with viral or bacterial respiratory tract infection, were used as control group.

Pre-specified exclusion criteria were withdrawal of consent or patients unable to consent and delay of life-saving diagnostic or therapeutic procedures by inclusion into the study (which did not occur in this study cohort). Researchers involved in the qPCR analysis were blinded with respect to group allocation/SARS-CoV-2 test status. As some patients were referred to our hospital ED from general practitioners with existing COVID-19 diagnosis, nurses and physicians drawing blood and taking clinical data could not always be blinded to SARS-CoV-2 status, also for personnel safety considerations. However, all patients included into the study were treated equally with regard to diagnostic testing, timing of the testing, and safety precautions. The current study was approved by the Ethics Committee of the University of Regensburg. Informed consent was obtained from all patients.

After consent, clinical baseline characteristics and vital signs were documented for each patient. Furthermore, outcome and complications during hospital stay were assessed. Severe disease was defined as subsequent need for mechanical ventilation, admission to an intensive care unit, or death. Otherwise, patients were classified as moderate disease. An overview of inclusion procedures and patient stratification can be found in Figure 1.

For qPCR analysis, patient blood was drawn by venepuncture from each consenting patient immediately after admission to the ED and inactivated using Trifast (Ambion, Waltham, MA, USA). RNA was extracted using trichloromethane–chloroform solution and isopropanol solution. RNA was purified using the RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands) and transcribed to cDNA. Quantitative analysis of NHE1 and GLUT1 RNA was performed using the respective primers (NHE1: Hs00300047_m1, GLUT1: Hs00892681_m1, Applied Biosystems, Waltham, MA, USA) on a TaqManMan apparatus (Applied Biosystems), and expression was normalized to β-actin or GAPDH (β-actin: Hs00357333_g1, GAPDH: Hs02786624_g1, Applied Biosystems). An extended description of the methodology can be found in the Supporting Information. We chose whole-blood analysis for its easy applicability, requiring no separation stages of cells or plasma, and for safety considerations, as SARS-CoV-2 can easily be inactivated using Trifast without opening the blood tubes, thus avoiding potentially hazardous aerosol generation.

To strengthen our RNA data from whole blood of patients with COVID-19, we also obtained left ventricular cardiac tissue from patients who died of COVID-19 and analysed NHE1 and GLUT1 RNA in this cardiac tissue. Because of ethical and legal concerns, these deceased patients were not all from our ED study. However, infection had been verified by PCR from respiratory material in all of these patients, and all patients had symptomatic illness, which was determined to be the cause of death. As control tissue is extremely rare, we used a combination of left ventricular tissues from patients who had died from other respiratory infections or from patients whose hearts were destined for heart donations that ultimately could not be performed. No clinical data are available regarding the control group. Of note, there was no difference between the entities comprising the control group.

As we had better experience with using GAPDH as a housekeeper for NHE1 in cardiac tissue, we used this instead of β-actin for NHE1; however, to exclude this as confounder,
For SARS-CoV-2 testing, our facility mainly tested throat rinse water by PCR, but external test facilities also used throat swabs and sputum, which was accepted for our study if performed by a certified laboratory.

For statistical testing, normality was tested using the Kolmogorov–Smirnov test. For data with normal distribution, a Student’s t-test was performed in case of two groups without pairing. When testing multiple groups, an ANOVA was performed. For data for which normality could not be assumed, a Mann–Whitney U test was performed in case of two groups without pairing. Otherwise, a Kruskal–Wallis test was used. The respective post-tests adjusting for multiple testing are referenced in the figure legends. Categorical data were tested using Fisher’s exact test. The significance level was taken to 5% (two-sided P). Data are presented as mean ± SEM, if not otherwise indicated. No data were excluded from the analysis.

Statistics were performed using GraphPad Prism v8 (GraphPad Software, San Diego, CA, USA) and SPSS 26. Original data can be made available in a blinded manner upon reasonable request.

Results

For this study, 43 patients were included in our analysis of whole-blood RNA. In 21 of these patients (48.8%), SARS-CoV-2 infection could be confirmed. Clinical characteristics of these patients can be found in Table 1.

Patients in the study were mostly male, and the mean age was 53.6 years, which was not significantly different between patients with confirmed SARS-CoV-2 infection and control. Body mass index was increased but similar in both groups. The median time from symptom onset to presentation in the ED differed significantly between the SARS-CoV-2 positive
Table 1 Patient characteristics

| Characteristic                        | Control (n = 22) | SARS-CoV-2 (n = 21) | Statistics |
|---------------------------------------|------------------|---------------------|------------|
| **Baseline characteristics**          |                  |                     |            |
| Age (years)                           | 57.1 ± 4.1       | 49.9 ± 3.6          | P = 0.2a   |
| Sex, % male (n)                       | 59.1 (13)        | 57.1 (12)           | P > 0.9a   |
| BMI (kg/m²)                           | 26.9 ± 1.1       | 27.8 ± 1.2          | P = 0.6c   |
| Smokers (continued), % (n)            | 9.1 (2)          | 9.5 (2)             | P > 0.9b   |
| Diabetes, % (n)                       | 27.3 (6)         | 4.8 (1)             | P = 0.09b  |
| Hypertension, % (n)                   | 50 (11)          | 28.6 (6)            | P = 0.2b   |
| Coronary artery disease, % (n)        | 40.9 (9)         | 4.8 (1)             | P = 0.009b |
| Chronic kidney disease, % (n)         | 13.6 (3)         | 9.5 (2)             | P > 0.9b   |
| COPD, % (n)                           | 18.2 (4)         | 0 (0)               | P = 0.1b   |
| Asthma, % (n)                         | 13.6 (3)         | 0 (0)               | P = 0.2b   |
| **Baseline medication**               |                  |                     |            |
| Baseline medication, % (n)            | 68.2 (15)        | 61.9 (13)           | P = 0.8b   |
| ACE/AT1-inhibitors, % (n)             | 40.9 (9)         | 19.1 (4)            | P = 0.2b   |
| Beta-blockers                         | 27.3 (6)         | 19.1 (4)            | P = 0.7b   |
| Aspirin (100 mg/day), % (n)           | 36.4 (8)         | 14.3 (3)            | P = 0.2b   |
| Statins, % (n)                        | 40.9 (9)         | 4.8 (1)             | P = 0.009b |
| Metformin, % (n)                      | 13.6 (3)         | 0 (0)               | P = 0.2b   |
| DPP4-inhibitors, % (n)                | 9.1 (2)          | 0 (0)               | P = 0.5b   |
| Sulfonylurea, % (n)                   | 0 (0)            | 0 (0)               | P = 0.9b   |
| Gliflozins, % (n)                     | 0 (0)            | 0 (0)               | P > 0.9b   |
| Incretins, % (n)                      | 0 (0)            | 0 (0)               | P > 0.9b   |
| Insulin, % (n)                        | 4.6 (1)          | 4.8 (1)             | P > 0.9b   |
| Immunosuppressants, % (n)             | 13.6 (3)         | 9.5 (2)             | P > 0.9b   |
| **Symptoms**                          |                  |                     |            |
| Onset of symptoms to presentation (days), median (95% CI) | 3.5 (1; 8) | 8 (5; 13) | P = 0.01c |
| Fatigue, % (n)                        | 90.9 (20)        | 100 (22)            | P = 0.5b   |
| Fever, % (n)                          | 72.7 (16)        | 77.3 (17)           | P > 0.9b   |
| Dyspnoea, % (n)                       | 63.6 (14)        | 66.7 (14)           | P > 0.9b   |
| Coughing, % (n)                       | 68.2 (15)        | 71.4 (15)           | P > 0.9b   |
| Chills, % (n)                         | 59.1 (13)        | 61.9 (13)           | P > 0.9b   |
| **Physical markers**                  |                  |                     |            |
| Body temperature (°C)                 | 37.6 ± 0.2       | 37.7 ± 0.2          | P = 0.8c   |
| SpO₂ (%)                              | 95.3 ± 0.6       | 94.9 ± 0.8          | P = 0.7a   |
| Respiratory rate (/min)               | 20.5 ± 1.3       | 22 ± 1.5            | P = 0.4a   |
| Heart rate (b.p.m.)                   | 89.2 ± 4.5       | 95.6 ± 3.3          | P = 0.3a   |
| Systolic blood pressure (mmHg)        | 134.2 ± 5.2      | 132.9 ± 4.3         | P = 0.95c  |
| Diastolic blood pressure (mmHg)       | 83.0 ± 2.9       | 80.4 ± 2.9          | P = 0.5a   |
| NEWS-2 score                          | 3.1 ± 0.5        | 3.8 ± 0.6           | P = 0.4a   |

ACE, angiotensin-converting enzyme; AT1, angiotensin II-receptor-subtype 1; BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; NEWS-2, National Early Warning Score-2.

*P*-test.
*1* Fisher’s exact test.
*2* Mann–Whitney *U* test.

The cohort and the control group. Clinical symptoms, vital signs, or National Early Warning Score-2 (NEWS-2) scoring on admission were not significantly different between both groups. In venous blood gas testing, pH, standard base excess, standard bicarbonate, and pCO₂ did not differ between SARS-CoV-2 and control groups (Supporting Information, Table SJ). In a non-parametric correlation analysis, there was no significant association between NHE1 expression and pH in the SARS-CoV-2 or the control group.

In the SARS-CoV-2 group, one patient (4.8%), compared to six patients in the control group (27.3%), had diabetes mellitus, which was numerically different but did not reach statistical significance. Patients in the control group suffered significantly more often from coronary artery disease. Regarding drug therapy, only the usage of statins differed significantly between both groups.

NHE1 expression from whole blood was significantly elevated in patients with SARS-CoV-2 in comparison with control (*P* = 0.01; Figure 2A). There was no difference between male and female patients (*P* = not significant). RNA expression of GLUT1 was not significantly different between control group and COVID-19 patients (Figure 2B).

The ratio of GLUT1 and NHE1 whole-blood RNA expression in SARS-CoV-2-positive patients, however, was significantly reduced compared with control (Figure 2C).

In a stepwise multivariate linear regression analysis, only SARS-CoV-2 status was significantly associated with NHE1 expression (*P* = 0.015), in contrast to diabetes, gender, age, coronary artery disease, heart failure, obesity, arterial hypertension, hyperlipidaemia, smoking, and statin usage, which were non-significant covariates (each *P* = not significant).
Figure 2 Sodium proton exchanger 1 (NHE1) and glucose transporter 1 (GLUT1) expression in the blood of COVID-19 patients. (A) NHE1 expression is significantly increased in whole blood of patients with SARS-CoV-2 infection (SARS-CoV-2 positive) at first contact in the emergency department compared with control (CTRL; \( P = 0.01 \), Mann–Whitney U test). (B) GLUT1 expression is not significantly altered in whole blood of patients with SARS-CoV-2 infection compared with CTRL (\( P = 0.29 \), Mann–Whitney U test). (C) The ratio of GLUT1 and NHE1 is significantly decreased in patients with SARS-CoV-2 infection compared with CTRL (\( P = 0.0039 \), Mann–Whitney U test). (D and E) In patients with COVID-19 at first contact in the emergency department, (D) NHE1 or (E) GLUT1 expression is not significantly different in patients who later develop moderate disease (MD) compared with patients later requiring intubation and/or dying [severe disease (SD); (D): \( P = 0.99 \), t-test; (E): \( P = 0.30 \), t-test]. (F) GLUT1 to NHE1 ratio is significantly decreased in patients with SD compared with patients with MD \( (P = 0.036 \), Welsh t-test).
significantly altered in left ventricular myocardium of deceased COVID-19 patients. The ratio of GLUT1 and NHE1 in the myocardium of deceased COVID-19 patients was significantly lower, resembling the lower ratio of GLUT1 and NHE1 in the blood of COVID-19 patients in the ED, who are subsequently at risk of intubation or death. This suggests that NHE1 and GLUT1 may be critically involved in the disease progression of SARS-CoV-2 infection.

In COVID-19, increased inflammatory markers such as C-reactive protein and interleukin 6 have been reported, especially in severe COVID-19 cases. Cardiac complications, such as myocarditis, arrhythmias, acute coronary syndrome, or acute heart failure, are known complications in severe disease courses. Furthermore, elevated levels of cardiac markers, like cardiac troponin or brain natriuretic peptide, are common in these patients as surrogate of myocardial damage due to inflammation or ischaemia. A potential explanation of the increased cardiovascular morbidity in COVID-19 is the binding of SARS-CoV-2 to the host receptor angiotensin-converting enzyme 2, which is highly expressed in the human heart and vascular cells, but exact pathophysiological processes of myocardial damage are still not fully understood.

NHE is an ion transporter protein family, which plays a critical role in regulation of intracellular pH or cell volume, and NHE1 is the predominant isofrom of the NHE family in myocardial and vascular cells. Previous studies found an increased activity of NHE1 associated with hypertrophy, cardiac fibrosis, and heart failure. Therefore, NHE1 has been identified as a potential target in therapy of heart failure. Interestingly, NHE1 activity seems to be involved in inflammatory response and leucocyte function. In this study, we found elevated NHE1 expression in whole blood of patients with COVID-19 in comparison with other bacterial or viral infections. This elevated expression was also present in myocardial tissue of deceased patients with COVID-19. Of note, inflammatory parameters like interleukin 6, C-reactive protein, or white blood cell count did not differ significantly between both groups (Supporting Information, Table S3). A potential confounder due to increased inflammatory response in the SARS-CoV-2 group cannot be excluded but seems therefore unlikely. Under the aspect of increased cardiovascular complications due to COVID-19, the elevated expression of NHE1 could potentially be involved in pathophysiological pathways of cardiovascular damage due to COVID-19. Increased NHE1 expression and/or activity could also be involved in or be dependent on altered platelet function in COVID-19, as NHE1 activation contributes to platelet activation. Interestingly, platelet activation can also be modulated by SGLT2 inhibitors. In our COVID-19 cohort, the platelet count is not significantly different between the control group and the COVID-19 group (Supporting Information, Table S3). COVID-19 patients with moderate disease and severe disease show no difference in platelet counts (moderate disease: 195.2 ± 14.84 vs. severe disease: 144.6 ± 35.25, t-test P = 0.14, data expressed as mean ± SEM). Pearson correlations for platelet counts and NHE1 expression in the blood show neither a significant correlation in the control or COVID-19 group nor when looking at COVID-19 patients with moderate vs. severe disease. However, platelet count and platelet activity may of course differ, but as we used lysed whole blood for our experiments (safety for our personnel),

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we cannot determine platelet activity in our study cohort, as this would require fresh samples with high safety precautions. Nevertheless, after strong platelet activation, platelets aggregate and thus cannot be measured in the bloodstream; thus, platelet count is at least an indirect measure of platelet activity.26

Interestingly, it has been shown that NHE1-deficient mice show strongly increased cardiac GLUT1 expression.13 GLUT1 is a glucose transporter of the GLUT family and ubiquitously expressed in almost all tissues. Primary function of GLUT1 is the basal cellular glucose uptake independent from insulin. Increased GLUT1 expression was shown in animal models of myocardial ischaemia or systemic inflammatory response syndrome,27,28 suggesting a compensatory mechanism due to stress and hypoxia. While hypoxia can also increase NHE1 expression, among others, via Sgk1,8,9 activity of the kinase has also been shown to alter GLUT1 activity. Further, GLUT1 expression was correlated with left ventricular contractility in that system inflammatory response syndrome animal model. Interestingly, a reduced myocardial GLUT1 expression was found in patients with end-stage heart failure in one study.29

In our study, GLUT1 whole-blood RNA expression was not significantly different between COVID-19 patients or control, but the ratio of GLUT1 to NHE1 was lower in patients with COVID-19 in comparison with control. In patients with severe COVID-19, the ratio was even lower than in patients with moderate disease. These altered expressions on NHE1 and GLUT1 were similar in myocardial tissue of deceased patients with COVID-19. Furthermore, GLUT1 was significantly lower expressed in the myocardial tissue of patients who deceased due to COVID-19. This pattern might reflect a maladaptation to inflammatory stress and myocardial ischaemia in patients with COVID-19 and unfavourable disease progression. It may also hint at disturbed cellular ion handling and pH regulation, as both low and high sodium levels can alter GLUT1 expression,12 and regulation of cellular pH is dependent on both GLUT1 expression and NHE1 expression and vice versa.11

Further research regarding the role of these transporters is urgently warranted, especially regarding the potential effects of glioflozin in COVID-19, which have been reported to affect both NHE1 and GLUT1.4,7

Potential limitations of our study include the low number of diabetic patients in the COVID-19 group, as diabetic patients are at increased risk of mortality when contracting SARS-CoV-2. Also, we could not investigate the effects of glioflozin on GLUT1 and NHE1 in COVID-19 patients, which will need to be addressed in the future. Furthermore, an additional desirable control group for the analysis of cardiac expression of NHE1 and GLUT1 in deceased COVID-19 patients would arguably be tissue from patients who have recovered from COVID-19; however, this is of course not reasonably obtainable. In addition, these findings from a limited number of patients from only one site have to be validated in larger patient cohorts in the near future, also exploring whether reduced GLUT1/NHE1 ratio is an independent risk factor for mortality in COVID-19.

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Conflict of interest

The authors state that no conflict of interest exists.

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Author contributions

J.M., J.H., and C.J. designed the study, gathered and analysed data, performed statistical analysis, wrote the manuscript, and are responsible for the integrity of the work as a whole. F.H., K.E., M.E., and C.M. gathered data and revised the manuscript for critical intellectual content. S.W., S.S., and L.S.M. analysed data and revised the manuscript for critical intellectual content.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Venous blood gas analyses.
Table S2. Clinical data for deceased COVID-19 patients with left ventricular tissue donation.
Table S3. Laboratory parameters.
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