Lethal diquat poisoning manifests as acute central nervous system injury and circulatory failure: A retrospective cohort study of 50 cases

Jia-Ning Zhou,[a,b] *and Yuan-Qiang Lu* [a,b]*

[a]Department of Emergency Medicine, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, People's Republic of China
[b]Key Laboratory for Diagnosis and Treatment of Aging and Physic-chemical Injury Diseases of Zhejiang Province, Hangzhou 310003, Zhejiang, People's Republic of China

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Summary

Background The mortality rate of patients with diquat (DQ) poisoning is extremely high due to insufficient understanding of DQ-induced injury. This study aimed to summarize the characteristics of DQ poisoning as well as analyse the correlation between plasma DQ concentration and patient outcomes, thus providing a new strategy for diagnosis and treatment.

Methods This single-centre retrospective cohort study was conducted at the Emergency Department of the First Affiliated Hospital, Zhejiang University School of Medicine, China, between Oct 9, 2019 and March 10, 2022. 50 patients, whose plasma or urine samples tested positive for diquat and negative for paraquat by high performance liquid chromatography-tandem mass spectrometry, were included in the study.

Findings The mortality rate of acute DQ poisoning was 25 (50%) of 50. Compared with the survival group, the death group presented significantly higher initial plasma DQ concentration (Cp_1), aspartate aminotransferase, alanine aminotransferase, serum creatinine, and creatine kinase-MB ($P < 0.05$). We found that six (24.0%) patients died of central nervous system injury, six (24.0%) patients died of refractory circulatory failure, and 13 (52.0%) patients died of central nervous system injury combined with circulatory failure. Receiver operator characteristic curve analysis showed that the area under the curve of Cp_1 was 0.967 (95% CI: 0.911, 1.000), and the cut-off value was 3516.885 ng/mL (sensitivity, 90.9%; specificity, 96.0%).

Interpretation Lethal DQ poisoning is primarily associated with serious brain and vascular injury, as well as a high rate of mortality. Further research into the mechanisms of refractory circulatory failure and central nerve system damage could help reduce mortality.

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Keywords: Diquat poisoning, plasma diquat concentration; Central nervous system injury; Circulatory failure; Neuroimaging; High-performance liquid chromatography-mass spectrometry

Introduction

As people who attempt suicide via paraquat (1, 1'-dimethyl-4, 4'-bipyridinium, PQ) ingestion have an extremely high mortality rate, PQ has already been banned in China.[1,2] Its substitute, diquat (1, 1'-ethylene-2, 2'-bipyridinium, DQ), has become a widely used herbicide in agriculture.[3,4] As the ingredient lists labelled on many pesticide bottles are mostly unreliable, we have to be especially vigilant that DQ and PQ may be sold as a mixture.[5] The reported mortality rate of acute DQ poisoning in the USA was <1.0%–3.0%. Among them, occupational factors accounted for 8%-44%, while the factors of dying by suicide accounted for only 1%-9%.[6,7] Statistic has shown that the mortality rate from acute DQ poisoning in China ranged from 16.7% to 60.0%, and the route of exposure was oral.[8] Comparing the management of herbicides between the

*Correspondence author at: 79 Qingchun Road, Hangzhou 310003, People's Republic of China.
E-mail address: luyuanqiang@zju.edu.cn (Y.-Q. Lu).

Research in context

Evidence before this study

Diagnosis, characterisation, and therapeutic decision-making in patients with acute diquat (DQ) poisoning remain a challenge. We searched PubMed and Cochrane Library without date restrictions for evidence that fatal DQ poisoning manifested central nervous system (CNS) injury and circulatory failure using the search terms "diquat", "herbicide", "poisoning or intoxication", "acute central nervous system injury", "toxic encephalopathy", and "circulatory failure" in Oct 1, 2021. The retrieved literature was published from Jan 1, 1983 to Oct 1, 2021. From the retrieval results we found that in recent years, several case reports had reported cases of DQ-induced CNS injury.

Added value of this study

Previous studies on DQ-induced CNS injury were case reports. However, this study found that the incidence of CNS injury was high in patients who died of acute DQ poisoning. In addition, we also found that most patients who died of acute DQ poisoning were complicated by circulatory collapse. This study summarised the characteristics of DQ poisoning as well as analyzed the correlation between plasma DQ concentration and patient outcomes, thus providing a new strategy for diagnosis and treatment.

Implications of all the available evidence

Our findings support that lethal DQ poisoning is primarily associated with serious brain and vascular injury, as well as a high rate of mortality. Our work paves the way for future studies exploring the mechanisms of refractory circulatory failure and CNS damage, which could help reduce mortality in patients with acute DQ poisoning.

two countries, we found that the production and sale of herbicides had a strict legal system in the USA, while the Chinese government's supervision in this area was still inadequate. We would like to raise public awareness of the high mortality rate from acute DQ poisoning. For clinical diagnosis and treatment, understanding the characteristics and risk factors of DQ poisoning is essential.

Though the chemical structure of DQ is similar to that of PQ, the clinical manifestations of acute DQ poisoning significantly differ from PQ poisoning. PQ results in acute lung injury in the early stages and pulmonary fibrosis in the later stages.[9,10] Several case reports showed that acute high-dose DQ poisoning usually affected the kidneys, liver, brain, and heart.[11–14] Current toxicology studies indicate that DQ does not covalently bind to macromolecules (i.e., lipids, proteins and nucleic acids), but it can generate reactive oxygen species (ROS) in cells through an oxidation–reduction cycle process, resulting in increased phospholipid membrane permeability and subsequent cell membrane rupture.[5] Recent research has shown that lethal DQ poisoning can cause central nervous system injury, as evidenced by variable CT imaging, including cerebral ischaemia, cerebral haemorrhage, and brain swelling.[1,3,12,15] However, the specific mechanism of DQ-induced central nervous system injury is yet to be elucidated. In addition, some patients with DQ poisoning had obscure symptoms in the early stages or were using haemoperfusion, so emergency physicians could hardly be aware of circulatory failure in time.[7,16] Because of the complicated clinical symptoms and insufficient understanding of intoxication mechanisms, the overall efficacy of treatment under standard guidelines is limited. We analysed the clinical characteristics of patients with acute DQ poisoning in a retrospective and observational study of 50 patients with DQ poisoning. The objective of this research was to learn more about DQ and discover novel therapeutic treatment options.

Methods

Study design and setting

This research was a single-centre retrospective cohort study. Between Oct 9, 2019 and March 10, 2022, 124 patients with suspected DQ poisoning were subjected to quantitative analysis of DQ and PQ by high performance liquid chromatography-mass spectrometry (HPLC-MS/MS). DQ has been tested positive in the plasma or urine of 58 patients. Patients accompanied by PQ poisoning (n = 1) and patients without complete clinical records (n = 7) were excluded (eFigure 1). To detect the all-cause mortality of patients with acute DQ poisoning, with null hypothesis proportion 0.3, true proportion 0.5, type I error rate 5%, and a power of 80%, a sample size of 49 patients in this study was necessary. All enrolled patients had no history of severe brain, heart, liver, or kidney diseases. The primary endpoint of this study was in-hospital mortality. For clinical characteristics analysis, we divided patients into a survival group and a death group according to their outcome.

The secondary outcomes were the occurrence of fatal complications after admission, which included central nervous system damage and circulatory collapse as measured by the following. We employed the following diagnostic criteria for DQ-induced central nervous system injury and refractory circulatory failure in combination with the diagnostic criteria for toxic encephalopathy or circulatory failure used in other studies.[17–19] The diagnosis of DQ-induced central nervous system injury was based on: brain parenchymal lesions shown by head CT, disturbance of consciousness or cognitive impairment, and signs of brain parenchymal damage. Refractory circulatory failure is defined as the inability to maintain blood pressure at 90/60 mmHg with high

doses of vasoactive drugs or even veno-arterial extracorporeal membrane oxygenation (V-A ECMO). Then all patients were divided into four groups according to the existence of fatal complications. Group A, B, C, and D stand for patients without central nervous system injury and circulatory failure, patients with central nervous system injury, and circulatory failure, and patients who suffered these two fatal complications, respectively.

Ethics statement

This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, with approval number IIT20210873A. The study title is "Clinical Study of Acute Diquat Poisoning Injuries." A written informed consent exemption from the Institutional Review Board was obtained. The principles of the Helsinki Declaration guided the treatment protocols.

Data collection

The data of all patients were collected in the medical record system, including (a) demographic parameters such as age and gender; (b) route of exposure, estimated DQ intake volume, plasma/urinary DQ concentrations, time from DQ exposure to visiting our hospital, clinical manifestations, and treatment; (c) dynamic laboratory changes during hospitalization including white blood cell, neutrophil count, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (SCr), lactic dehydrogenase (LDH), creatine kinase-MB (CK-MB), partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), actual base excess (ABE), lactic acid (Lac), activated partial thromboplastin time (APTT), prothrombin time, and thrombin time (TT).[20]

HPLC-MS/MS detection procedures

Blood samples were placed in EDTA tubes after collection, and urine samples were placed in conventional urine cups. These samples were stored at 4°C and tested for DQ at the earliest. Quantitative analysis of DQ and PQ was performed using an HPLC system coupled to a triple quadrupole MS/MS instrument (Agilent 6494; Palo Alto, USA). 1)Patient sample/ quality control (QC) sample processing: blood was centrifuged at 4000 r/min for 8 min, and 100 μL of plasma samples/ QC sample was accurately measured and placed in 1.5 mL EP tubes. Then 50.0 μL of internal standard working solution (1000 ng/mL) and 450 μL methanol were added and vortexed for 2 min to mix. The mixture was centrifuged for 10 min at 13,000 r/min. 400 μL supernatant and 200 μL ultrapure water were drawn into the injection vial. A 5 μL sample was injected for HPLC-MS/MS analysis and the chromatograms record. The retention time of PQ and PQ-d8 was 2.11 min. The retention time of DQ and DQ-d4 was 2.59 min; 2)Chromatographic conditions: Chromatographic column: Waters XBridge BEH HILIC column (2.5 μm, 2.1 mm × 100 mm), column temperature 40°C. The mobile phase A1 was an aqueous solution containing 200 mmol/L ammonium formate and 0.1% formic acid. The mobile phase B1 was 100% pure acetonitrile. The mobile phase A2 was deionized water. Gradient injection was carried out at a flow rate of 0.4 mL/min. The initial mobile phase consists of 30% A1 and 70% B1. After running for 2 min, it was changed to 60% A1 and 40% B1. The mobile phase was maintained at this ratio for 3 min and returned to 30% A1 and 70% B1 in 1 min, then equilibrated the column to the pre-injection state for 4 min. After the sample detection was completed, the system was flushed with 60% A2 and 40% B1 for 10 min, and finally, the system was flushed with 100% B2 for 10 min to store the column in acetonitrile; 3) the PQ/DQ concentration of each sample analysing: if the analysis result had only the internal standard peak but no target peak, PQ/DQ was considered negative; if both the target peak and the internal standard peak appeared, the PQ/DQ concentration in the sample could be calculated by the ratio of peak area (eFigure 2).

Sample collection

Blood and urine samples of DQ poisoning patients were collected immediately upon admission and thereafter, on a routine basis until DQ was found to be negative in blood and urine.[21] The accuracy of DQ detection by HPLC-MS/MS ranged from 50 ng/mL to 10000 ng/mL. In the statistical analysis, we defined DQ concentrations less than 50 ng/mL but greater than 0 ng/mL as 50 ng/mL, and concentrations greater than 10000 ng/mL as 10000 ng/mL. After admission, the initial plasma DQ concentration was defined as Cp_1, the second DQ concentration as Cp_2, and the initial urinary DQ concentration was defined as Cu_1. We calculated the clearance rate of plasma DQ concentration according to the following formula: $R = 100 \times (Cp_2 - Cp_1)/Cp_1$ (%).

Treatments

The primary treatment for acute DQ poisoning in this study was to terminate DQ absorption, and the main measures included gastric lavage, absorption, and catharsis. Using a large amount of physiological saline at the earliest, and glycol for catharsis. Molten stone powder can reduce the absorption of gastrointestinal poisons. Then, by augmenting diuresis and/or haemoperfusion, DQ was eliminated.[22,23] Mannitol to prevent and reduce cerebral edema, corticosteroids to scavenge inflammatory mediators, and vitamin C as an antioxidant to scavenge oxygen free radicals were among the pharmacological therapies. When the patient's oxygen saturation dropped below 90%, a venturi mask or even

mechanical ventilation was employed to keep the blood oxygen saturation at a safe level. On the basis of fluid resuscitation, various vasoactive drugs were continuously pumped to prevent persistent hypotension. If the preceding efforts failed, we used V-A ECMO to maintain the patient's circulation and oxygenation.[9]

Statistical analysis

Continuous variables were expressed as mean ± SD or median (interquartile range) and qualitative variables as numbers and percentages. Continuous variables were compared using a t-test or Mann–Whitney U test. Fisher's stringent test compared the categorical variables. The receiver operating characteristic (ROC) curve was used to determine the power of predicting all-cause mortality by the Cp_1 and Cu_1. The Kaplan-Meier survival curves were depicted for fatal complications. The end time of observation of survival events of all patients was the time of discharge. All P values were two-tailed, and $P < 0.05$ was significant. Statistical analysis was performed using the SPSS Statistical Software (Version 26.0, SPSS Inc., IBM, USA).

Role of the funding source

There was no funding source for this study. Both authors had full access to all data in the study and accept responsibility to submit for publication.

Results

General characteristics

50 patients were selected for this study, which contained 17 men and 33 women. The average age was 30.28 ± 1.45 years old. The mortality rate of acute DQ poisoning was 50.0%. Thirty patients tested positive for DQ through plasma and urine samples, 14 patients tested positive for DQ in plasma, and 6 patients tested positive for DQ in urine samples. The median time to hospital after DQ poisoning was 15.00 (7.00, 39.00) hours in the survival group and 9.00 (6.00, 22.50) hours in the death group. The estimated ingestion volume of the death group was more than that of the survival group [100.00 (100.00, 150.00) ml vs. 20.00 (10.00, 50.00) ml, $P < 0.001$] (Table 1). Patients with acute DQ poisoning presented with burning pain in the throat (30 of 50, 60.0%), nausea and vomiting (25 of 40, 50.0%), limb swelling (5 of 50, 10.0%), anuria (3 of 50, 6.0%), and unclear consciousness (2 of 50, 4.0%). As shown in Table 1, the mean AST, ALT, SCr, $PaCO_2$ and ABE of the survival group were much higher than those of the death group, whereas Lac was significantly lower than the death group (all $P < 0.01$). The PO_2, BUN, and PLT in the survival group and death group at admission were in the normal range, and there was no statistical significance (all $P > 0.05$). However, some parameters showed great variation among individuals, such as AST, ALT, BUN, and SCr, which are involved in, respectively, liver and kidney function.

DQ concentration

As shown in the Figure 1a, the average Cp_1 of the death group was significantly higher than that of the survival group ($P < 0.001$). Cu_1 of the two groups could not be compared because the urine samples were not completely acquired. Correlation analysis revealed a negative correlation between the plasma DQ concentration and the test time ($P < 0.001$) (Figure 1b).

All patients with acute DQ poisoning were treated with haemoperfusion after admission to our hospital, and we observed a rapid decline in plasma DQ concentrations after admission. Given that part of the values for Cp_1 and Cp_2 were above the accurate range, we only counted the clearance rate between Cp_1 and Cp_2 in 27 patients. We found that the change in concentration per unit of time was positively related to the initial concentration ($r = 0.951$, $P < 0.001$) (Figure 1c).

Dynamic monitoring of laboratory indexes during hospitalization

According to statistics, most of the patients died in the acute phase, which was from the first to the fifth day after DQ poisoning. Therefore, we did a dynamic analysis of the laboratory indicators from day 1 to day 5. As shown in Figure 2, the liver enzymes of the survival group were within the normal range. The ALT of the death group was significantly higher than that of the survival group from day 1 to day 4 (all $P < 0.001$), which peaked on day 2 after poisoning [580.00 (280.50,862.00) U/L]. The CK-MB of the death group was significantly higher than that of the survival group, which reached a peak on day 2 [139.50 (61.50, 258.25) U/L, $P < 0.001$]. The SCr of the death group was significantly higher than that of the survival group ($P < 0.05$). The median SCr was 252.50 (197.25, 325.75) μmol/L, which peaked on day 5 after poisoning. The median Lac of the death group was 186.00 (115.25, 275.00) mmol/L, which peaked on day 1 after poisoning. The APTT and TT of the death group were significantly prolonged after DQ poisoning. There was no significant difference in PCO_2 in the two groups ($P > 0.05$), while the PO_2 of the death group was lower than that of the survival group ($P < 0.01$). The Lac of the death group was significantly higher than that of the survival group ($P < 0.001$).

Fatal complications of DQ poisoning

Following DQ poisoning, 22 patients developed symptoms of central nervous system injury, including mania, seizures, somnolence, and coma. At the same time, evident indications such as bilateral pupil dilatation and loss of light reflexes were present. The mean time to the

Parameters	Survivor (n = 25)	Death (n = 25)	P value
Gender (male, %)	6 (24.0%)	11 (44.0%)	0.136
Gender (female, %)	19 (76.0%)	14 (56.0%)	0.136
Age (mean ± SD)	29.00 ± 9.69	30.92 ± 12.91	0.697
Time to the hospital (h)	15.00 (7.00, 39.00)	9.00 (6.00, 22.50)	0.144
Length of hospitalization (d)	12.00 (8.25, 18.50)	1.00 (1.00, 3.00)	<0.001***
Estimated ingestion volume (ml)	20.00 (10.00, 50.00)	100.00 (100.00, 150.00)	<0.001***
Admission			
AST (IU/L)	17.13 ± 6.22	373.83 ± 570.07	0.008**
ALT (IU/L)	18.04 ± 11.30	268.00 ± 389.92	0.004**
GGT (IU/L)	18.83 ± 14.02	22.94 ± 15.94	0.452
TBil (μmol/L)	11.21 ± 6.66	15.23 ± 6.77	0.017*
DBil (μmol/L)	4.32 ± 2.26	6.17 ± 3.15	0.009**
Alb (g/L)	44.44 ± 5.95	44.34 ± 7.93	0.575
SCr (μmol/L)	69.42 ± 29.31	199.56 ± 229.84	0.009**
BUN (mmol/L)	5.47 ± 3.95	8.34 ± 6.63	0.080
LDH (U/L)	294.80 ± 421.48	996.40 ± 1075.94	0.005**
CK-MB (U/L)	22.00 ± 14.91	113.43 ± 187.29	0.023*
PaO_2 (mmHg)	82.03 ± 22.21	90.76 ± 29.89	0.960
$PaCO_2$ (mmHg)	36.81 ± 6.12	25.51 ± 7.38	<0.001***
HCO_3^- (mmol/L)	23.44 ± 3.66	15.22 ± 3.58	<0.001***
ABE (mmol/L)	-0.46 ± 3.64	-7.69 ± 4.68	<0.001***
Lac (mmol/L)	1.21 ± 0.48	4.98 ± 2.74	<0.001***
WBC (10E9/L)	12.73 ± 4.86	24.04 ± 7.93	<0.001***
PLT (10E9/L)	286.78 ± 93.58	267.67 ± 87.15	0.762
APTT (s)	26.55 ± 2.96	28.82 ± 5.69	0.097
PT (s)	12.36 ± 2.94	13.03 ± 2.92	0.880
TT (s)	28.40 ± 32.16	65.14 ± 59.32	<0.001***

***Table 1*: Comparisons of baseline characteristics between the survivor and death groups with acute DQ poisoning.**
DQ: diquat; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase; TBil: Total bilirubin; DBil: Direct bilirubin; Alb: Albumin; SCr: the serum creatinine concentration; BUN: Blood urea nitrogen; LDH: Lactic dehydrogenase; CK-MB: Creatine Kinase-MB; PaO_2: partial pressure of oxygen; $PaCO_2$: partial pressure of carbon dioxide; ABE: Actual base excess; Lac: lactic acid; WBC: White blood cell count; PLT: Platelet count; INR: International normalized ratio; APTT: Activated partial thromboplastin time; PT: prothrombin time; TT: thrombin time.

onset of central nervous system injury was 23.76 ± 23.28 hours after DQ poisoning. Due to the rapid progression of central nerve injury, we only recorded eight head CT images with significant abnormalities. We found that patients with DQ-induced central nervous system injury showed abnormal low-density shadows, shallow sulci, and ventriculomegaly on plain CT scans. Among these abnormal CT images with hypodensity, 6 (75%) of 8 were located in the midbrain and pons, 5 (62.5%) of 8 were located in the bilateral thalamus, 3 (37.5%) of 8 were located in the bilateral basal ganglia, and 1 (12.5%) of 8 were located in the bilateral cerebellar hemispheres (Figure 3a-d). Among these 22 patients, six patients experienced an aggravation of central nervous system injury and died, three patients recovered after treatment but were discharged with mild limb motor dysfunction, and 13 patients subsequently developed refractory circulatory failure.

At the end stage of lethal DQ poisoning, 19 individuals exhibited significant signs and symptoms of circulatory failure, including tachycardia, clammy and piebald extremities, swollen extremities, oliguria, elevated blood lactate, and persistent hypotension. The mean time for refractory circulatory failure to manifest following DQ poisoning was 28.32 ± 21.12 h. We observed rapid progression to refractory circulatory failure in all 19 patients. Even with continued vasopressor therapy and V-A ECMO support, the patient's blood pressure dropped below 90/60 mmHg. As mentioned above, we found that central nervous system injury invariably preceded circulatory failure in 13 patients who developed central nervous system injury combined with refractory circulatory failure.

As a result, we gradually identified central nervous system injury and refractory circulatory failure as potentially fatal complications of lethal DQ poisoning. We found that six (24.0%) patients died of central nervous system injury, six (24.0%) patients died of refractory circulatory failure, and 13 (52.0%) patients died of central nervous system injury complicated with refractory circulatory failure (Figure 4a). Patients with central nervous system injury had a median Cp_1 of 9644.30 (3378.60,

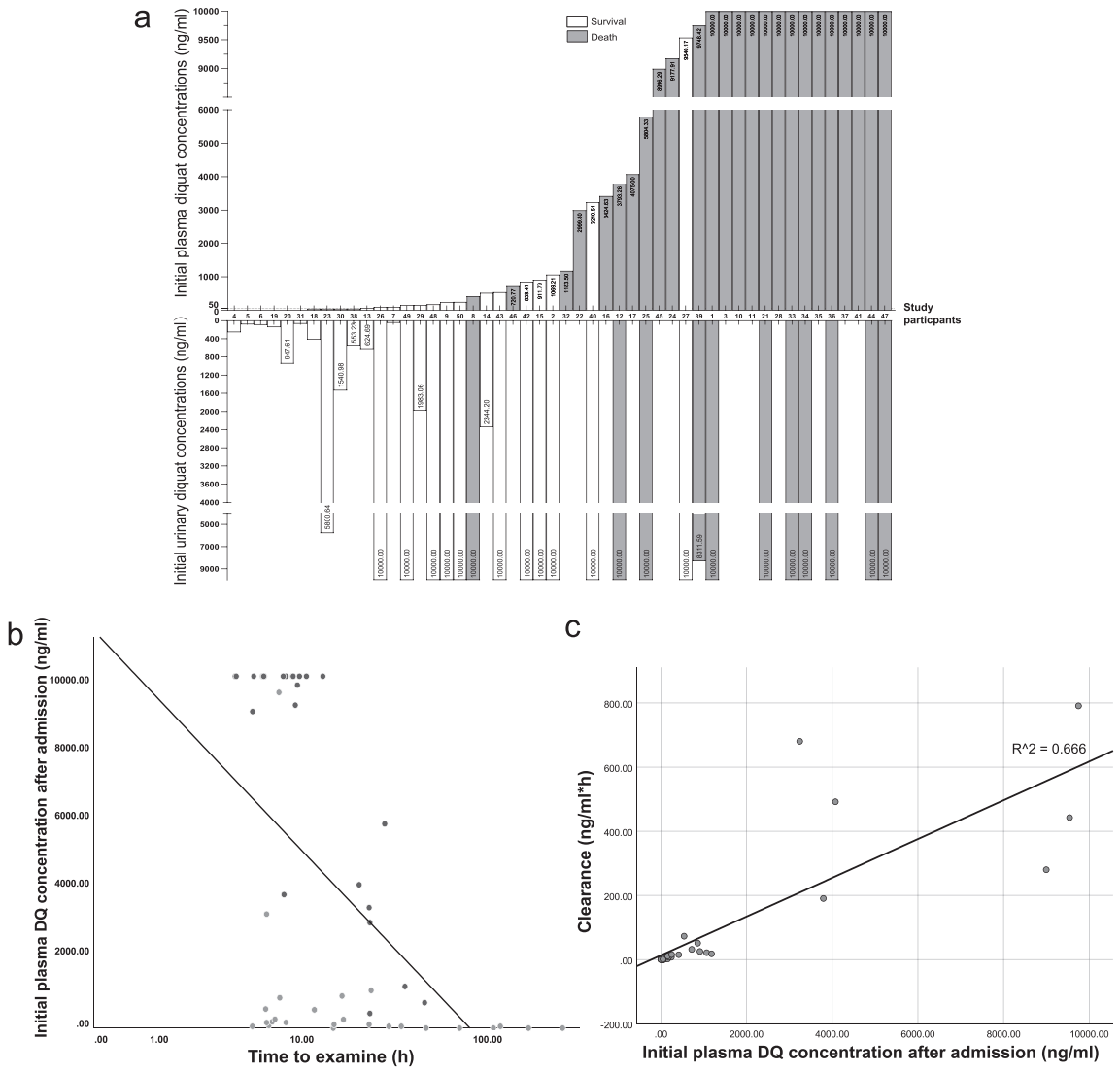

Figure 1. The prognosis of acute diquat (DQ) poisoning is related to the initial plasma DQ concentration (Cp_1). (A) Based on the prognosis, the gray bar symbolises patients in the death group, and the white bar symbolises patients in the survival group. Then it was sorted according to the initial plasma and urinary concentration of DQ; (B) Correlational analysis between Cp_1 and test time; (C) We calculated the clearance rate of plasma DQ concentration according to the following formula: $R = 100 \times (Cp_2 - Cp_1)/Cp_1$ (%). The correlational analysis between the clearance rate of plasma DQ concentration and Cp_1.

10000.00) ng/mL. Patients with refractory circulatory failure had a median Cp_1 of 10000.00 (5804.33, 10000.00) ng/mL.

ROC curve and survival analysis

In the early stage of data analysis of this study, we conducted univariate analysis on various possible confounding factors (including gender, age, hospitalization time, region, etc.). Statistical analysis found that these confounders were not significantly associated with death. Eventually, only Cp_1 and Cu_1 were included in the ROC analysis. To further evaluate the predictive ability of Cp_1 and Cu_1 on the mortality of DQ poisoning, ROC curves were drawn (Figure 4b). The results showed that the area under curve (AUC) of Cp_1 was 0.967 (95% CI: 0.911, 1.000), and that of Cu_1 was 0.760 (95% CI: 0.607, 0.913). The cut-off value of Cp_1 was 3516.885 ng/ml (sensitivity, 90.9%; specificity, 96.0%). We plotted Kaplan-Meier survival curves to analyse the mortality of different fatal complications, including four groups (Group A, B, C, and D) and all

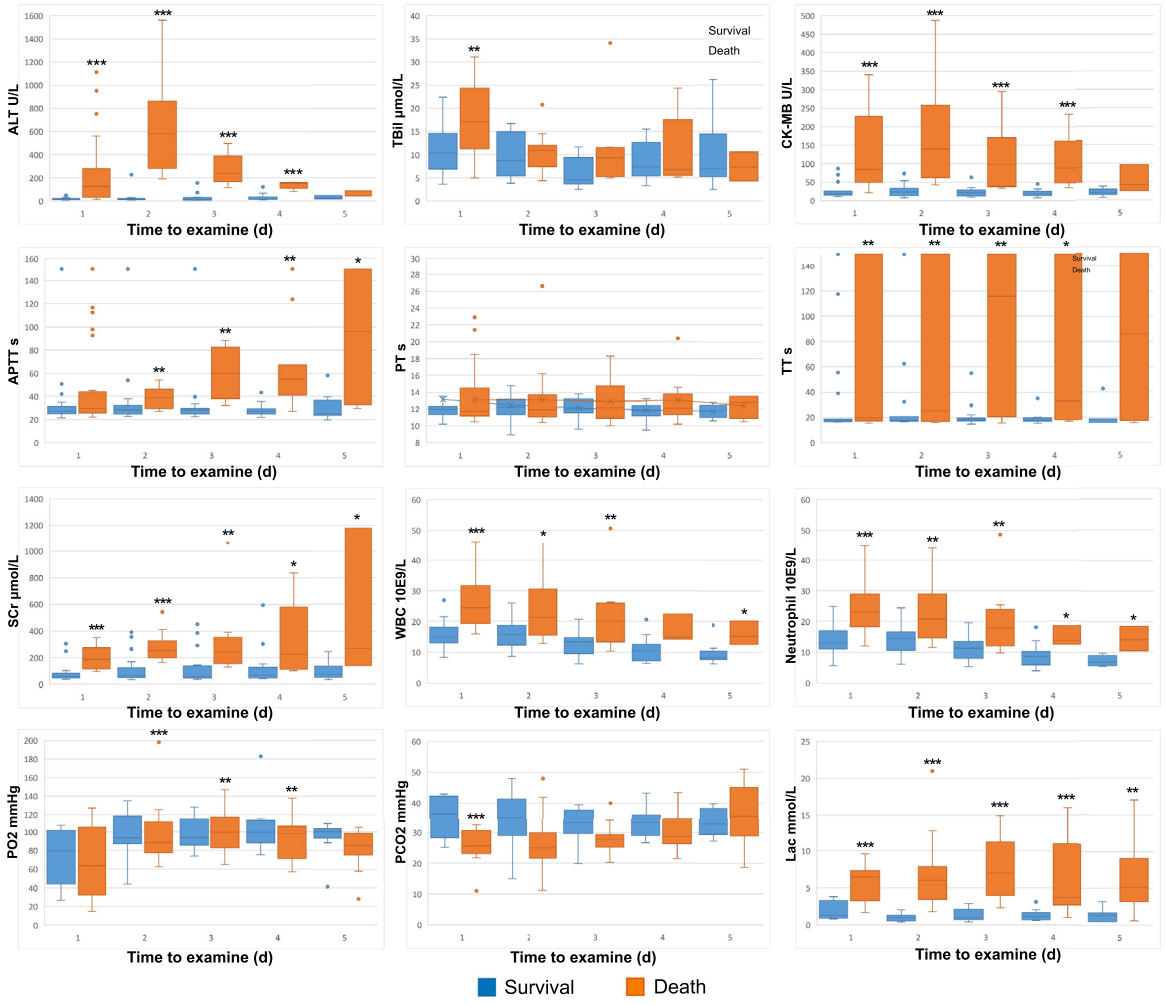

Figure 2. Clinical characteristics of acute DQ poisoning. Dynamic laboratory indexes between survival and death groups from day 1 to day 5 of DQ poisoning, includes ALT, TBil, CK-MB, APTT, PT, TT, SCr, WBC, Neu, PO_2, PCO_2, and Lac. (compared with survival group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

DQ: diquat; ALT: alaninetransaminase; TBil: total bilirubin; CK-MB: creatine kinase-MB; APTT: activated partial thromboplastin time; PT: prothrombin time; TT: thrombin time; SCr: serum creatinine; WBC: white blood cell; Neu: neutrophils; PO_2: artial pressure of oxygen; PCO_2: artial pressure of carbon dioxide; Lac: actic acid.

patients. As shown in Figure 4c, we found a significant difference in survival probability among them ($P < 0.001$). There was no doubt that Group A (patients with no fatal complications) had the highest survival probability and median survival time of the four groups. In the remaining three groups, Group B (patients with a central nervous system injury) had the highest survival probability, whereas Group C (patients with circulatory failure) and Group D (patients with two fatal complications) had similarly low survival probabilities. We found that the median survival time of Group B was 5 days, which was the longest. And the median survival time of Group C (day 3) was longer than that of Group D (day 2).

Discussion

We found that the intake doses from the medical history might not correspond to actual intake doses, which is related to causes such as false recall, vomiting after taking medication, and chaotic pesticide production regulation. Recent research has reported new detection techniques for UPLC-MS/MS for DQ detection in cowpea samples.[24] Then DQ concentrations of human plasma and urine samples were quantified by HPLC-MS/MS.[25,26] However, as a relatively new technology, HPLC-MS/MS is merely applied in clinical studies related to DQ poisoning.[27] 50 cases in our study were transferred to the hospital after receiving routine

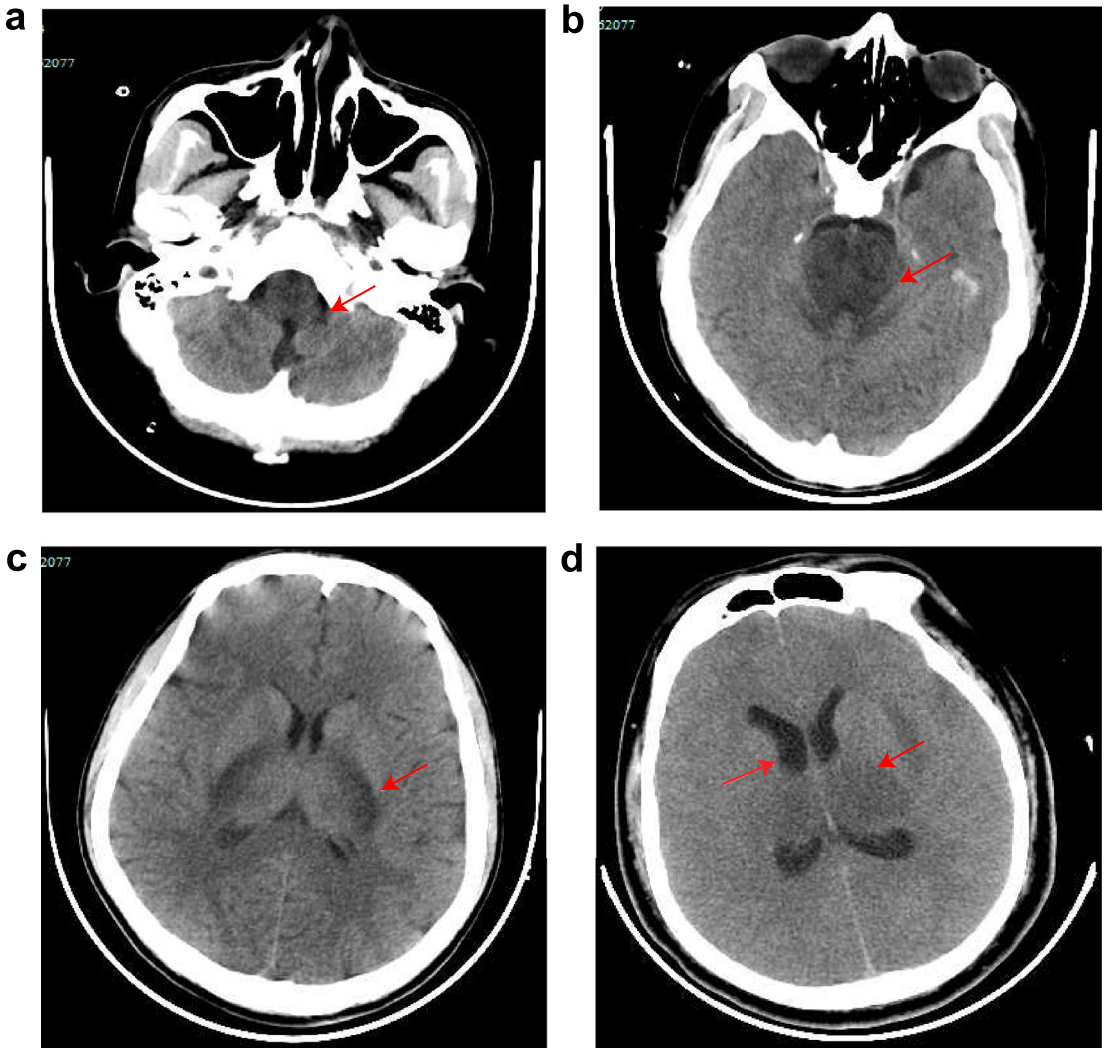

Figure 3. Abnormal head CT imaging. Head CT appearance of the medulla oblongata (A), the midbrain (B), the basal ganglia (C), and the thalamus (D) was the low-density image, as well as the brain tissue was diffuse swelling.

treatment from local hospitals. The median time to our hospital was more than 9 hours. Although the peak concentration of DQ had passed at that time, we believed that the Cp_1 remained a well-detected indicator of the daily measured DQ concentration. DQ was excreted through the kidneys, with the result that the urinary concentration of DQ was often outside of the measurable range in our study.[28,29] Furthermore, patients with DQ-induced acute kidney injury may experience oliguria or anuria, making continuous monitoring of changes in urinary DQ concentrations difficult. Therefore, at this stage, we believe that Cp_1 is the most suitable indicator for evaluation.

We have expected that haemoperfusion could reduce the high mortality associated with acute DQ poisoning. Therefore, we calculated the clearance rate of plasma DQ concentration according to the following formula: $R = 100 \times (Cp_2 - Cp_1)/Cp_1$ (%). We observed a significant decrease in plasma DQ concentration after haemoperfusion clinically. Moreover, we found that the clearance rate of DQ was significantly correlated with the concentration before haemoperfusion. However, due to the limitations of medical ethics, we could not divide all patients into the haemoperfusion treatment group and the non-treatment group for comparative analysis, so as to judge the impact of haemoperfusion on mortality. We hope to analyse the effect of haemoperfusion therapy by further studying the time point and the times of haemoperfusion in the next exploration.

Vanholder, R. et al. reported marked necrosis of the pontine capillary wall and perivascular haemorrhage in patients with DQ poisoning by pathological examination.[30] Several case reports have also discovered the

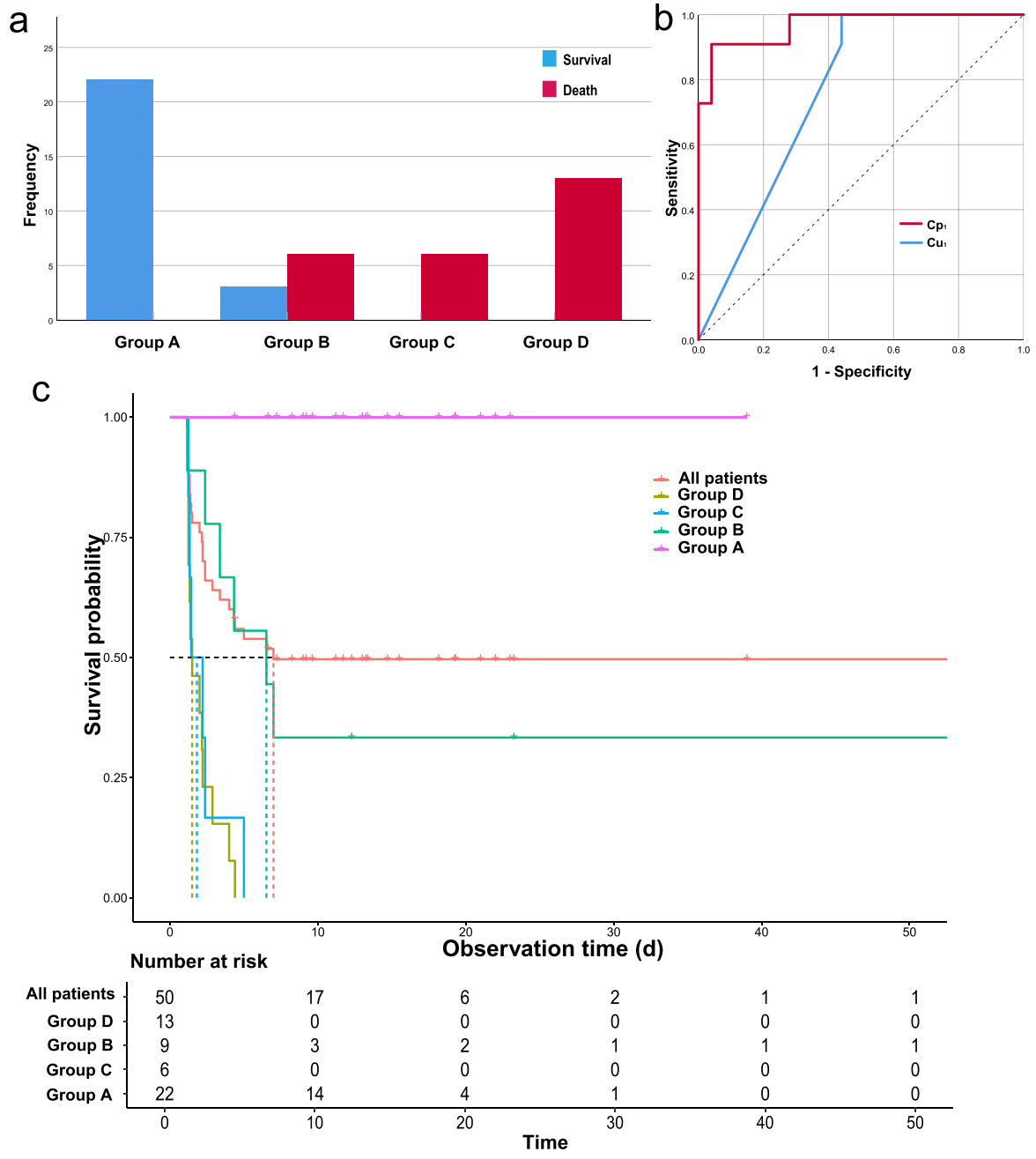

Figure 4. Statistical analysis chart of patients with lethal DQ poisoning.

Histogram of frequency distribution based on fatal complication; (B) Receiver operating characteristic curves for predicting all-cause mortality based on initial plasma DQ concentration (Cp_1) and initial urinary DQ concentration (Cu_1); (C) Survival analysis was performed based on the occurrence of fatal complications; Patients in Group A had no central nervous injury and no refractory circulatory failure; patients in Group B had central nervous injury; patients in Group C had refractory circulatory failure; and patients in Group D had both central nervous injury and refractory circulatory failure.

symptoms of encephalopathy such as mania, epilepsy, lethargy, and coma after DQ poisoning.[1,3,31] Actually, we have noticed that a portion of the cases in our study showed abnormal head CT images. The results showed that the most common sites of damage were the midbrain and pons, which was partly consistent with previous reports.[32] The midbrain and pons are crucial elements of the brainstem, which is the main control

centre for breathing and circulation. This could explain why patients who have suffered a central nervous system injury have a higher mortality rate than those who have not. Furthermore, we verified the existence of DQ in cerebrospinal fluid in 6 cases with central nervous system injury and successfully detected DQ concentrations by HPLC-MS/MS (data not shown), suggesting that DQ concentration in cerebrospinal fluid might be a potential indicator for DQ-induced encephalopathy. We thought that DQ could directly penetrate the blood-brain barrier through a specific and unknown mechanism, thereby directly injuring the surrounding brain tissue such as the midbrain, pons, bilateral thalamus, and bilateral basal ganglia. A preliminary study only found that carbon monoxide could cross the blood-brain barrier by suppressing the expression of the zonula occludens-1 (ZO-1) and aquaporin-4 (AQP-4) axis.[33] This may have some hints for the exploration of the underlying mechanism.

Our findings showed that lethal DQ poisoning causes multiple organ dysfunction, especially in the circulatory system. To the best of our knowledge, no study reported the clinical symptoms and detailed mechanisms of DQ poisoning. On the one hand, high concentrations of DQ in cerebrospinal fluid directly cause irreversible damage to brain tissue, especially in the brainstem region, this partly explains why central nervous system injury always preceded refractory circulatory failure in patients with two fatal complications. On the other hand, DQ was distributed widely and could shuttle between peripheral circulation and tissues, leading to changes in capillary permeability. Moreover, we observed elevations of myocardial enzymes in some patients during the early stages of intoxication, implying that circulatory failure might also be due to myocardial injury. The ROC curve and survival analysis demonstrated that, once refractory circulatory failure occurred, the survival probability and survival time of patients were significantly reduced. Due to the difficulty in saving patients clinically, it is crucial to further explore the mechanism of DQ poisoning.

The majority of cases of acute DQ poisoning occur in rural settings with limited medical resources. As a result, we thought that simple indicators reflecting patient status and early prognostic assessment would benefit the treatment. This is the first study to show a direct relationship between Cp_1 and patient outcomes. However, there are several limitations to this research. Larger study cohorts will be needed in the future to corroborate these findings, especially since predictive models based on these parameters may help to form the correct clinical consensus on acute DQ poisoning.

Our study confirms a positive correlation between Cp_1 and mortality in patients with acute DQ poisoning. The first cause of increased mortality in patients with acute DQ poisoning is refractory circulatory failure, and the second is central nervous system injury, in the brainstem region. Thus, it is the best preventive measure to reduce the mortality rate of DQ poisoning to educate the public about its dangers.

Contributors

YL conceived the study and verified the underlying data. JZ analysed the data, wrote the first and successive drafts of the manuscript, and verified the underlying data. Both authors read, discussed, and approved the final version of the manuscript. The corresponding author attest that both listed authors meet the authorship criteria and that no others meeting the criteria have been omitted, had full access to all data in the study, and had the final responsibility to submit it for publication.

Data sharing statement

De-identified data can be made available from the corresponding author upon reasonable request. Contact information for the corresponding author is included on the title page.

Declaration of interests

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101609.

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