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Original Article/Liver

The characteristics of liver injury induced by *Amanita* and clinical value of α-amanitin detection

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ABSTRACT

Background: *Amanita* poisoning as a foodborne disease has raised concerning mortality issues. Reducing the interval between mushroom ingestion and medical intervention could greatly influence the outcomes of *Amanita* poisoning patients, while treatment is highly dependent on a confirmed diagnosis. To this end, we developed an early detection-guided intervention strategy by optimizing diagnostic process with performing α-amanitin detection, and further explored whether this strategy influenced the progression of *Amanita* poisoning.

Methods: This study was a retrospective analysis of 25 *Amanita* poisoning patients. Thirteen patients in the detection group were diagnosed mainly based on α-amanitin detection, and 12 patients were diagnosed essentially on the basis of mushroom consumption history, typical clinical patterns and mushroom identification (conventional group). *Amanita* poisoning patients received uniform therapy, in which plasmapheresis was executed once confirming the diagnosis of *Amanita* poisoning. We compared the demographic baseline, clinical and laboratory data, treatment and outcomes between the two groups, and further explored the predictive value of α-amanitin concentration in serum.

Results: Liver injury induced by *Amanita* appeared worst at the fourth day and alanine aminotransferase (ALT) rose higher than aspartate aminotransferase (AST). The mortality rate was 7.7% (1/13) in the detection group and 50.0% (6/12) in the conventional group ($P = 0.030$), since patients in the detection group arrived hospital much earlier and received plasmapheresis at the early stage of disease. The early detection-guided intervention helped alleviate liver impairment caused by *Amanita* and decreased the peak AST as well as ALT. However, the predictive value of α-amanitin concentration in serum was still considered limited.

Conclusions: In the management of mushroom poisoning, consideration should be given to the rapid detection of α-amanitin in suspected *Amanita* poisoning patients and the immediate initiation of medical treatment upon a positive toxin screening result.

Introduction

Fungi have had a long history of collection as a delicacy to add to our food diversity, whereas lethal mushroom poisoning cases happen worldwide every year because certain toxic mushrooms resemble edible species and even experienced collectors might misjudge the edibility of mushrooms [1,2]. In China, nearly 70% of deaths related to mushroom poisoning result from collecting members of the genus *Amanita* [3], which shows an annual upward trend in incidence [4,5].

Following severe gastrointestinal symptoms of nausea, vomiting, abdominal pain and choleraic diarrhea, which are obvious within 12-48 h after mushroom ingestion, *Amanita* poisoning is characterized as a life-threatening fulminant liver failure that occurs 72-96 h after mushroom ingestion [6]. A large proportion of *Amanita* poisoning patients might suffer multiple organ dysfunction syndrome (MODS) or death [6,7]. Therefore, a comprehensive strategy of treating *Amanita* poisoning has evolved, which includes the application of possible antidotes, the prevention of absorption, the elimination of absorbed toxins such as plasmapheresis (plasma exchange, PE) and diuresis, supportive therapy, and the final choice of liver transplantation [2]. However, the therapeutic effect of comprehensive treatment is still far from satisfactory not

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only due to the paucity of specific antidotes and the rare chance of liver transplantation [1,2], but also the tendency to delay the intervention, since the diagnosis of *Amanita* poisoning is occasionally inadequately made on admission manifestations. The diagnosis is based on typical disease patterns [8], challenging clinicians to make an early diagnosis [9]. The accurate diagnosis of *Amanita* poisoning would be more viable if based on mushroom identification supported by experienced mycologists when patients are admitted. However, in most occasions, clinicians are not experienced in distinguishing fungi and mushrooms of *Amanita* are seldom well-kept - abandoned or rotten - for identification. On the other hand, the transient remission of gastrointestinal symptoms before liver failure might predispose the patient to misdiagnosis and untimely discharge [2]. Liver function that rapidly deteriorates in the late stage would indicate the passing of the best rescue time and subsequent poor prognosis, which pressures researchers to find an early and precise diagnostic method for *Amanita* poisoning.

Advances in technology have made toxin detection an important supportive method for diagnosis. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) has proved to be capable of quickly detecting subtypes of amatoxins in the blood, urine, or other body fluids with high specificity and sensitivity [7,10,11]. Consistent with the investigation of lethal *Amanita* species distribution in China in 2016 [12], we collected poisonous mushrooms at sites where patients had picked them before and identified them as *Amanita rimosa* and *Amanita fuliginea* by genetic sequencing. Additionally, the overall detection of toxin content discovered that the toxin with the highest content in these *Amanita* mushrooms in Asia was the subtype α-amanitin, which is obviously higher as compared to that in *Amanita* of Europe and North America [13]. Therefore, we applied the policy of detecting α-amanitin by LC-MS/MS on admission to guarantee or exclude the diagnosis of lethal *Amanita* poisoning in mushroom poisoning patients as quick as possible. Once diagnosed, the immediate initiation of detoxification was performed by plasmapheresis. Dramatically, patients achieved a higher survival rate as compared to the conventionally diagnosed patients. Therefore, we conducted this retrospective analysis to seek out more clinical information of *Amanita* poisoning in Asian people and explore the clinical value of α-amanitin detection on admission.

Methods

Subjects

This retrospective study enrolled *Amanita* poisoning patients who were admitted or referred to the First Affiliated Hospital of Zhejiang University, School of Medicine from June 1, 2011 to June 30, 2021. The diagnosis of *Amanita* poisoning was made on the basis of epidemiological history of suspected mushroom consumption and typical patterns of acute liver failure following a remission of gastrointestinal symptoms. When liver impairment was not presented on admission, i.e., in the early stage of this disease, diagnosis would be considered definite if the mushrooms were identified as *Amanita* by at least two experienced mycologists and clinicians. Therefore, patients and their family members were required to provide photographs or remains of the relevant mushroom or if absent, some mushrooms were collected exactly where previously picked (Fig. 1A). Reference to the mushroom atlas would be the last option to identify the mushroom species. In the worst occasions, the morphological identification of poisonous mushroom was impossible to achieve due to the absence of photos and failed search, thus the diagnosis of *Amanita* poisoning was not made until the appearance of severe liver impairment. Consequently, starting from 2019, immediate α-amanitin detection in the blood and/or urine samples was applied in mushroom poisoning patients to help diagnosis on the basis of the above-mentioned conventional diagnostic process. A positive toxicology screen in blood and/or urine would confirm the diagnosis of *Amanita* poisoning.

Patients with viral hepatitis, hepatic cirrhosis, autoimmune liver disease, and taking hepatotoxic drugs like acetaminophen, or other conditions that might influence liver function, pregnancy or life-threatening diseases, such as heart failure, end-stage renal disease, sepsis and advanced malignant tumor, were excluded. Patients younger than 16 years of age or those with obscure medical history records before admission to our hospital were excluded.

Twenty-five *Amanita* poisoning patients were finally enrolled in this study. Thirteen patients in the detection group who were admitted between 2019 and 2021 were diagnosed mainly on the basis of detection before obtaining the result of liver function and morphological identification. Twelve patients in the conventional group were diagnosed on the basis of the conventional diagnostic procedure and admitted in previous years (Fig. 1B). Only 4 patients in the conventional group provided photographs of the mushrooms they had eaten when they arrived at the hospital. The study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, China (IIT20200005A).

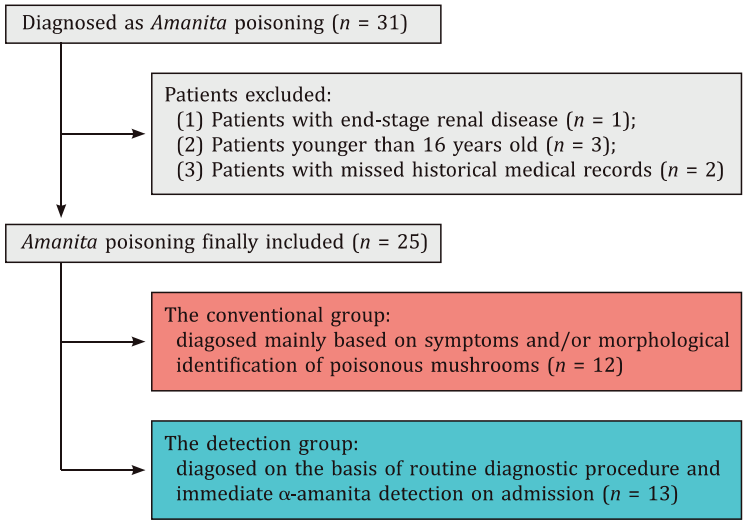
Treatment

All patients were subjected to a uniform protocol which included immediate detoxification, potential antidotes and supportive therapy. Extracorporeal detoxification with plasmapheresis was initiated once the diagnosis of *Amanita* poisoning was confirmed according to our formerly mentioned diagnostic procedure. Gastric lavage was not considered as no mushroom debris had remained in the gastrointestinal tract of patients when they arrived. Potential antidotes of silymarin and N-acetylcysteine were applied. Moreover, patients with dehydration and electrolyte disturbance due to gastrointestinal symptoms received intravenous fluid support. In severe cases, patients were given supplements such as human albumins, cryoprecipitate and platelets. Vasoactive drugs and mechanical ventilation were necessary in cases of respiratory and circulatory failure.

Sample collection and toxin detection

The first blood sample was collected within 30 min after arriving at the emergency room, and blood samples were routinely taken in the morning during hospitalization to detect liver function and other laboratory indices. Regarding specimens for toxin detection, these were collected at the same time of the first sample and stored at 4 °C before α-amanitin qualification and quantification in patients with a history of suspected mushroom consumption, no matter whether they presented liver injury on admission. Taken that α-amanitin has a short half-life in circulation and is mainly excreted through the kidneys, blood and urine specimens were collected as early as possible. For patients referred to our hospital, their blood/urine samples stored in the first visit hospital were mobilized for detection so as to increase the positive rate. We regarded the detection result from urine as a qualitative analysis to confirm the existence of α-amanitin, and its concentration in circulation as a semi-quantitative analysis to predict the severity of this disease. Supported by staffs from Zhejiang Provincial Center for Disease Control and Prevention, the α-amanitin detection procedure was performed using the online SPE-LC-MS/MS system built on an 8060 LC–MS instrument equipped with an ESI source (Shimadzu, Kyoto, Japan), which has been regarded as a highly sensitive method at the pg/mL level in blood for diagnosis [14]. All samples for detection were stored at -20 °C before extraction. The

A



B

Fig. 1. Different species of *Amanita* and the flow chart of *Amanita* poisoning patients' enrollment. Photographs of *Amanita rimosa* (above) and *Amanita fuliginea* (below), recorded respectively before cooking and collection at the original picking site after admission (**A**). Flowchart of the enrollment of *Amanita* poisoning patients for the current retrospective study (**B**).

entire collection and detection procedure for each patient could be finished within 3 h after admission to the hospital.

Primary data collection and secondary data processing

The recorded data of patients included the following: (1) demographic baseline: age, sex, comorbidities, hepatitis B virus (HBV) infection status and smoking/drinking status; (2) clinical manifestations on admission: abdominal pain, diarrhea, vomiting, jaundice, coma, and melena; (3) clinical data and laboratory values: vital signs of basal body temperature (T), heart rate (HR), respiratory rate (RR), and mean artery pressure (MAP) on admission, arterial pH, arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$), bicarbonate ions (HCO_3^-), serum sodium, serum potassium and left ventricular ejection fraction (LVEF) on admission; aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total bilirubin (TB), gamma-glutamyl transferase (GGT), albumin (ALB), international normalized ratio (INR) for prothrombin time, activated partial thromboplastin time (APTT), lactate (Lac), fasting blood glucose (FBG), lactate dehydrogenase (LDH), serum creatinine (Scr), glomerular filtration rate (GFR), creatine kinase isoenzyme MB (CK-MB), C-reactive protein (CRP), white blood cell count (WBC), neutrophil percentage (N), platelet count (PLT) and hemoglobin level (HB) on admission and their peak/trough values during hospitalization; all results of AST, ALT and TB were recorded; (4) time information: estimated *Amanita* ingestion time (I), admission time (A), first time record of detoxification with plasmapheresis (D), the collection time of blood samples for α-amanitin detection, as well as of the important laboratory tests of AST, ALT and TB, especially the recorded maximum values; (5) treatment data related with plasmapheresis and data of outcomes: total sessions of plasmapheresis, mean volume of plasma used in plasmapheresis for each patient, survival and length of hospitalization.

The model for end-stage liver disease (MELD) scores were calculated using patients' values of serum bilirubin, Scr, and the INR to predict prognosis [15]. The I-A durations consisted of the interval between *Amanita* ingestion and admission. The I-D durations was the exposure time of toxin, defined as the interval between *Amanita* ingestion and extracorporeal detoxification initiation. The A-D durations represented the reaction and preparation time of plasmapheresis from admission. Considering that α-amanitin was quickly cleared from the blood, we calculated the interval between *Amanita* ingestion and detection as the I-Detection durations. The liver function might change dramatically within one or two days, and was closely monitored in the first several days of hospitalization. In addition, the interval between mushroom ingestion and each blood collection varied among patients. Therefore, we selected the patients' highest AST, ALT and TB values every 24 h from mushroom ingestion to show the time trend of liver function, and calculated the appearance time of the highest AST, ALT and TB from mushroom ingestion.

Statistical analysis

Most numerical variables were found to be non-normally distributed after normality testing (Shapiro-Wilk test). As a result, these data were presented as median (interquartile range, IQR) and compared with nonparametric tests (Wilcoxon rank-sum test and Wilcoxon signed-rank test, respectively, for unpaired and paired comparisons), while some normally distributed data were expressed as mean ± standard deviation (SD) and compared with Student's *t*-test. The categorical variables expressed as count (proportion) were compared with Fisher's exact test. A log-rank test was applied for survival analysis. Some laboratory tests were not implemented in some patients, and these consequently missing values were not handled by any methods due to limited sample size. The repeated measure analysis of covariance based on a generalized linear model, as implemented under mixed models, which is suitable when missing values exist, was applied to analyze the liver function change from baseline as the dependent variable, and the grouping factor of detection-guided-intervention, time, and their interaction as independent variables [16]. A two-sided $P < 0.05$ was considered statistically significant. Statistical analysis and data visualization were performed using the R software (version 4.1.0, R Foundation for Statistical computing, Vienna, Austria) and Adobe Illustrator (version CC 2018, California, CA, USA).

Results

Baseline characteristics, clinical manifestations and vital signs on admission

A total of 25 *Amanita* poisoning patients with a mean age of 41.36 ± 14.53 years were included in our study. Of them, 16 (64.0%) were male. These patients were otherwise healthy; there

Table 1
Demographic and admission clinical characteristics of patients.

Parameters	All patients (n = 25)	The conventional group (n = 12)	The detection group (n = 13)	P value
Demographic characteristics				
Male	16 (64.0%)	8 (66.7%)	8 (61.5%)	1.000
Age (yr)	41.36 ± 14.53	38.42 ± 17.15	44.08 ± 11.46	0.339
Hypertension	1 (4.0%)	1 (8.3%)	0	0.480
Smoker	6 (24.0%)	3 (25.0%)	3 (23.1%)	1.000
Drinker				0.551
Current	5 (20.0%)	2 (16.7%)	3 (23.1%)	
Former	2 (8.0%)	0	2 (15.4%)	
Never	18 (72.0%)	10 (83.3%)	8 (61.5%)	
Admission clinical characteristics				
Diarrhea	25 (100%)	12 (100%)	13 (100%)	1.000
Vomiting	24 (96.0%)	12 (100%)	12 (92.3%)	1.000
Abdominal pain	17 (68.0%)	10 (83.3%)	7 (53.8%)	0.202
Jaundice	8 (32.0%)	5 (41.7%)	3 (23.1%)	0.411
Melena	2 (8.0%)	2 (16.7%)	0	0.220
MAP (mmHg)	108.33 (98.00-122.67)	108.16 (96.58-123.00)	108.33 (98.67-122.67)	0.568
T (°C)	37.20 (36.90-37.40)	37.25 (36.82-37.40)	37.20 (36.90-37.40)	0.978
HR (beats/min)	82.00 (73.00-90.00)	78.00 (67.75-93.00)	86.00 (75.00-90.00)	0.478
RR (breaths/min)	16.00 (14.00-20.00)	16.50 (13.00-20.00)	16.00 (15.00-20.00)	0.680

Numerical variables were expressed as mean ± standard deviation or median (interquartile range). Categorical variables were expressed as count (percentage). MAP: mean arterial pressure; T: temperature; HR: heart rate; RR: respiratory rate.

was only 1 (4.0%) hypertension and no case of diabetes. As for HBV infection status, all patients were tested negative for the surface antigen of the hepatitis B virus (HBsAg). Six (24.0%) patients were regular smokers. Five (20.0%) patients were regular drinkers and 2 (8.0%) were former drinkers. In the 25 enrolled patients, the most common symptoms were diarrhea (100%), vomiting (96.0%), and abdominal pain (68.0%). Eight (32.0%) patients were presented with jaundice on admission. Two (8.0%) patients exhibited a severe melena when transferred to the hospital. Most patients had stable vital signs of T, HR, RR and MAP.

No differences in sex, age, rate of smoking and drinking, or common comorbidities were found between the conventional and detection groups (all $P > 0.05$). The clinical manifestations and vital signs also showed no statistical differences between the two groups (all $P > 0.05$) (Table 1).

Changes in laboratory results of Amanita poisoning

Liver failure is the most threating complication of *Amanita* poisoning, therefore, the laboratory analysis primarily focused on liver enzymes, bilirubin, protein synthesis function indicated by ALB level, as well as coagulation function. The time intervals of reaching the peak values of AST, ALT and TB from mushroom ingestion for all patients was 81.96 (71.97-132.50) h, 95.37 (78.51-156.50) h and 89.25 (79.06-136.65) h, respectively. With regards to the peak values of AST and ALT, the former was lower than the latter (1060.00 vs. 2132.00 U/L, $P < 0.001$). As patients received supplements of albumin or cryoprecipitate, trends of ALB and coagulation functions were not analyzed. Additionally, among the 7 deceased patients, the phenomenon of separation in enzyme and bilirubin trends was observed, and this might appear on the sixth day (121.08-131.62 h), after mushroom ingestion.

Then we compared laboratory results between the detection group and the conventional group to explore the effects of the detection and intervention (Table S1, Fig. 2A and Fig. 3). The median AST, ALT, TB, ALB, INR and APTT values were all worse in the conventional group than in the detection group upon admission (2024.00 vs. 40.00 U/L, $P = 0.006$; 2592.00 vs. 45.00 U/L, $P = 0.002$; 76.25 vs. 16.90 μmol/L, $P = 0.001$; 37.90 vs. 44.50 g/L, $P = 0.002$; 2.98 vs. 1.13, $P = 0.003$; and 40.00 vs. 24.00 s, $P = 0.001$, respectively) and during hospitalization, except ALB (2729.00 vs. 544.00 U/L, $P = 0.008$; 4075.50 vs. 863.00 U/L, $P = 0.003$; 123.60 vs. 25.10 μmol/L, $P = 0.008$; 31.20 vs. 34.10 g/L, $P = 0.082$; 3.96 vs. 1.19, $P = 0.003$; and 66.25 vs. 33.00 s, $P = 0.003$, respectively). The GGT level showed no difference between the two groups on admission and during hospitalization. The MELD scores on admission were significantly lower in the detection group (24.70 vs. 7.90, $P = 0.002$), indicating a milder injury in the early stage of disease. We further drew the trend of liver function during the first week after *Amanita* ingestion. As shown in Fig. 2B, AST and ALT showed an up-and-down change during the first week in both groups, while the TB of the conventional group kept elevating. The repeated analysis of covariance revealed that AST, ALT and TB were higher in the conventional group during the first week. The effect of time, and the interaction of time and the detection procedure were significant regarding AST and ALT, while they were not significant regarding TB, indicating the earlier arriving of peak liver enzyme values in the detection group.

Amanita poisoning also influenced the function of other systems, as shown in Table S1 and Fig. 3. In terms of hemostasis, the Lac level was significantly higher in the conventional group during hospitalization ($P = 0.045$), but not on admission ($P = 0.087$). The FBG showed no difference when comparing the conventional group with the detection group, but it decreased after admission in the detection group (Fig. 3A). We did not find differences in the results of arterial blood gas analysis on admission (all $P > 0.05$). The serum potassium ($P = 0.018$) and sodium ($P = 0.009$) were found to be lower in the conventional group, as these patients suffered longer from gastrointestinal symptoms (Fig. 3B). The admission and peak LDH levels were significantly higher in the conventional group, indicating a wider range of tissue injury (Fig. 3C). The levels of Scr and GFR, which are indicators of kidney function, showed no statistical difference between the two groups (all $P > 0.05$, Fig. 3D). The admission CK-MB was slightly higher in the conventional group ($P = 0.039$), while no patient complained of chest pain or tightness. The LVEF of all patients were normal on admission (Fig. 3E). Interestingly, the CRP level was inconsistent with the other two inflammation indicators (Fig. 3F), namely, it was significantly higher in the conventional group upon hospital admission ($P = 0.001$) and during hospitalization ($P = 0.039$), while the WBC count ($P = 0.002$) and neutrophil percentage ($P = 0.044$) were higher in the detection group upon hospital admission. *Amanita* poisoning also influenced the hematopoiesis of PLT and HB. Although in the normal range, the PLT count was

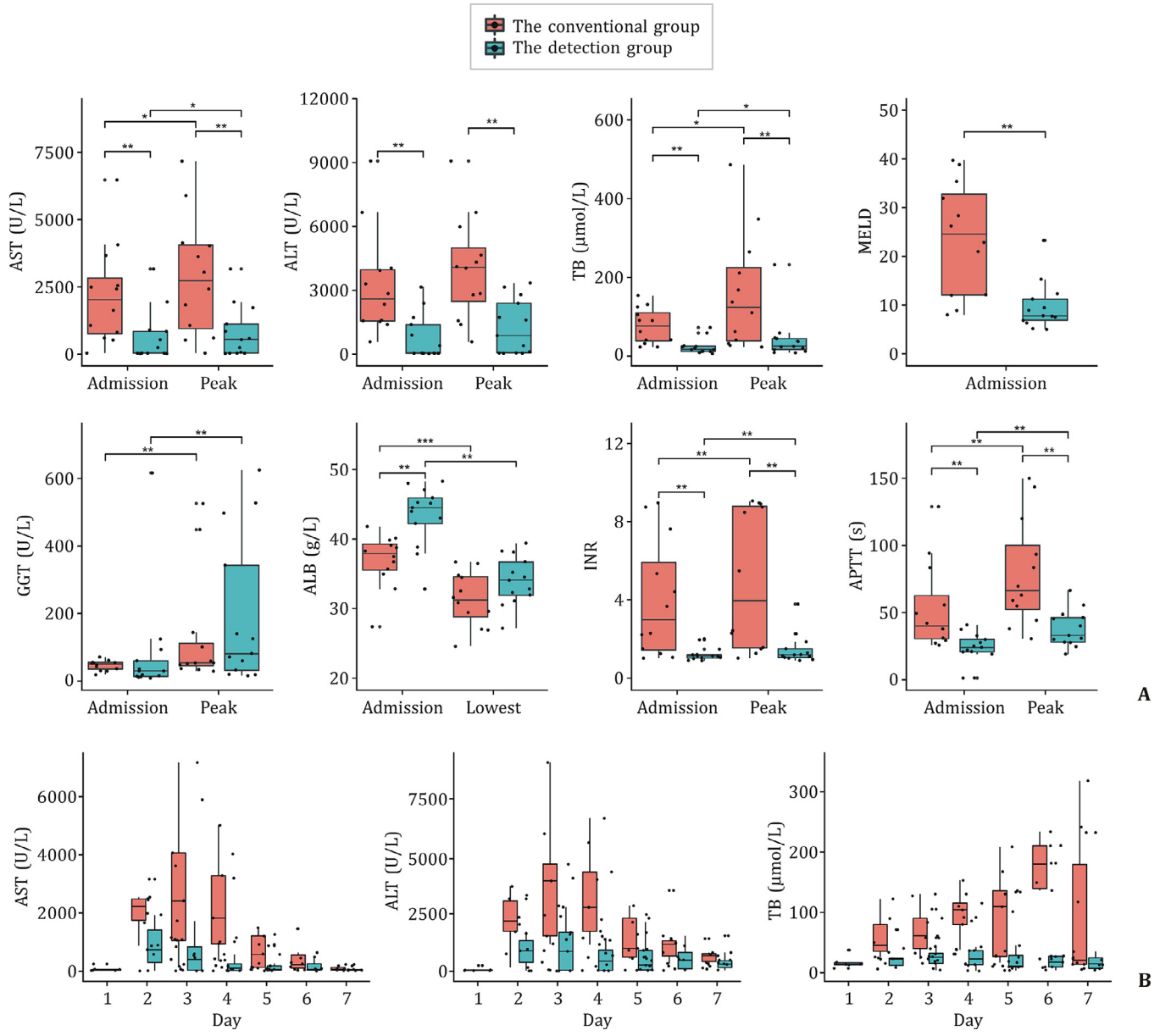


Fig. 2. Comparison of patient liver function on admission and its peak values between the detection and conventional groups, and the time trend within the course of diseases. The admission values and peak values of the hepatic function index of AST, ALT, TB, GGT, ALB, INR, APTT, and MELD scores on admission were calculated to reflect the liver function. The liver function was worse on admission in the conventional group and liver impairment was alleviated in the detection group during hospitalization (**A**). The time trend of AST, ALT and TB during the first week after *Amanita* ingestion for the two groups (**B**). *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. AST: aspartate aminotransferase; ALT: alanine aminotransferase; TB: total bilirubin; MELD: model for end-stage liver disease; GGT: gamma-glutamyl transferase; ALB: albumin; INR: International normalized ratio of prothrombin time; APTT: activated partial thromboplastin time.

higher in the detection group on admission ($P = 0.041$) and during hospitalization ($P = 0.011$). As shown in Fig. 3G, patients in both groups suffered a significant drop in PLT and HB levels, hence the supplement of PLT and coprecipitation were needed in severe cases.

Time of intervention with plasmapheresis and outcomes of patients

As shown in Table S1 and Fig. 4, the patients reached our hospital at a median time of 73.14 (58.21-80.79) h in the conventional group and 35.18 (29.03-48.58) h in the detection group, with a significant statistical difference after mushroom ingestion ($P < 0.001$, Fig. 4A). Consequently, the initiation time of plasmapheresis for detoxification from *Amanita* ingestion varied significantly, which was 80.25 (77.17-82.73) h and 53.00 (44.13-56.52) h, respectively, in the conventional and the detection groups ($P = 0.002$, Fig. 4B). The detection procedure neither accelerated or delayed the initiation of plasmapheresis after admission (Fig. 4C), nor influenced the frequency of plasmapheresis execution (Fig. 4D). The average plasma volume used in the conventional group was significantly higher than that in the detection group (2067.28 vs. 1597.28 mL, $P = 0.006$, Fig. 4E).

Although there was no difference in terms of the length of hospitalization (Fig. 4F), the mortality dramatically dropped from 50.0% (6/12) in the conventional group to 7.7% (1/13) in the detection group ($P = 0.030$). The survival curves depicted in Fig. 4G revealed that the detection group had a higher probability of survival, demonstrating that α-amanitin detection might benefit the prognosis of *Amanita* poisoning ($P = 0.025$).

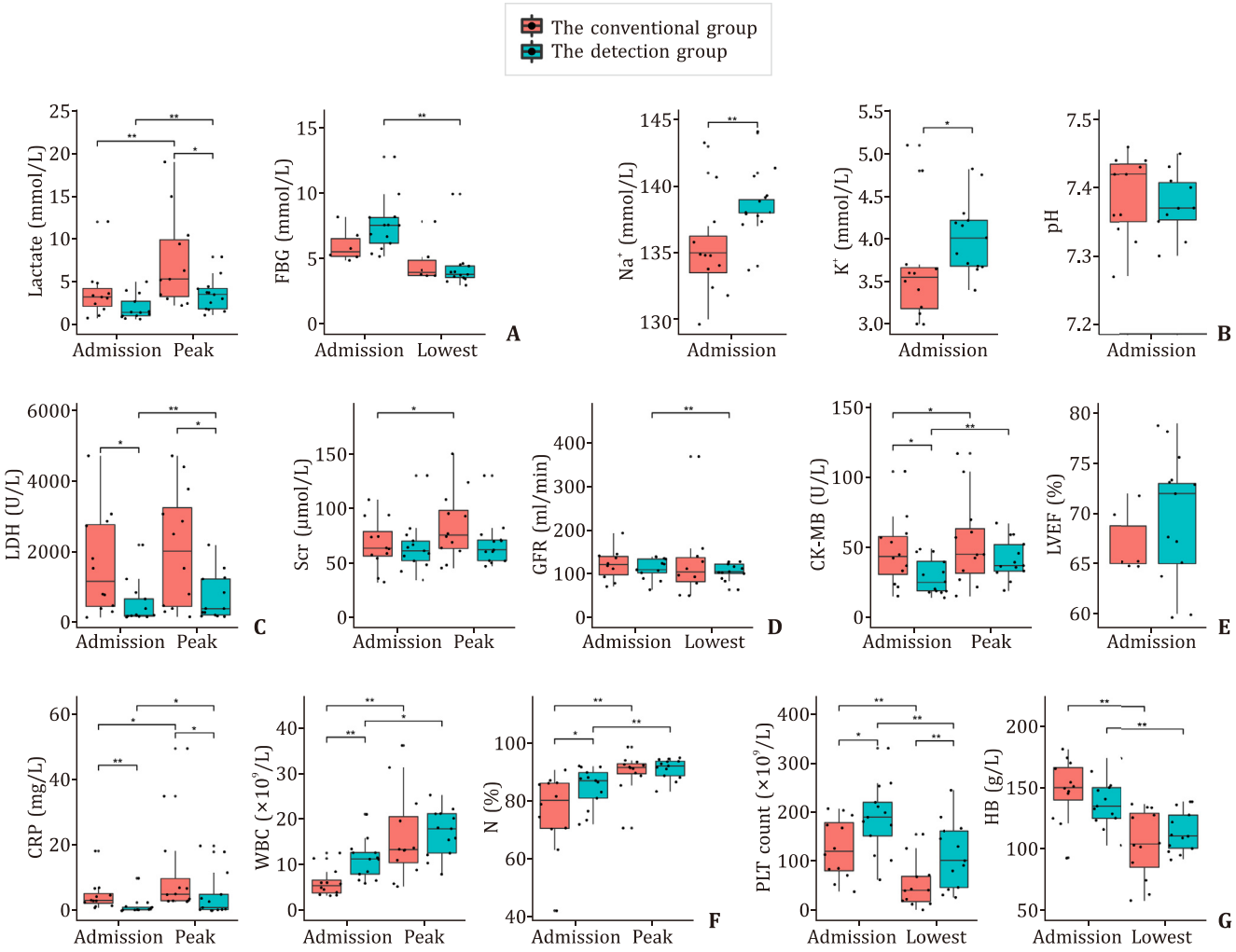


Fig. 3. Laboratory values on admission, and the peak values of important systems except the liver in the detection and conventional groups. The internal environment indicators of admission and peak lactate, and fasting blood glucose (FBG) (**A**), serum sodium (Na), potassium (K), and pH (**B**) were compared, and the disturbance of hemostasis was milder in the detection group. The admission and highest LDH levels (**C**) assessing organ injury revealed wider tissue injury on admission and during hospitalization in the conventional group. The kidney function was assessed by Scr and GFR on admission and for peak value (**D**), and showed no differences between the two groups. The comparisons of admission and highest CK-MB and admission LVEF levels revealed damaged myocardial function (**E**). The CRP levels, WBC count and neutrophil (N) percentage showed the inflammation and infection status of the two groups (**F**). The hematogenetic function assessed through PLT count and HB (**G**) showed worse hematopoietic function in the conventional group. *: $P < 0.05$; **: $P < 0.01$. LDH: lactate dehydrogenase; Scr: serum creatinine; GFR: glomerular filtration rate; CK-MB: creatinine kinase isoenzyme MB; LVEF: left ventricular ejection fraction; CRP: C reactive protein; WBC: white blood cell; PLT: platelet count; HB: hemoglobin.

Furthermore, follow-up telephone interviews were made concerning the survivals of *Amanita* poisoning patients discharged in former years, and found these patients in good health with no observable complications.

α-amanitin concentrations and detection time in the detection group

According to our qualitative and quantitative analyses of blood and urine samples of each patient in the detection group upon hospital admission, the median concentrations of α-amanitin were 0.079 (0.000-0.192) ng/mL in serum and 2.640 (1.712-8.417) ng/mL in urine. We found a higher sensitivity of toxin detection in urine, as evidenced by the higher concentrations of α-amanitin in urine compared with blood collected at the same time. α-amanitin detection had a negative result for six patients' blood collected at more than 30 h after mushroom ingestion, whereas their urine was positive. Traces of α-amanitin were found in urine in two cases after 2 days (Fig. 5A). As the serum concentration of α-amanitin quickly changes over time, we combined the information of concentration with detection time and tried to explore their relationship with liver function, treatment and outcomes. However, limited by sample size, we failed to find a significant regular distribution of heterogeneous points or a gathering trend of homogeneous points by following the bubble charts (Fig. 5B).

Discussion

In this research, the mortality rate due to *Amanita* poisoning in the detection group was lower than previously reported in the range of 30%-50% [1,17]. This variation of mortality rate between studies could be partially due to the heterogeneity of subject groups across studies, mushroom species, or a varying availability of medical resources [18]. The outcome for *Amanita* poisoning patients depends on the level of toxin intake, the therapy used, and the interval between mushroom ingestion and admission to hospital [8,19]. Patients diagnosed with *Amanita* poisoning received uniform therapy in our medical center, thus the decreased mortality in the detection group might relate to their earlier clinical intervention since toxin exposure. Underlying differences in poison dose between the two groups might work as a confounding fac-

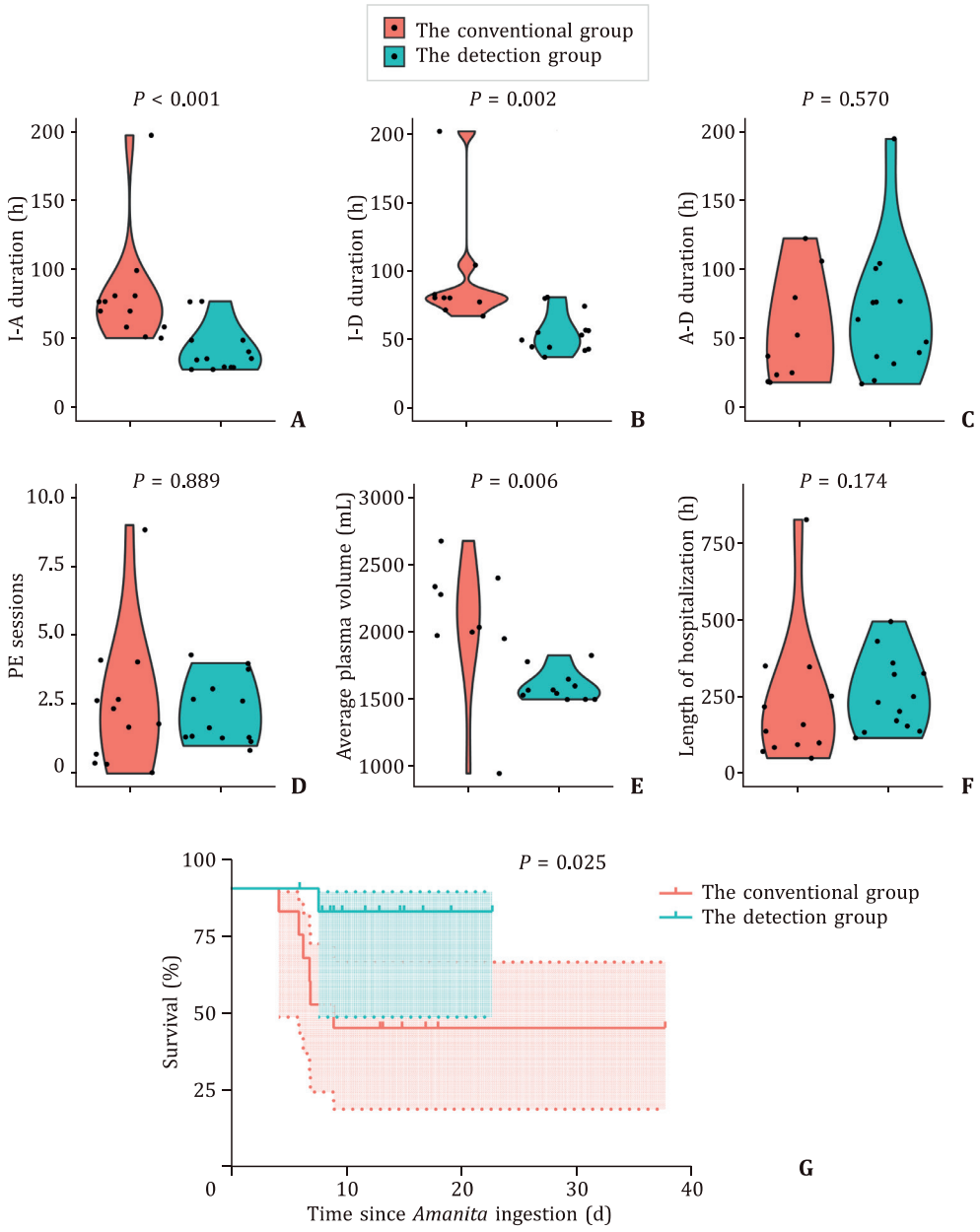


Fig. 4. Comparisons of time, intervention data and outcomes between the detection and conventional groups. The intervals of *Amanita* ingestion to admission (**A**) and intervals of ingestion to detoxification initiation with plasmapheresis (**B**) were shorter in the detection group, while the intervals of admission to detoxification initiation (**C**) had no difference between the two groups. The number of plasmapheresis (PE) sessions undertaken (**D**) showed no difference, but the average plasma volume consumed per plasmapheresis session (**E**) was higher in patients with *Amanita* poisoning in the conventional group. These patients shared the same length of hospitalization (**F**), but the survival curves (**G**) revealed that patients in the detection group had a higher survival rate. I-A duration: intervals of *Amanita* ingestion to admission; I-D duration: intervals of *Amanita* ingestion to extracorporeal detoxification; A-D duration: intervals of admission to extracorporeal detoxification.

tor because the amount of poisonous mushroom that each patient ingested was hard to trace. It is reasonable that the milder liver injures we observed in the detection group was due to their actual lower poison dose or their earlier admission, or a combination of these two factors. Regardless, our research revealed the diagnostic benefit of α-amanitin detection in early recognition and timely treatment of *Amanita* poisoning could be associated with improved outcomes.

The toxicity of *Amanita* mainly comes from hepatotoxic and nephrotoxic cyclopeptides named amatoxins, among which α-amanitin is the richest subtype in content and the most studied one [13]. The water-soluble α-amanitin, weighing 900 dalton, is heat, acid, and alkali resistant, therefore, cooking procedures cannot inactivate the toxins but make mushroom soup even more poisonous [1]. After ingestion, the toxin passes along and readily absorbs into the gastrointestinal tract, and quickly distributes into the circulation after first passing through the liver. This toxin is known to combine with the RNA polymerase II and damage cells by blocking protein synthesis [20,21], especially in those with active metabolism such as gastrointestinal epithelial cells, hepatocytes, or renal tubular cells [7,21]. In addition, p53- and caspase-3-dependent apoptosis and oxidative stress mediate the cell injury induced by amatoxins. Also, organic anion-transporting polypeptide 1B3 (OATP1B3), localized in the sinusoidal membranes of human hepatocytes, accelerates the transport of α-amanitin into hepatocytes [22] and contributes to the enrichment of the toxin in

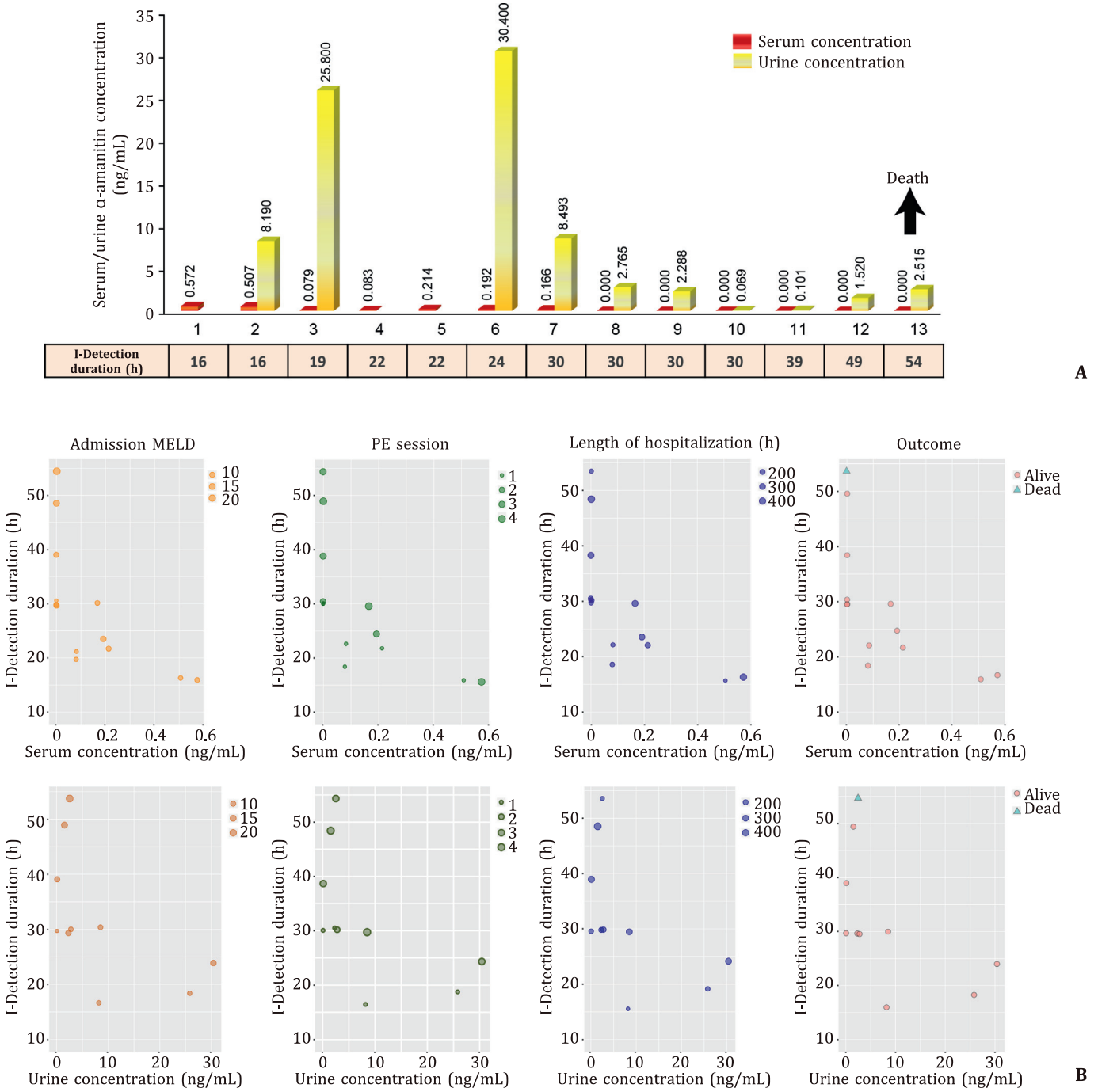


Fig. 5. Urine and serum α-amanitin concentrations and the exploration of their correlation with liver function, detoxification, and outcomes. Histogram of serum and urine α-amanitin concentrations (ng/mL) collected at the same time (**A**). The urine specimens of three patients had not been collected. Six patients tested negative for α-amanitin in blood samples (concentrations = 0.000 ng/mL). The time intervals of *Amanita* ingestion and detection for each patient was recorded in the chart below. The bubble charts showed the combined influence of serum or urine concentrations and detection times on admission MELD scores, PE session, length of hospitalization, and survival (**B**). I-Detection duration: time intervals of *Amanita* ingestion and detection; MELD: model for end-stage liver disease; PE: plasmapheresis.

the vulnerable liver and its enterohepatic circulation. Recently, α-amanitin was discovered to induce mitochondrial dysfunction in hepatocytes [23]. These mechanisms explain the impaired hepatic function, wider tissue injury and greater decrease in hemoglobin and platelet levels observed in conventionally diagnosed *Amanita* poisoning patients.

Existing reviews have evaluated the medical interventions to treat *Amanita* intoxications in detail [2], and the efficacy of a possible antidote [24], polymyxin B that was newly found with an in silico method [21,25], as well as biliary drainage, but it still needs more high-quality evidence [18,26,27]. Extracorporeal detoxification treatment is highly valued, just as plasmapheresis, molecular adsorbent recirculating system (MARS) [28,29] and fractionated plasma separation and absorption (FPSA) [30,31], among which plasmapheresis could be carried out in the intensive care unit of multi-level hospitals. Early intervention by plasmapheresis seems to be effective for both pediatric and adult amatoxin poisoning patients [32–34] by eliminating not only amatoxins and harmful endogenous metabolites from cellular necrosis, but also supplying albumin, coagulation factors and mineral salts to maintain

the stability of the internal environment [2]. The above-mentioned rapid clearance of amatoxins from circulation indicates the significance of early diagnosis and treatment in the enhanced elimination of toxins [10], and some even question the necessity and effectiveness of invasive management, such as that of systemic detoxification 36 h after *Amanita* ingestion [18,26]. In the detection group, these survivors of *Amanita* poisoning underwent plasmapheresis 2-3 days after mushroom consumption, indicating that plasmapheresis might be worthwhile even if executed after 36 h.

In our retrospective comparison, the detection procedure might have lengthened the initiation time of plasmapheresis from admission, possibly because some patients in the conventional group could get a definitive diagnosis based on their elevated liver enzymes and bilirubin when they came to the hospital. However, in the detection group, timely detection identified the existence of α-amanitin in patients before the appearance of liver injury, especially in patients who could not provide the photographs of mushrooms they had consumed. Plasmapheresis was consequently performed one day earlier, at a stage when liver impairment had not happened or become severe, in the detection group than the conventional group. Additionally, the fact should not be neglected that early intervention is highly dependent on early admission. Possibly, the popular medical science has arisen the public awareness of timely consultation and led to an earlier admission. Considering that *Amanita* poisoning often occurs at family gatherings, one confirmation of α-amanitin in one patient's blood/urine sample could support the diagnosis for the rest of concurrent patients. The α-amanitin identification by detection did help to confirm diagnosis, thus provided the clinicians with evidence to execute plasmapheresis for detoxification at an early stage in accordance with 'the earlier the better' policy of Pillukat et al. in 2016 [28]. Patients would always refuse aggressive treatment at the stage of gastrointestinal symptoms, however, our positive detection results persuaded them to accept treatment before liver failure would become irreversible. In remote areas, both members of the public and clinicians lack an understanding and the necessity of prompt diagnosis of highly lethal *Amanita* poisoning. Therefore, detection should be tailored as much as possible to local conditions to aid with timely diagnosis, which is of urgent need to establish and popularize an easy, fast and sensitive test method to use in emergency room.

The early collection and analysis of blood and urine samples is of great value, while the cost-effective detectable time limit for collection is short. Jaeger et al. carried out a fundamental study on the toxicokinetics of α-amanitin in 45 human patients [10], while more researchers focused on animal models [3,25,26]. These studies revealed that most toxins were eliminated through the feces, while others absorbed from gastrointestinal tract readily accumulated and remained in vital organs like the liver and kidneys for extended periods of time [10], and were detectable in urine in 90-120 min after ingestion [1]. The absorbed α-amanitin does not undergo metabolism in humans [35]. However, due to its low protein binding ratio [36], it will stay in plasma for only a very short time period (it is usually detectable in plasma within 36 h) [3], and its concentration will quickly change over time. Approximately 60% of absorbed toxins will be excreted into the bile and enter enterohepatic circulation [37] (detectable in the bile within 6 days after ingestion) [18], while the kidneys help with the final removal of more than 85% of amatoxins [38] (detectable in urine within 4 days) [10]. In addition, the clinical value of detection will fade as organ failure will appear and become irreversible after 72 h [39]. Based on the existing toxicokinetic investigation, as well as the detection results acquired in this study, we recommend that blood and urine samples are collected within 72 h, the earlier, the better, to assure reliable detection results.

The detection of α-amanitin in blood and urine samples has both qualitative and semi-quantitative significance. To date, a positive detection result has the stronger clinical guiding significance than the underlying predictive value of toxin concentration. The published literatures, however, fail to conclude whether the serum or urine concentrations have a relationship with liver function and outcome in *Amanita* poisoning cases, and we did not find the subtype-dose-time-outcome relationship in our small-scale study, either. The fact that different species of mushrooms within the *Amanita* genus contain other types of toxins, as well as the individual differences in susceptibility, will also make it more difficult to investigate this relationship. Some researchers have proposed the establishment of a standardized international registry system of *Amanita* poisoning cases to guide predictions and effective interventions in the future [40]. We recommend that clues of amatoxins, more specifically, their concentrations in body fluids and collection time should be recorded in this public database once launched. Ideally, fulfilling this database might help us find the subtype-dose-time-outcome formula and contribute to prognosis prediction, instruct individualized treatment, and save medical resources at the same time. Furthermore, it is necessary to describe a standard amatoxin detection process to guarantee the homogeneity of results among different laboratories.

The limitations of our study are obvious. First and foremost, limited by sample size, the effects of underlying physiological factors were difficult to control. The inevitable Berkson's bias in our single-center retrospective study also restricted the generalizability of our results. Additionally, we did not detect other subtypes of amatoxins or other kinds of toxins, therefore, the influence of such unknown toxins could not be evaluated. The α-amanitin concentrations in both blood and urine of some patients were not redetected after plasmapheresis sessions, making it less likely to evaluate the predictive value of α-amanitin concentration.

In conclusion, for mushroom poisoning patients, regardless of whether liver function injury has occurred at the time of consultation at an emergency department, the early detection of α-amanitin attributes to confirming diagnosis and promoting early medical intervention. A timely plasmapheresis under the guidance of detection can improve the prognosis of *Amanita* poisoning by alleviating the damage of liver and other organs. The detection of amatoxins is recommended to be established according to local conditions in different level hospitals, especially for high-risk areas where people have a general habit of eating self-collected mushrooms or mushrooms bought from non-standard markets. Further randomized controlled clinical trials of larger sample size are of urgent demand in finding better treatment options for *Amanita* poisoning.

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CRediT authorship contribution statement

Li-Ying Lin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Ya-Ling Tong**: Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Yuan-Qiang Lu**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology,

Project administration, Writing - original draft, Writing - review & editing.

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Ethical approval

This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (IIT20200005A).

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hbpd.2022.01.007.

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