Prevalence, severity, duration and resolution of cholestasis after acute liver failure

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ABSTRACT

Objective Persistent cholestasis may follow acute liver failure (ALF), but its course remains unknown. We aimed to describe the prevalence, onset, severity, duration and resolution of post-ALF cholestasis.

Design Cohort of 127 adult patients with ALF at a liver transplantation centre identified using electronic databases. We obtained laboratory data every 6 hours for the first week, daily until day 30 and weekly, when documented, until day 180.

Results Median age was 40.7 (IQR 31.0–52.4) years, median peak alanine aminotransferase level was 5494 (2521–8819) U/L and 87 (68.5%) cases had paracetamol toxicity. Overall, 12.6% underwent transplantation (3.4% for paracetamol vs 32.5% for non-paracetamol; p<0.001). Ninety-day mortality was 20.7% for paracetamol versus 32.5% for non-paracetamol; p<0.001.

Conclusion Persistent cholestasis may follow acute liver failure and may be associated with several biochemical abnormalities.

INTRODUCTION

Acute liver failure (ALF) is a rapidly progressive severe illness typically leading to intensive care unit (ICU) admission. ALF carries a high mortality without emergency liver transplantation (ELT). Nonetheless, with intensive supportive care, some patients (especially after paracetamol overdose) recover sufficient function without transplantation. However, in all cases, in the absence of transplantation, improvement can be slow and may be associated with several biochemical abnormalities.

Almost all studies of the biochemical abnormalities associated with ALF have focused on aminotransferase levels on admission to hospital, and/or have followed biochemical recovery for just 1 week from admission. No studies have provided a prolonged detailed assessment of post-ALF cholestasis.

Summary box

What is already known on this topic
► Following major injury, liver recovery is complex and can be slow.
► No studies have provided prolonged detailed assessment of post-ALF cholestasis.

What this study adds
► We have described the prevalence, severity, duration and time to normalisation of markers of blood purification of factors that lead to jaundice (cholestasis) in such patients admitted to intensive care.
► Only a minority of patients achieve normal cholestasis markers by hospital discharge, and some even fail to return to normal at subsequent longer follow-up.
► How this study might affect research, practice and/or policy
These findings inform clinical expectations of the natural course of this condition and help with both diagnosis and prognosis.
► Importantly, they can be of assistance during conversations with patients and families regarding realistic expectations post-ALF jaundice and its likely duration.
## Table 1. Baseline characteristics of the included patients considering only survivors and patients who did not undergo transplantation

|                            | Overall (n=85) | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
|-----------------------------|---------------|---------------------|------------------------|---------|
| **Age, years**              | 38.6 (28.3–51.2) | 38.6 (29.5–51.6) | 38.7 (27.3–46.8) | 0.906   |
| **Male gender, n (%)**      | 11 (12.9) | 6 (9.0) | 5 (27.8) | 0.050   |
| **Body mass index, kg/m²**  | 24.8 (21.6–27.8) | 24.6 (22.2–28.0) | 25.2 (21.3–27.1) | 0.451   |
| **Severity of illness**     |               |                     |                       |         |
| APACHE II                   | 14.0 (10.0–18.0) | 14.0 (9.5–18.0) | 15.5 (10.0–20.8) | 0.438   |
| APACHE III                  | 61.0 (46.0–80.0) | 59.0 (45.5–78.0) | 63.5 (50.2–81.8) | 0.788   |
| ANZROD                      | 0.09 (0.04–0.26) | 0.09 (0.04–0.26) | 0.09 (0.05–0.22) | 0.974   |
| **MET call admission, n (%)** | 3 (3.5) | 1 (1.5) | 2 (11.1) | 0.112   |
| **Acute liver failure aetiology, n (%)** | < 0.001 |                     |                       |         |
| Paracetamol                 | 67 (78.8) | 67 (100.0) | 0 (0.0) |         |
| Unknown                     | 6 (7.1) | 0 (0.0) | 6 (33.3) |         |
| Other drugs                 | 2 (2.4) | 2 (2.4) | 2 (11.1) |         |
| Vascular                    | 1 (1.2) | 1 (1.2) | 1 (5.6) |         |
| Amanita phalloides          | 3 (3.5) | 0 (0.0) | 3 (16.7) |         |
| Viral                       | 2 (2.4) | 2 (2.4) | 2 (11.1) |         |
| Alcohol                     | 2 (2.4) | 2 (2.4) | 2 (11.1) |         |
| Autoimmune                  | 1 (1.2) | 0 (0.0) | 1 (5.6) |         |
| NAFLD of pregnancy          | 1 (1.2) | 0 (0.0) | 1 (5.6) |         |
| **ICU source of admission, n (%)** | 0.104 |                     |                       |         |
| Other hospital              | 63 (74.1) | 52 (77.6) | 11 (61.1) |         |
| ICU from other hospital     | 15 (17.6) | 10 (14.9) | 5 (27.8) |         |
| Emergency department        | 4 (4.7) | 4 (6.0) | 0 (0.0) |         |
| Ward                        | 3 (3.5) | 1 (1.5) | 2 (11.1) |         |
| **Hospital source of admission, n (%)** | 0.425 |                     |                       |         |
| Other hospital              | 63 (74.1) | 50 (74.6) | 13 (72.2) |         |
| ICU from other hospital     | 13 (15.3) | 8 (11.9) | 5 (27.8) |         |
| Home                        | 5 (5.9) | 5 (7.5) | 0 (0.0) |         |
| Emergency department from other hospital | 3 (3.5) | 3 (4.5) | 0 (0.0) |         |
| Nursing home                | 1 (1.2) | 1 (1.5) | 0 (0.0) |         |
| **Hepatic encephalopathy, n (%)** | 0.780 |                     |                       |         |
| 1                           | 26 (30.6) | 20 (29.9) | 6 (33.3) | 0.968   |
| 2                           | 15 (17.6) | 13 (19.4) | 2 (11.1) |         |
| 3                           | 9 (10.6) | 7 (10.4) | 2 (11.1) |         |
| 4                           | 6 (7.1) | 5 (7.5) | 1 (5.6) |         |
| **Acute kidney injury at ICU admission, n (%)** | 0.772 |                     |                       |         |
| Renal replacement therapy   | 56 (65.9) | 45 (67.2) | 11 (61.1) |         |
| Mechanical ventilation      | 37 (43.5) | 29 (43.3) | 8 (44.4) |         |
| **Coexisting disorders, n (%)** | 0.999 |                     |                       |         |
| Chronic respiratory disease | 0 (0.0) | 0 (0.0) | 0 (0.0) | ---     |
| Chronic cardiovascular disease | 0 (0.0) | 0 (0.0) | 0 (0.0) | ---     |
| Chronic kidney disease      | 0 (0.0) | 0 (0.0) | 0 (0.0) | ---     |
| Immune disease              | 1 (1.2) | 1 (1.5) | 0 (0.0) | 0.999   |

Continued
Accordingly, in order to define the natural course of such cholestasis in the absence of liver transplantation, we aimed to study the prevalence, severity, duration and resolution of postinjury cholestasis in ALF patients overall and according to the aetiology of ALF (paracetamol vs non-paracetamol).

METHODS

Study design

We performed a single-centre retrospective observational study in an academic tertiary teaching hospital referral centre for liver transplantation (Austin Hospital, Heidelberg, Australia).

Patients

All adult patients (age >16 years) with an ICU admission diagnosis of ALF were eligible for inclusion. We excluded patients with chronic liver disease (either on historical, clinical, laboratory, radiographic and/or histopathologic results) based on agreement between two investigators (SW and CM). For patients who were readmitted to the ICU, only the first admission was included.

Data collection and definitions

We used the Australian and New Zealand Intensive Care Society Adult Patient database for patient screening and selection, and retrieval of patient demographics and biochemical and in-hospital outcomes. We obtained additional data, including aetiology of hepatic insult, hepatic encephalopathy scores on admission according to West Haven criteria (independently assessed by two investigators), 30-day and 90-day survival, vital signs, supportive therapies (including sedation, mechanical ventilation, renal replacement therapy) and laboratory test results from the hospital’s electronic medical records. Laboratory data were obtained every 6 hours for 7 days while in ICU, daily (when available) until day 30 and weekly (when available) until day 180, or until time of death or LT. Day 1 was the first day of ICU admission.

We defined normalisation of laboratory tests as two consecutive measurements within the normal laboratory range: ALT ≤ 40 U/L, AST ≤ 35 U/L, GGT ≤ 50 U/L, an ALP ≤ 110 U/L and bilirubin ≤ 21 µmol/L.

 Overall (n=85)  | Type of liver failure
|-----------------|-----------------|-----------------|-----------------|----------|
|                 | Overall (n=85)  | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
| Immunosuppression | 3 (3.5)         | 3 (4.5)          | 0 (0.0)         | 0.999   |
| Leukaemia       | 0 (0.0)         | 0 (0.0)          | 0 (0.0)         | ---     |

Vital signs at ICU admission

|                       | Overall (n=85) | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
|-----------------------|----------------|--------------------|------------------------|---------|
| Highest temperature, °C | 37.0 (36.5–37.5) | 37.0 (36.5–37.5)  | 37.0 (36.5–37.3)       | 0.293   |
| Highest heart rate, bpm | 115 (100–130)   | 112 (101–130)     | 117 (101–134)          | 0.901   |
| Lowest mean arterial pressure, mm Hg | 67 (62–75) | 67 (62–75) | 70 (63–78) | 0.401   |
| Highest respiratory rate, breaths/min | 22 (18–28) | 22 (16–28) | 21 (18–27) | 0.816   |
| Urine output, mL       | 1247 (369–1824) | 1070 (323–1650)  | 1650 (1102–2150)       | 0.061   |

Laboratory tests at ICU admission

|                     | Overall (n=85) | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
|---------------------|----------------|--------------------|------------------------|---------|
| Lowest albumin, g/L | 27 (24–29)     | 26 (23–29)         | 27 (25–29)             | 0.267   |
| pH                  | 7.41 (7.30–7.46) | 7.43 (7.30–7.46)  | 7.39 (7.31–7.41)       | 0.242   |
| PaO2/FiO2           | 424 (293–533)  | 414 (300–537)     | 443 (221–500)          | 0.839   |
| PaCO2, mm Hg        | 33 (29–37)     | 33 (28–37)         | 33 (30–39)             | 0.546   |
| Bilirubin, µmol/L   | 81 (50–127)    | 81 (50–127)       | 85 (50–143)            | 0.683   |
| Highest creatinine, µmol/L | 132 (75–257) | 129 (76–186) | 182 (69–348.2) | 0.444   |
| Lowest glucose, mmol/L | 5.3 (4.6–6.5) | 5.3 (4.7–6.3)  | 5.3 (4.2–6.8)          | 0.743   |
| Lowest haemoglobin, g/L | 102 (87–114) | 102 (87–112) | 103 (86–121) | 0.519   |
| Highest white blood cell count, x10^9/L | 10.8 (6.7–16.0) | 10.8 (6.6–15.3) | 11.9 (7.1–18.6) | 0.543   |
| Lactate, mmol/L     | 4.1 (3.2–5.9)  | 4.1 (3.4–5.8)     | 4.1 (2.9–8.9)          | 0.826   |
| Lowest platelets, x10^9/L | 107 (70–162) | 100 (70–162) | 126 (70–165) | 0.607   |
| Urea, mmol/L        | 5.4 (3.3–9.9)  | 5.0 (3.2–9.9)     | 6.3 (4.5–17.0)         | 0.202   |

Data are median (IQR) or N (%).

ANZROD, Australian and New Zealand risk of death; APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit; MET, medical emergency team; NAFLD, non-alcoholic fatty liver disease.
### Laboratory tests in the included patients considering only survivors and patients who did not undergo transplantation

| Transaminases | | | | | |
|---|---|---|---|---|
| | Overall (n=85) | Type of liver failure | | | |
| | | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
| Alanine aminotransferase | | | | | |
| Peak, U/L | 5594 (3463–8868) | 6529 (3862–9356) | 4468 (2314–5992) | 0.018 |
| Days between ICU admission and peak | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 0.0 (0.0–0.6) | 0.004 |
| Days until normalisation* | 21.5 (17.5–29.5) | 20.0 (18.0–26.5) | 45.0 (29.0–80.0) | 0.373 |
| Patients with normalisation at latest follow-up | 18/62 (29.0) | 15/46 (32.6) | 3/16 (18.8) | 0.355 |
| Median time until last measurements, days | 16.0 (10.0–30.0) | 15.0 (9.0–29.0) | 19.0 (12.0–30.8) | 0.438 |
| Number of measurements after hospital DC | 1.6±2.7 | 1.4±2.2 | 2.5±4.2 | 0.133 |
| Patients with normalisation until hospital DC | 12/85 (14.1) | 11/67 (16.4) | 1/18 (5.6) | 0.446 |
| Aspartate aminotransferase | | | | | |
| Peak, U/L | 7343 (3808–11 010) | 7711 (4987–11 010) | 5984 (2954–11 160) | 0.407 |
| Days between ICU admission and peak | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 0.0 (0.0–0.5) | 0.034 |
| Days until normalisation* | 12.0 (11.0–14.0) | 12.0 (11.0–14.0) | 13.0 (11.0–15.0) | 0.999 |
| Patients with normalisation at latest follow-up | 13/50 (26.0) | 9/37 (24.3) | 4/13 (30.8) | 0.719 |
| Median time until last measurements, days | 13.0 (8.2–22.8) | 13.5 (8.2–23.0) | 13.0 (10.0–19.0) | 0.625 |
| Number of measurements after hospital DC | 0.8±1.0 | 0.7±0.9 | 1.1±1.3 | 0.139 |
| Patients with normalisation until hospital DC | 9/78 (11.5) | 7/62 (11.3) | 2/16 (12.5) | 0.999 |
| Coagulation | | | | | |
| APTT | | | | | |
| Peak, s | 44.0 (38.0–60.0) | 44.0 (38.0–55.0) | 57.5 (40.2–69.5) | 0.177 |
| Days between ICU admission and peak | 0.6 (0.0–4.6) | 0.6 (0.0–4.6) | 2.0 (0.1–4.6) | 0.444 |
| INR | | | | | |
| Peak | 4.5 (3.1–5.8) | 4.6 (3.2–5.9) | 3.7 (2.3–4.7) | 0.090 |
| Days between ICU admission and peak | 0.0 (0.0–0.5) | 0.0 (0.0–0.0) | 0.0 (0.0–0.6) | 0.245 |
| Days until normalisation* | 7.5 (6.0–11.0) | 9.0 (5.8–13.2) | 7.0 (6.0–7.0) | 0.090 |
| Patients with normalisation at latest follow-up | 42/68 (61.8) | 32/52 (61.5) | 10/16 (62.5) | 0.999 |
| Median time until last measurements, days | 13.0 (9.0–27.0) | 15.0 (8.0–27.5) | 12.5 (10.2–18.5) | 0.539 |
| Number of measurements after hospital DC | 1.0±1.4 | 1.0±1.5 | 0.9±0.9 | 0.846 |
| Patients with normalisation until hospital DC | 36/85 (42.4) | 27/67 (40.3) | 9/18 (50.0) | 0.592 |
| Renal | | | | | |
| Creatinine | | | | | |
| Peak, µmol/L | 210.0 (111.0–451.0) | 200.0 (106.5–450.0) | 295.0 (139.2–476.5) | 0.423 |
| Days between ICU admission and peak | 4.5 (0.0–9.6) | 4.6 (0.0–10.1) | 3.5 (0.1–9.3) | 0.952 |
| Days until normalisation* | 1.0 (1.0–3.0) | 1.0 (1.0–3.0) | 2.0 (1.0–5.0) | 0.160 |
| Patients with normalisation at latest follow-up | 69/81 (85.2) | 56/63 (88.9) | 13/18 (72.2) | 0.126 |
| Median time until last measurements, days | 15.0 (9.0–31.0) | 15.0 (9.0–30.5) | 16.0 (11.2–30.8) | 0.698 |
| Number of measurements after hospital DC | 1.6±2.9 | 1.4±2.3 | 2.5±4.4 | 0.158 |
| Patients with normalisation until hospital DC | 66/85 (77.6) | 54/67 (80.6) | 12/18 (66.7) | 0.217 |
| Urea | | | | | |
| Peak, mmol/L | 12.5 (7.6–18.5) | 11.6 (7.4–17.6) | 14.0 (10.0–22.2) | 0.280 |
| Days between ICU admission and peak | 6.5 (0.5–10.5) | 6.5 (0.6–11.5) | 5.6 (0.1–8.3) | 0.491 |
| Others | | | | | |
| Ammonia | | | | | |
| Peak, L | 123.0 (92.0–178.0) | 123.0 (96.0–184.5) | 119.0 (75.8–143.2) | 0.346 |

Continued
the test, as described above. The peak value was defined as the highest value within the follow-up period.

**Statistical analysis**

Continuous variables are reported as median (quartile 25% to quartile 75%) or means with 95% CIs as appropriate and categorical variables as number (percentage). We divided patients according to the aetiology of ALF into paracetamol or non-paracetamol-related ALF. We compared continuous variables using the Wilcoxon rank-sum test and, for categorical variables, using Fisher’s exact test.

We plotted laboratory tests (when performed) over time after excluding measurements after ELT, stratified according to aetiology and presented as mean with 95% CI. We compared groups using mixed-effect linear models with the group, time (as a continuous variable) and interaction between group×time included as fixed effect, with patients included as random effect to account for repeated measurements. Two p values are reported: (1) p value for the group difference, reflecting the overall test for differences between groups across the follow-up; and (2) p values for the group×time interaction, evaluating whether change over time differed by group. All analyses were performed using the software R (R Core Team, 2016, Vienna, Austria), and, given the multiplicity of comparisons and variables, a p value<0.01 was considered statistically significant.

**RESULTS**

**Patients**

We studied 127 ALF patients admitted to the study ICU between 1 January 2010 and 30 June 2020. Their baseline characteristics are reported in online supplemental table 1. Median age was 40.7 (31–52) years, 84.3% were women, and the most frequent ALF aetiology was paracetamol overdose (68.5%). Patients in the paracetamol group were more often women, had higher ALT and AST and more prolonged international normalised ratio (INR) values, but lower bilirubin levels at ICU admission. All other laboratory tests and vital signs were similar.

On the day of ICU admission, 90.3% were treated with continuous renal replacement therapy and 55.1% with mechanical ventilation. ALT, bilirubin and ALP differed between transplanted patients, non-transplanted survivors and non-transplanted non-survivors at presentation (online supplemental etable 2).

**Clinical outcomes**

Overall, 12.6% of patients underwent ELT, with fewer patients receiving ELT in the paracetamol group (3.4% vs 32.5%; p<0.001). Overall, 30-day mortality was 20.5% (18.4% paracetamol vs 25% non-paracetamol; p=0.478) and 90-day mortality was 23.6% (20.7% paracetamol vs 30% non-paracetamol; p=0.268) (online supplemental etable 3). After excluding transplantation and non-survivors, 85 non-transplanted survivors remained for analysis of post-ALF cholestasis (table 1). The duration of stay in ICU and hospital for such survivors is presented in online supplemental etable 4.

**Laboratory tests**

Laboratory tests for markers of organ injury including time to peak level and time to normalisation in survivors
are presented in table 2. Patients exhibited major abnormalities of coagulation, ammonia, lactate and renal function tests, with greater abnormalities of ALT and AST and earlier onset of abnormalities for paracetamol overdose. Overall, ALT levels returned to normal in 14.1% of patients (16.4% paracetamol vs 5.6% non-paracetamol) with a similar pattern for AST. At hospital discharge, the INR had normalised in approximately half of ALF patients, and the serum creatinine in three-quarters of patients.

### Cholestasis markers
Among non-transplanted survivors, the peak level, days to peak level after ICU admission and days to normalisation and number and percentage of patients achieving normalisation are presented in table 3. The median peak bilirubin level was 169 µmol/L, peaking at day 3.5 days. Although the bilirubin level was lower at baseline for paracetamol, it rapidly increased to equivalent levels to non-paracetamol ALF by day 4 (figure 1). In the patients where normalisation occurred at last follow-up (n=30), the median number of days to normalisation was 14.

Changes in bilirubin were accompanied by an ALP increase to a median peak of 183 U/L after a median of 4.5 days, and GGT to 341 U/L by 4.5 days (table 3). The courses of ALT, ALP, GGT and bilirubin levels in the week

| Laboratory tests results for markers of cholestasis considering only survivors and patients who did not undergo transplantation |
|-------------------------------------------------------------------------------------------------------------|
| **Table 3**                                                                                     | Overall (n=85) | Type of liver failure | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
|-------------------------------------------------------------------------------------------------------------|
| **Cholestatic markers**                                                                                   |               |                       |                   |                          |         |
| **Alkaline phosphatase**                                                                                  |               |                       |                   |                          |         |
| Peak, U/L                                                                                                 | 183.0 (140.0–243.0) | 179.0 (134.5–244.5) | 207.0 (167.0–225.8) | 0.501                   |
| Days between ICU admission and peak                                                                      | 4.5 (1.5–8.5)   | 4.5 (1.5–8.6)         | 5.1 (2.1–8.3)      | 0.666                   |
| Days until normalisation*†                                                                                 | 6.0 (4.5–12.5)  | 6.0 (4.2–12.0)        | 12.0 (10.0–13.0)   | 0.306                   |
| Patients with normalisation at latest follow-up                                                         | 31 / 69 (44.9) | 26 / 52 (50.0)        | 5 / 17 (29.4)      | 0.168                   |
| Median time until last measurements, days                                                                | 16.0 (10.0–30.0) | 15.0 (9.0–29.0)       | 19.0 (12.0–30.8)   | 0.438                   |
| Number of measurements after hospital DC                                                                | 1.6±2.7         | 1.4±2.2               | 2.4±4.2            | 0.154                   |
| Patients with normalisation until hospital DC                                                            | 29 / 72 (40.3)  | 26 / 56 (46.4)        | 3 / 16 (18.8)      | 0.081                   |
| **Gamma-glutamyl transferase**                                                                           |               |                       |                   |                          |         |
| Peak, U/L                                                                                                 | 341.0 (223.0–492.0) | 338.0 (228.0–500.5) | 354.0 (195.5–473.8) | 0.936                   |
| Days between ICU admission and peak                                                                      | 4.6 (3.5–8.6)   | 4.5 (3.5–8.6)         | 5.6 (4.5–9.0)      | 0.511                   |
| Days until normalisation*†                                                                                 | 15.0 (8.5–40.0) | 12.5 (4.8–24.2)       | 28.0 (24.0–43.5)   | 0.153                   |
| Patients with normalisation at latest follow-up                                                         | 11 / 62 (17.7)  | 8 / 45 (17.8)         | 3 / 17 (17.6)      | 0.999                   |
| Median time until last measurements, days                                                                | 16.0 (10.0–30.0) | 15.0 (9.0–29.0)       | 19.0 (12.0–30.8)   | 0.438                   |
| Number of measurements after hospital DC                                                                | 1.6±2.7         | 1.4±2.2               | 2.4±4.2            | 0.154                   |
| Patients with normalisation until hospital DC                                                            | 7 / 84 (8.3)    | 6 / 67 (9.0)          | 1 / 17 (5.9)       | 0.999                   |
| **Bilirubin**                                                                                             |               |                       |                   |                          |         |
| Peak, µmol/L                                                                                               | 169.0 (80.0–320.0) | 181.0 (78.0–313.5) | 136.5 (88.8–396.5) | 0.855                   |
| Days between ICU admission and peak                                                                      | 3.5 (1.5–10.6)  | 3.6 (1.0–10.6)        | 2.5 (1.5–8.1)      | 0.553                   |
| Days until normalisation*†                                                                                 | 14.0 (5.0–50.5) | 17.0 (5.0–66.0)       | 11.0 (8.5–14.8)    | 0.541                   |
| Patients with normalisation at latest follow-up                                                         | 30 / 64 (46.9)  | 22 / 48 (45.8)        | 8 / 16 (50.0)      | 0.781                   |
| Median time until last measurements, days                                                                | 16.0 (10.0–30.0) | 15.0 (9.0–29.0)       | 19.0 (12.0–30.8)   | 0.438                   |
| Number of measurements after hospital DC                                                                | 1.6±2.7         | 1.4±2.2               | 2.4±4.2            | 0.153                   |
| Patients with normalisation until hospital DC                                                            | 16 / 85 (18.8)  | 13 / 67 (19.4)        | 3 / 18 (16.7)      | 0.999                   |

Data are median (IQR) or N (%).
*Days until normalisation applies only to those patients who achieved normalisation. Normalisation was defined as two consecutive measurements with alkaline phosphatase<110 U/L or gamma-glutamyl transferase<50 35 U/L or bilirubin≤21 µmol/L.†Considering the time after the first abnormal value until normalisation.
DC, discharge; ICU, intensive care unit.
following ICU admission are shown in figure 2. When followed to 30, 60, 90 and 180 days, as shown in figure 3, ALT levels were essentially down to normal levels by day 15. In contrast, all markers of cholestasis remained abnormal for much longer (table 4). Patients with follow-up measurement of bilirubin after hospital discharge did not appear to differ from those without such follow-up (table 5).

**DISCUSSION**

**Key findings**

We studied the prevalence, severity, duration and time to normalisation of biochemical markers of cholestasis (bilirubin, ALP, GGT) after ALF in patients who survived without liver transplantation. We found that all patients developed biochemical evidence of cholestasis, which peaked between 3 and 5 days after ICU admission. Moreover, at hospital discharge, only one in five patients had achieved normal bilirubin levels and, at last follow-up, despite ALT levels returning to normal measurement at a median of 16 days, less than half had normal bilirubin levels. Finally, similarly, patterns of failed normalisation at hospital discharge or last follow-up also applied to ALP and GGT levels.

**Relationship to previous studies**

To the best of our knowledge, this is the only study detailing changes in bilirubin and liver enzymes in critically ill patients with ALF until hospital discharge and subsequent follow-up. In particular, it is the only one to report changes in biochemical markers of cholestasis over such an extended period. Previous studies have documented laboratory results in ALF patients, but typically only at the time of ICU admission or peak values.3–12 Kumar et al found predictive value in combined assessment of binary trends of ammonia, bilirubin and INR (elevated, increased or decreased) but only over the first 3 days.13 Only three studies, extended their observations to 1 week. Kim et al noted that a raised bilirubin and activated partial thromboplastin time were associated with mortality after poisonous mushroom ingestion, but of 93 study patients, only 23 had ALF and 10 died.14 Koch et al reported lower bilirubin and ALP levels in 7 patients with ALF secondary to paracetamol versus 16 non-paracetamol patients but no laboratory follow-up beyond 7 days.15 Finally, Li et al grouped 380 paediatric patients with indeterminate liver failure (only 48 had severe disease) according to biochemical (INR, bilirubin) and
clinical data in the first 7 days of enrolment into common subgroups. However, only three or more laboratory values of any one measurement were required, missing data points were imputed based on observed data and linear trajectories in biochemical changes were assumed.\textsuperscript{16}

**Implications**

Our findings imply that, in ALF patients, cholestasis is ubiquitous at presentation and peaks between 3 and 5 days. Moreover, they indicate that the cholestatic phase of injury is long. Finally, they demonstrate that less than one

### Table 4 Liver enzyme levels at different follow up periods

|                   | Overall (n=85) | Paracetamol (n=67) | Non-paracetamol (n=18) | P value |
|-------------------|----------------|--------------------|------------------------|---------|
| Follow-up time    |                |                    |                        |         |
|                   | 16.0 (10.0–31.0)| 15.0 (9.0–30.5)    | 19.0 (12.0–30.8)       | 0.491   |
| Day 7             |                |                    |                        |         |
| Alanine aminotransferase | 701.6 (455.5–1013.0) | 701.6 (455.5–1009.9) | 680.4 (476.5–1230.8) | 0.799   |
| Aspartate aminotransferase | 85.3 (68.8–116.6) | 83.8 (68.5–115.2) | 97.2 (75.2–214.0) | 0.212   |
| Alkaline phosphatase | 134.8 (108.2–181.3) | 133.0 (103.7–182.2) | 151.3 (118.8–175.0) | 0.566   |
| Gamma-glutamyl transferase | 210.3 (135.9–340.9) | 218.8 (135.9–320.9) | 204.9 (161.8–449.4) | 0.524   |
| Bilirubin          | 119.2 (43.9–231.8) | 134.7 (56.5–238.0) | 75.8 (38.3–201.5) | 0.448   |
| Day 15             |                |                    |                        |         |
| Alanine aminotransferase | 89.8 (61.1–121.4) | 88.7 (70.3–116.3) | 104.3 (52.2–130.2) | 0.986   |
| Aspartate aminotransferase | 56.5 (37.4–77.8) | 56.5 (37.9–73.2) | 59.7 (35.4–85.7) | 0.909   |
| Alkaline phosphatase | 118.0 (105.0–160.4) | 116.7 (105.0–171.7) | 120.0 (108.8–145.7) | 0.901   |
| Gamma-glutamyl transferase | 129.3 (74.5–303.4) | 126.7 (75.0–269.0) | 253.7 (75.2–469.5) | 0.682   |
| Bilirubin          | 213.6 (27.9–300.8) | 242.7 (60.0–291.3) | 24.0 (18.5–212.3) | 0.130   |
| Day 30             |                |                    |                        |         |
| Alanine aminotransferase | 40.4 (30.0–59.5) | 51.7 (35.2–99.0) | 32.0 (20.0–32.0) | 0.061   |
| Aspartate aminotransferase | 86.0 (68.1–113.2) | 86.0 (68.1–113.2) | ---                   | ---     |
| Alkaline phosphatase | 123.8 (78.2–150.8) | 138.0 (110.7–175.8) | 67.0 (64.0–117.0) | 0.079   |
| Gamma-glutamyl transferase | 115.3 (65.2–173.2) | 146.5 (84.8–278.8) | 72.0 (39.0–80.0) | 0.157   |
| Bilirubin          | 130.0 (14.2–364.1) | 203.5 (74.0–373.8) | 9.0 (8.0–14.0) | 0.047   |
| Day 60             |                |                    |                        |         |
| Alanine aminotransferase | 61.0 (39.5–100.0) | 72.0 (52.8–108.5) | 23.0 (23.0–23.0) | 0.134   |
| Aspartate aminotransferase | 95.0 (52.0–144.8) | 95.0 (52.0–144.8) | ---                   | ---     |
| Alkaline phosphatase | 177.0 (86.5–202.0) | 179.0 (128.2–212.5) | 61.0 (61.0–61.0) | 0.207   |
| Gamma-glutamyl transferase | 196.0 (96.0–514.5) | 341.0 (157.0–528.8) | 44.0 (44.0–44.0) | 0.134   |
| Bilirubin          | 42.0 (19.5–123.0) | 50.0 (27.0–155.5) | 8.0 (8.0–8.0) | 0.134   |
| Day 90             |                |                    |                        |         |
| Alanine aminotransferase | 18.0 (12.0–19.9) | 18.0 (16.0–18.6) | 20.8 (14.9–26.6) | 0.999   |
| Aspartate aminotransferase | ---            | ---                | ---                   | ---     |
| Alkaline phosphatase | 81.2 (69.5–103.9) | 81.2 (64.6–105.6) | 88.5 (78.8–98.2) | 0.999   |
| Gamma-glutamyl transferase | 35.5 (25.2–57.2) | 51.2 (37.2–146.9) | 19.5 (17.8–21.2) | 0.064   |
| Bilirubin          | 9.3 (5.6–17.0) | 15.6 (11.0–21.4) | 4.8 (4.4–5.1) | 0.064   |
| Day 180            |                |                    |                        |         |
| Alanine aminotransferase | 20.0 (13.5–40.8) | 30.5 (14.5–48.0) | 17.5 (13.8–21.2) | 0.355   |
| Aspartate aminotransferase | 52.0 (52.0–52.0) | 52.0 (52.0–52.0) | ---                   | ---     |
| Alkaline phosphatase | 140.5 (103.5–166.2) | 160.5 (144.8–184.8) | 86.0 (82.0–90.0) | 0.064   |
| Gamma-glutamyl transferase | 133.5 (22.5–360.8) | 320.5 (186.8–418.8) | 18.5 (17.2–19.8) | 0.064   |
| Bilirubin          | 7.0 (3.2–11.5) | 6.5 (3.0–11.5) | 8.0 (6.0–10.0) | 0.639   |

Data are median (IQR).

Definitions: day 7 (between day 6 and 8); day 15 (between day 14 and 16); day 30 (between day 29 and 31); day 60 (between day 55 and 65); day 90 (between day 80 and 100); day 180 (between day 160 and 200).
Table 5  Baseline characteristics of the included patients according to the measurement or not of bilirubin after hospital discharge considering only survivors and patients who did not undergo transplantation

| Measurement of bilirubin after discharge | Yes (n=57) | No (n=28) | P value |
|----------------------------------------|-----------|-----------|---------|
| **Age, years**                          | 38.6 (31.1–48.0) | 39.0 (24.1–55.2) | 0.844   |
| **Male gender, n (%)**                  | 8 (14.0)  | 3 (10.7)  | 0.999   |
| **Body mass index, kg/m²**              | 24.2 (21.3–27.1) | 25.7 (22.9–29.4) | 0.284   |
| **Severity of illness**                 |           |           |         |
| APACHE II                               | 14.0 (9.0–18.0) | 14.0 (10.0–19.2) | 0.650   |
| APACHE III                              | 59.0 (45.0–78.0) | 63.0 (46.0–82.0) | 0.397   |
| ANZROD                                  | 0.1 (0.0–0.2) | 0.1 (0.0–0.3) | 0.466   |
| **MET call admission, n (%)**           | 1 (1.8)  | 2 (7.1)  | 0.251   |
| **Acute liver failure aetiology, n (%)**|           |           | 0.211   |
| Paracetamol                             | 41 (71.9) | 26 (92.9) |        |
| Unknown                                 | 6 (10.5)  | 0 (0.0)  |        |
| Other drugs                             | 1 (1.8)  | 1 (3.6)  |        |
| Vascular                                | 1 (1.8)  | 0 (0.0)  |        |
| Amanita phalloides                      | 3 (5.3)  | 0 (0.0)  |        |
| Viral                                   | 2 (3.5)  | 0 (0.0)  |        |
| Alcohol                                 | 2 (3.5)  | 0 (0.0)  |        |
| Autoimmune                              | 1 (1.8)  | 0 (0.0)  |        |
| NAFLD of pregnancy                      | 0 (0.0)  | 1 (3.6)  |        |
| **ICU source of admission, n (%)**      |           |           | 0.338   |
| Other hospital                          | 45 (78.9) | 18 (64.3) |        |
| ICU from other hospital                 | 9 (15.8)  | 6 (21.4)  |        |
| Emergency department                    | 2 (3.5)  | 2 (7.1)  |        |
| Ward                                    | 1 (1.8)  | 2 (7.1)  |        |
| **Hospital source of admission, n (%)** |           |           | 0.110   |
| Other hospital                          | 46 (80.7) | 17 (60.7) |        |
| ICU from other hospital                 | 8 (14.0)  | 5 (17.9)  |        |
| Home                                    | 2 (3.5)  | 3 (10.7)  |        |
| Emergency department from other hospital| 1 (1.8)  | 2 (7.1)  |        |
| Nursing home                            | 0 (0.0)  | 1 (3.6)  |        |
| **Hepatic encephalopathy, n (%)**       | 36 (63.2) | 20 (71.4) | 0.478   |
| 1                                       | 17 (29.8) | 9 (32.1)  | 0.578   |
| 2                                       | 10 (17.5) | 5 (17.9)  |        |
| 3                                       | 4 (7.0)   | 5 (17.9)  |        |
| 4                                       | 5 (8.8)   | 1 (3.6)   |        |
| **Acute kidney injury at ICU admission, n (%)** | 14 (24.6) | 8 (28.6) | 0.793   |
| **Organ support at ICU admission, n (%)** |           |           |         |
| Renal replacement therapy               | 37 (86.0) | 19 (86.4) | 0.999   |
| Mechanical ventilation                  | 22 (38.6) | 15 (53.6) | 0.246   |
| Coexisting disorders, n (%)              |           |           |         |
| Chronic respiratory disease             | 0 (0.0)   | 0 (0.0)   | ---     |
| Chronic cardiovascular disease           | 0 (0.0)   | 0 (0.0)   | ---     |
| Chronic kidney disease                  | 1 (1.8)   | 1 (3.6)   | 0.999   |
| Immune disease                          | 1 (1.8)   | 0 (0.0)   | 0.999   |

Continued
in five patients will normalise their cholestatic markers at hospital discharge and less than one in two patients will achieve recovery from cholestasis at last follow-up within 180 days. This implies that the true time to normalisation could not be determined in the majority of patients.

**Strengths and limitations**

This is the only study to report the changes in laboratory measurements over time after ALF. We have reported data on critically ill ALF survivors, in what is one of the largest reported cohorts of ALF patients in the literature. Additionally, for the first time we address the issue of post-ALF cholestasis, which has, to date, remained unexplored. Furthermore, for the first time, we provide granular biochemical information for a median follow of 16 days after ICU admission.

We acknowledge several limitations. This is a single-centre study with all the limitations inherent to such a study design. However, our ICU and transplantation centre has all the typical features of other similar centres in resource-rich countries, and our findings are likely generalisable to such centres. As the study is retrospective in nature, biochemical data collection was not protocolised. Thus, after discharge from the ICU, the measurement of blood biochemistry decreased with time and patients were discharged prior to normalisation of blood tests. As subsequent measurements were only dictated by clinical need and/or may have occurred outside of our electronic medical record system, our ability to report on biochemical variables beyond 30 days is limited. However, no data exist in the literature beyond day 7, making our report the most detailed representation of changes over time to date.

**CONCLUSION**

In summary, in ALF patients, postinjury cholestasis is ubiquitous and peaks after 3–5 days. This cholestatic phase of injury is prolonged, with only a minority of patients achieving normal cholestatic markers at hospital discharge and a median follow-up of 16 days, and, in some patients, failing to return to normal even at subsequent discharge.

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**Table 5 Continued**

| Measurement of bilirubin after discharge | Yes (n=57) | No (n=28) | P value |
|-----------------------------------------|-----------|-----------|---------|
| **Immunosuppression**                   | 3 (5.3)   | 0 (0.0)   | 0.548   |
| **Leukaemia**                           | 0 (0.0)   | 0 (0.0)   | ---     |
| **Vital signs at ICU admission**        |           |           |         |
| Highest temperature, °C                 | 37.1 (36.5–37.5) | 37.0 (36.5–37.1) | 0.203   |
| Highest heart rate, bpm                 | 110.0 (100.0–130.0) | 124.0 (109.5–140.0) | 0.064   |
| Lowest mean arterial pressure, mm Hg    | 67.0 (62.0–77.2) | 67.0 (59.8–72.5) | 0.326   |
| Highest respiratory rate, breaths/min   | 22.0 (18.0–28.0) | 20.0 (15.8–25.0) | 0.480   |
| Urine output, mL                        | 1150.0 (330.0–1932.5) | 1343.5 (431.8–1631.2) | 0.896   |
| **Laboratory tests at ICU admission**   |           |           |         |
| Lowest albumin, g/L                     | 27.0 (24.0–29.0) | 26.0 (22.2–29.8) | 0.974   |
| pH                                      | 7.4 (7.3–7.5) | 7.4 (7.3–7.5) | 0.918   |
| PaO₂/FiO₂                                | 423.8 (269.6–533.3) | 426.7 (359.5–532.1) | 0.630   |
| PaCO₂, mm Hg                            | 31.0 (29.0–36.5) | 34.5 (30.2–38.5) | 0.152   |
| Bilirubin, µmol/L                       | 84.0 (54.0–127.5) | 81.0 (44.8–121.2) | 0.730   |
| Highest creatinine, µmol/L              | 134.0 (75.5–265.5) | 124.0 (75.0–160.8) | 0.413   |
| Lowest glucose, mmol/L                  | 5.2 (4.6–6.5) | 5.4 (4.6–6.5) | 0.532   |
| Lowest haemoglobin, g/L                 | 102 (83–117) | 103 (89–111) | 0.907   |
| Highest white blood cell count, ×10⁹/L  | 9.6 (6.2–15.8) | 12.3 (8.2–15.7) | 0.470   |
| Lactate, mmol/L                         | 4.1 (3.4–6.5) | 4.6 (2.4–5.6) | 0.568   |
| Lowest platelets, ×10⁹/L                 | 100.0 (68.5–165.5) | 120.5 (76.2–159.2) | 0.927   |
| Urea, mmol/L                            | 6.3 (3.8–11.4) | 4.8 (3.2–9.3) | 0.299   |
| **Clinical outcomes**                   |           |           |         |
| ICU length of stay, days                | 6.2 (2.6–8.6) | 10.0 (4.4–19.8) | 0.027   |
| Hospital length of stay, day            | 11.6 (8.1–18.0) | 15.5 (8.6–6.9) | 0.051   |

Data are median (IQR) or N (%)
ANZROD, Australian and New Zealand risk of death; APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit; MET, medical emergency team; NAFLD, non-alcoholic fatty liver disease.
follow-up. These findings inform clinical expectations of the natural course of this condition and help with both diagnosis and prognosis. Importantly, they can be of assistance during conversations with patients and families regarding realistic expectations post-ALF jaundice and its likely duration. Further studies are needed to ascertain whether normalisation of post-ALF cholestasis does eventually occur in all survivors and, if so, when and to understand the impact of long-term cholestasis on future liver function and outcomes.

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