Research Article
Cholestasis and Autoimmune Diseases

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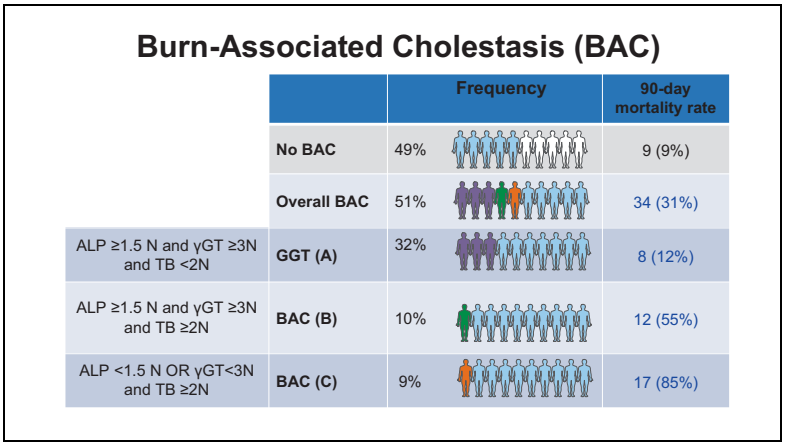
Contributing factors and outcomes of burn-associated cholestasis

Graphical abstract



Highlights

- In this cohort study, intrahepatic cholestasis was found in half of patients suffering from severe burn injuries.
- The presence of burn-associated cholestasis doubled the risk of death during the burn unit stay.
- There was a strong association between burn-associated cholestasis, bacteraemia and extrahepatic organ failure.
- Patients with elevated bilirubin levels without elevated ALP and GGT levels had the highest risk of mortality.
- Burn-associated cholestasis was associated with a long-term risk of sclerosing cholangitis.

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Lay summary

Cholestasis is common after burn injuries and is associated with burn severity, sepsis, organ failure and mortality. Patients with hyperbilirubinemia without elevated alkaline phosphatase and gamma-glutamyltransferase levels after the burn injury have a poor prognosis. Patients with burn-associated cholestasis may develop sclerosing cholangitis and secondary biliary cirrhosis.

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Contributing factors and outcomes of burn-associated cholestasis

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Background & Aims: Cholestasis often occurs after burn injuries. However, the prevalence of cholestasis and its effect on outcomes in patients with severe burn injuries are unknown. The aim of this study was to describe the course and the burden of cholestasis in a cohort of severely burned adult patients.

Methods: We investigated the relationship between burn-associated cholestasis (BAC) and clinical outcomes in a retrospective cohort of patients admitted to our unit for severe burn injuries between 2012 and 2015. BAC was defined as an increased level of serum alkaline phosphatase (ALP) ≥1.5x the upper limit of normal (ULN) with an increased level of gamma-glutamyltransferase (GGT) ≥3x ULN, or as an increased level of total bilirubin ≥2x ULN.

Results: A total of 214 patients were included: 111 (52%) patients developed BAC after a median (IQR) stay of 9 (5–16) days. At 90 days, the mortality rate was 20%, including 34 and 9 patients with and without BAC (p <0.001), respectively, which corresponded to a 2.5-fold higher (95% CI 1.2–5.2, p = 0.012) risk of 90-day mortality for patients with BAC. After being adjusted for severity of illness, patients with BAC, hyperbilirubinemia and without elevated ALP and GGT levels had a hazard ratio of 4.51 (95% CI 1.87–10.87) for 90-day mortality. BAC was associated with the severity of the burn injury, shock and bacteraemia. BAC was present in 38 (51%) patients at discharge, and 7 (18%) patients had secondary sclerosing cholangitis. These patients maintained elevated levels of ALP and GGT that were 5.8x (1.7–15) the ULN and 11x the ULN (4.5–22), respectively, 20 months (3.5–35) after discharge.

Conclusion: BAC is prevalent among patients with severe burn injuries and is associated with worse short-term outcomes, especially when total bilirubin levels were increased without elevated ALP and GGT levels. BAC survivors are at risk of developing sclerosing cholangitis.

Lay summary: Cholestasis is common after burn injuries and is associated with burn severity, sepsis, organ failure and mortality. Patients with hyperbilirubinemia without elevated alkaline phosphatase and gamma-glutamyltransferase levels after the burn injury have a poor prognosis. Patients with burn-associated cholestasis may develop sclerosing cholangitis and secondary biliary cirrhosis.

Introduction

Although the outcomes of severe burn injuries have improved over recent decades, morbidity and mortality remain high: almost 40% of patients develop acute respiratory distress syndrome,[1,2] up to 50% of patients develop acute kidney injury[3,4] and the reported mortality rates range from 15%[1,3,5] to 25–30%.[2,4] Liver necrosis and hepatic dysfunction after burn injuries have been reported since the late 1930s.[6] An autopsy series from the 1980s described increased liver size and lipid infiltrations after burn injuries.[7] Later studies showed that these infiltrations were prevalent among burn patients and correlated with the total body surface area (TBSA) of the burn[8] and were associated with liver dysfunction.[9] In a paediatric series, increases in alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) levels were reported 10 days after sustaining a burn injury.[10] Furthermore, cases of sclerosing cholangitis have been reported after burn injuries.[11] However, the

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prevalence of cholestasis and the relationship between cholestasis and outcomes in patients with burns is unknown. The purpose of this study was to describe the course and the burden of cholestasis in a cohort of severely burned adult patients. According to the standard definitions of cholestasis[12,13] based on ALP, GGT, and bilirubin levels, we propose the term/entity of burn-associated cholestasis (BAC) with a subtype classification according to the patterns of ALP, GGT, and total bilirubin levels and their associations with clinical outcomes.

Patients and methods

Study design

We conducted a retrospective, single-centre, cohort study among consecutive and non-selected patients (N = 735) admitted to the Burn Intensive Care Unit (BICU) of Saint Louis Hospital, Paris, France, between June 2012 (opening of the burn unit in the St. Louis Hospital) and December 2015. Patients were treated according to our local management protocols.[14] The study was approved by the local ethical committee (Comité de protection des personnes IV, St-Louis hospital; Institutional review board 00003835, protocol 2013/17NICB).

We selected patients with severe burn injuries, which were defined as burn injuries associated with at least 1 of the following criteria: total burn body surface area (BSA) ≥20%; full-thickness burn BSA ≥10%; or total burn BSA ≥10% with smoke inhalation injuries and organ support (*i.e.*, need for vasopressors or mechanical ventilation during the first 24 h). Patients with the following criteria were excluded from the analysis: no liver function test performed during the BICU stay; a length of stay in the BICU ≤48 h; an admission to the hospital more than 7 days from the burn injury; and cholestasis prior to BICU admission. The characteristics of the patients were collected from the patients' medical records. All biological measures, including liver tests, were retrieved from the biological database of the institution and merged in the patient characteristics dataset.

Definitions

BAC was defined as the development of intrahepatic cholestasis after a burn injury. All BAC patients had at least 1 liver imaging scan to exclude obstruction of the choledochal tract. Cholestasis was defined as an ALP level ≥1.5x the upper limit of normal (ULN) with a GGT level ≥3x the ULN[12] or a total bilirubin level ≥2x the ULN (34.2 μmol/L), which defines liver dysfunction in drug-induced liver injury[15] and is the cut-off of liver dysfunction in the sequential organ failure assessment (SOFA) score.[13] Three BAC subtypes were defined as follows: BAC (A) as a concomitant increase in ALP and GGT levels without hyperbilirubinemia; BAC (B) as a concomitant increase in ALP and GGT levels with hyperbilirubinemia during ICU stay; and BAC (C) as hyperbilirubinemia without an increase in ALP and GGT levels during the ICU stay. The BAC classification was based on physiological background information: total bilirubin level has been associated with mortality,[13] whereas increases in ALP and GGT levels have been reported to be protective.[16–18] We defined "sepsis-related cholestasis" as the occurrence of cholestasis within 7 days after sepsis.

Acute kidney injury (AKI) was defined with the Kidney Disease Improving Global Outcomes (KDIGO) criteria.[19] Acute respiratory distress syndrome (ARDS) was defined according to the Berlin definition.[1] Sepsis and septic shock were diagnosed according to the Sepsis-3 definition.[20] The diagnosis of bacteraemia was considered if at least 1 blood culture was positive in less than 24 h after the blood sampling. For coagulase-negative staphylococci, at least 2 positive samples from 2 different puncture sites with similar phenotypes were required. All episodes of bacteraemia were then prospectively recorded and validated by a multidisciplinary team on a weekly basis, including an infectious disease specialist, a bacteriologist and intensivists (*i.e.*, to exclude potential colonization or contamination).

Objectives

The main objective was to report the association between BAC and the 90-day mortality. The secondary objective was to identify risk factors for BAC.

Statistical analysis

Continuous variables were expressed as medians with interquartile ranges (IQRs) and were compared with the Mann-Whitney *U* test or with the Kruskal-Wallis test, as appropriate. Categorical variables were expressed as counts and percentages and were compared with the Fisher's exact test or the chi-square test, as appropriate. Time-to-event analyses were estimated with Kaplan-Meier analyses and Cox models. Multivariate associations were computed with binary logistic regression models. For all models, variables with nominal 2-tailed p values less than 0.2 were entered into the multivariate model, except for variables with obvious collinearity. The final models were selected using the backward stepwise regression based on the Wald test. Time-dependent Cox models were used to disentangle the relationships between BAC, sepsis and mortality. All statistical analyses were performed using SPSS software, version 21 (IBM, Armonk, NY USA). A p value <0.05 was considered statistically significant.

Results

Characteristics of patients

A total of 214 patients fulfilled the inclusion criteria (Fig. 1). Overall, 111 (52%) patients developed BAC 9 (5–16) days after sustaining a burn injury (Fig. 2 and Table 1). More patients with BAC had diabetes mellitus compared to patients without BAC (16% *vs.* 5%; $p = 0.008$). Patients with BAC had higher burn BSA (33% [20–55] *vs.* 23% [16–32]; $p = 0.001$), higher severity of illness (measured with the abbreviated burn severity index [ABSI] and the simplified acute physiology score II [SAPS II] severity scores) and required higher levels of supportive care during the first 24 h, including vasopressors (49% *vs.* 17%; p <0.001) and mechanical ventilation (75% *vs.* 46%; p <0.001), compared to patients without BAC.

The liver function tests were within normal ranges at admission, but patients with BAC had significantly higher AST levels, GGT levels, AST/ALT ratios, and total bilirubin levels than those without BAC. After admission, the patients with BAC developed higher peak AST and ALT levels, higher AST/ALT ratios, and lower prothrombin ratios than patients without BAC (p <0.001 for all comparisons). Total bilirubin and GGT levels reached peak values later in patients with BAC than in those without BAC (Table S1). At their peak values, patients with BAC had higher liver function test levels than those without. Patients with BAC required more enteral/parenteral feeding, more transfusions of red blood cells (p <0.001), developed more complications including sepsis (70% *vs.* 25%; p <0.001), AKI (59% *vs.* 25%;

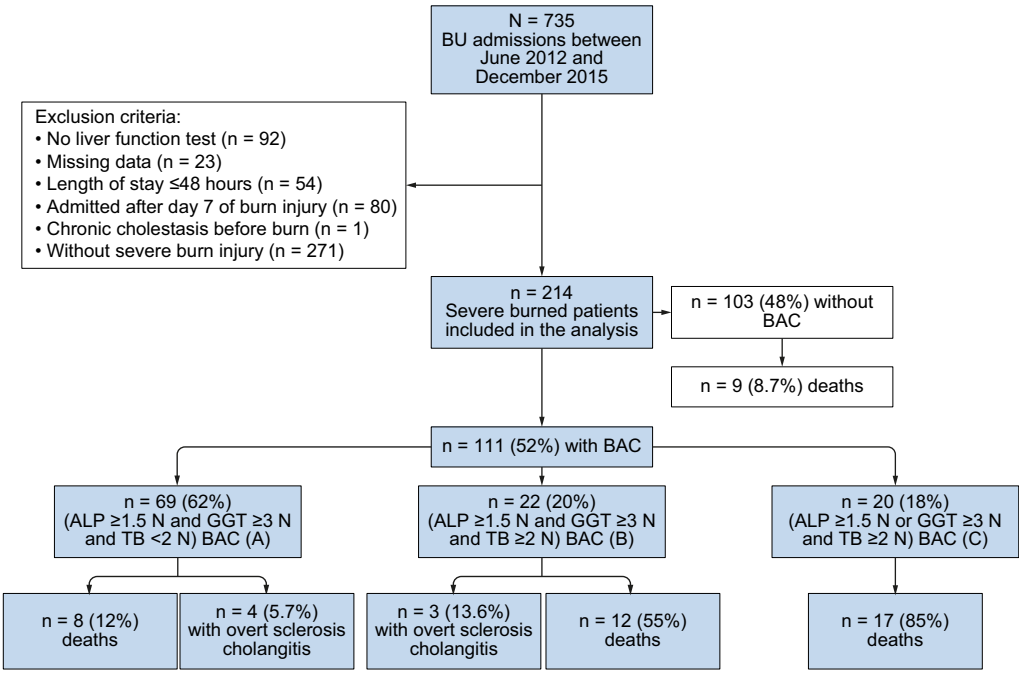

Fig. 1. Flowchart of the study. ALP, alkaline phosphatase; BAC, burn-associated cholestasis; BU, burn unit; GGT, gamma-glutamyltransferase; TB, total bilirubin; ULN, upper limit of normal.

p <0.001), and ARDS (45% *vs.* 16%; p <0.001) and had a longer BICU length of stay (38 days [22–65] *vs.* 22 days [13–37]; p <0.001) than patients without BAC.

Subtypes of BAC have different outcomes

There were 3 different profiles of BAC with different outcomes (Fig. 3). Patients with BAC (A) and BAC (B) had similar patterns of elevated GGT/ALP levels 1 week after the burn injury, peaking at day 17 for patients with BAC (A) and at day 39 for those with BAC (B) (Table S2). Patients with BAC (C) had a rapid increase in total bilirubin level that peaked at the end of the second week after the burn injury without GGT/ALP elevations (Fig. 3C), and BAC (C) patients had the highest peak AST levels and the highest AST/ALT ratios of all patients with BAC. A decrease in prothrombin ratios below 50% was observed in 82 (39%) patients, which occurred 3 (2–16) days after the burn injury and was more frequent among patients with BAC (B) and BAC (C) than BAC (A) (p <0.0001). All patients received vitamin K infusions. Normalization of the prothrombin ratios was observed within 2 (1–3) days and was independent of the BAC subtype. Compared to patients with BAC (A), those with BAC (B) and BAC (C) had higher severity scores, required more vasopressors, parenteral feeding and red blood cell transfusions and had more complications including sepsis, AKI, and ARDS. Patients with BAC (B) were roughly comparable to those with BAC (C) in terms of clinical characteristics, especially regarding alcohol use disorders and viral hepatitis, but cirrhosis was more prevalent among patients with BAC (C) than BAC (B) (Table S3). There was a gradual increase in mortality across BAC subtypes (Fig. 3D), reaching 17 (85%) deaths for patients with BAC (C).

Survival analyses

The recorded causes of death were initial multiple organ failure in 5 (12%) patients, cardiogenic shock in 2 (4%), mesenteric ischaemia in 4 (9%) and septic shock in 32 (75%) patients. Age, diabetes mellitus, cirrhosis, severity of illness, peak AST/ALT/ALP levels but not GGT levels, total bilirubin levels, nadir prothrombin ratio, sepsis and organ failure were associated with death (Table S4). The presence of BAC was associated with a 2.5 (95% CI 1.2–5.2; p = 0.012) times increased risk of 90-day mortality (Fig. 2D). After being adjusted for age and the presence of ABSI, AKI, ARDS and septic shock, the onset of BAC was associated with a hazard ratio of 2.28 (95% CI 1.06–4.92; p <0.001) for 90-day mortality, and the risk was limited to the BAC (C) subtype (Table 2). In multivariate analyses, after being adjusted for severity of illness and organ failure, the elevations in ALP and GGT levels appeared protective (Table S5). In contrast, the total bilirubin peak level was associated with mortality, with an area under the ROC curve of 0.89 (95% CI 0.83–0.94), which was similar to that of the severity score (Fig. S1).

Factors associated with BAC

ABSI (OR 1.2; 95% CI 1.0–1.4; p = 0.028), initial vasopressor requirement (OR 2.2; 95% CI 1.0–4.7; p = 0.041), and bacteraemia (OR 4.2; 95% CI 2.1–8.2; p <0.001) were independent risk factors for developing BAC (Table S6). A total of 73 (66%) patients with BAC developed bacteraemia (Fig. S2A), and the proportion of bacteraemia cases differed across BAC subtypes (BAC (A), 57%; BAC (C), 80% and BAC (B), 100%, p = 0.001) (Fig. S2B). The onset of BAC increased the risk of bacteraemia by more than 50% (Table S7). A total of 55 (50%) patients developed sepsis-related cholestasis. The difference in mortality was not statistically significant between patients with and without sepsis-related cholestasis (Fig. S3).

BAC evolution

Of the 74 patients with BAC who survived, a total of 38 (51%) patients had persistent cholestasis at discharge. The duration of cholestasis was 9 (6–9) days in patients with resolutive BAC and 30 (13–62) days for patients with BAC at discharge.

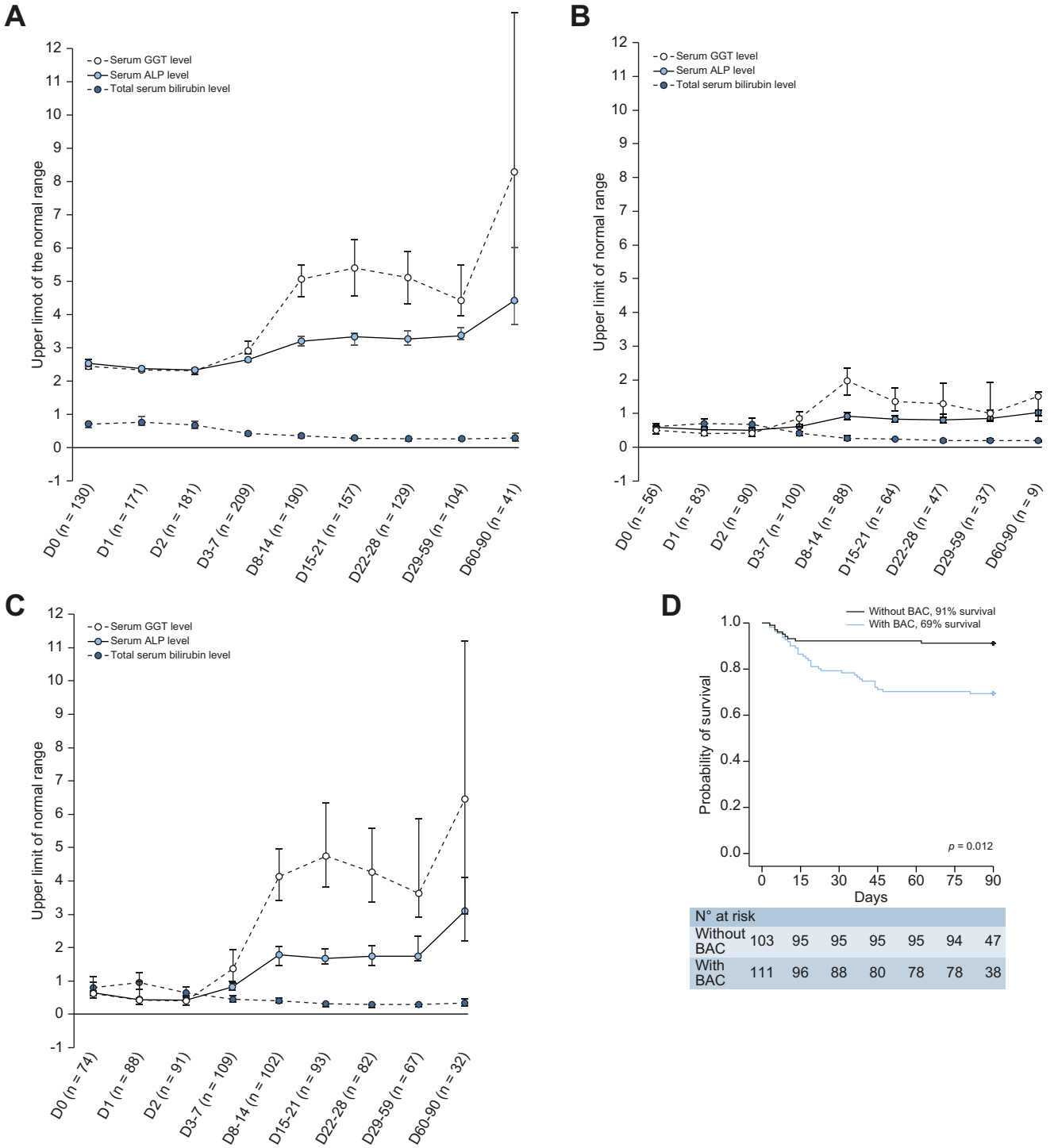

Fig. 2. Burn-associated cholestasis and outcomes in a retrospective cohort of patients with severe burn injuries (n = 214). (A) Median (95% CI) variations in liver function test results during the burn unit stay (with number at risk) in a cohort of severely burned patients (n = 214). (B) Median (95% CI) variation in liver function test results during the burn unit stay (with number at risk) among patients without BAC (n = 103). (C) Median (95% CI) variation in liver function test results during the burn unit stay (with number at risk) among patients with BAC (n = 111). (D) Probability of survival among severely burned patients with and without BAC (N = 214). The risk (panel D) was computed with the log-rank test. BAC was defined as an increase in ALP level ≥1.5x the ULN and GGT level ≥1.5x the ULN and/or an increase in total bilirubin level ≥2x the ULN. ALP, alkaline phosphatase; BAC, burn-associated cholestasis; GGT: gamma-glutamyl-transferase; ULN: upper limit of normal.

Seven patients were discharged with morphological signs of sclerosing cholangitis including dilatations and thickening of the common bile duct visible with ultrasound (n = 2), CT scan (n = 6), and magnetic resonance imaging (MRI) (n = 4) (Fig. 4). One patient had undergone an endoscopic retrograde cholangiopancreatography, which showed typical signs of cholangitis. The patients with BAC-associated sclerosing cholangitis were those with BAC (A) in 4 cases and BAC (B) in 3 cases; they had

Table 1. Characteristics of the patients.

Characteristics	Overall population (n = 214)	Without BAC (n = 103; 48%)	With BAC (n = 111; 52%)	*P* Value
Age, years	48 [32–61]	44 [30–59]	50 [35–64]	0.140
Male sex	145 (68)	74 (72)	71 (64)	0.243
BMI, kg/m^2	25 [23–28]	24 [22–27]	26 [23–29]	0.015
Presence of comorbidities				
Heart failure	13 (6)	5 (5)	8 (7)	0.472
Diabetes mellitus	23 (11)	5 (5)	18 (16)	0.008
Alcohol use disorders	62 (29)	28 (27)	34 (31)	0.550
Viral hepatitis	5 (2)	1 (1)	4 (3.6)	0.37
Cirrhosis	6 (3)	1 (1)	5 (4.5)	0.214
Burn characteristics				
Thermal burn	201 (94)	96 (93)	105 (95)	0.670
Electrical burn	10 (5)	5 (5)	5 (5)	1
Chemical burn	3 (1)	1 (1)	2 (2)	1
Inhalation injury	82 (38)	32 (31)	50 (45)	0.049
Severity scores				
Burn BSA, %	27 [18–45]	23 [16–32]	33 [20–55]	0.001
Full-thickness burn BSA, %	10 [2–25]	9 [0–16.5]	18 [5–39.5]	0.001
ABSI	7 [6–10]	6 [6–8]	8 [7–11]	0.001
SAPSII	26 [15–41]	21 [15–33]	31 [22–49]	0.001
Treatments in the first 24H				
Crystalloid infusion, ml/kg/%	3 [1.6–4.4]	2.4 [1.4–4]	3.4 [2.2–4.7]	<0.001
Norepinephrine	72 (34)	18 (17)	54 (49)	<0.001
Albumin infusion	57 (27)	14 (14)	43 (39)	<0.001
Hydroxocobalamin	28 (13)	8 (8)	20 (18)	0.040
Mechanical ventilation	130 (61)	47 (46)	83 (75)	<0.001
Liver function tests at admission				
AST, x ULN	1 [0.7–1.3]	0.9 [0.6–1.2]	1.1 [0.7–1.7]	0.007
ALT, x ULN	0.6 [0.5–1.1]	0.6 [0.5–1]	0.7 [0.5–1.2]	0.162
AST/ALT ratio	1.7 [1.3–2.3]	1.6 [1.2–2.1]	1.8 [1.4–2.5]	0.013
GGT, x ULN	0.5 [0.4–1.4]	0.5 [0.3–1.0]	0.6 [0.4–1.7]	0.024
ALP, x ULN	0.6 [0.4–0.8]	0.6 [0.4–0.8]	0.6 [0.5–0.8]	0.288
Total bilirubin, x ULN	0.7 [0.5–1.1]	0.6 [0.5–0.9]	0.8 [0.4–1.3]	0.011
Prothrombin ratio, %	84 [67–95]	85 [76–95]	81 [63–94]	0.082
Peak values of liver function tests				
AST, x ULN	2.2 [1.1–5.5]	1.1 [0.8–1.8]	4 [2.3–7.9]	<0.001
ALT, x ULN	2.6 [1.2–5.4]	1.3 [0.7–2.6]	3.8 [2.5–6.7]	<0.001
AST/ALT ratio	2 [1.3–2.7]	1.6 [0.7–3.3]	2.4 [1.7–3.5]	<0.001
GGT, x ULN	3.9 [1.5–8.9]	1.6 [0.7–3.2]	8.5 [4.7–14]	<0.001
ALP, x ULN	1.6 [0.9–3.2]	0.87 [0.6–1.2]	3.0 [2.1–5.3]	<0.001
Total bilirubin, x ULN	0.9 [0.6–1.6]	0.7 [0.5–1.0]	1.3 [0.7–2.6]	<0.001
Nadir prothrombin ratio, %	60 [39–75]	70 [54–81]	48 [29–66]	<0.001
Prothrombin ratio <50%,	82 (39)	19 (19)	63 (57)	<0.001
Time to prothrombin ratio <50%, days	3 [2–16]	3 [2–9]	3 [2–16]	0.755
Time to recover from prothrombin ratio <50%, days	2 [1–4]	2 [1–3]	2 [1–4]	0.379
Treatments during ICU stay				
Enteral feeding	145 (68)	53 (51)	92 (83)	<0.001
Parenteral feeding	18 (8)	1 (1)	17 (15)	0.001
Transfusion of RBCs	113 (52)	35 (34)	78 (70)	0.001
Outcomes				
Nosocomial infection	134 (63)	48 (47)	86 (78)	<0.001
Number of nosocomial infections	1 [0–2]	0 [0–1]	2 [1–4]	<0.001
Bacteraemia	97 (45)	24 (23)	73 (66)	<0.001
Sepsis	95 (44)	25 (24)	70 (63)	<0.001
Septic shock	68 (32)	11 (11)	57 (51)	<0.001
AKI	91 (43)	26 (25)	65 (59)	<0.001
RRT	44 (21)	7 (7)	37 (33)	<0.001
ARDS	68 (32)	17 (16)	51 (45)	<0.001
BICU length of stay, days	29 [15–47]	22 [13–37]	38 [22–65]	<0.001
90-day mortality	43 (20)	9 (9)	34 (31)	<0.001
ICU mortality	46 (22)	9 (9)	37 (33)	<0.001

Continuous variables are expressed as median and interquartile range (IQR) and were compared using the Mann-Whitney *U* test. test. Categorical variables are expressed as n (%) and were compared with Fisher's exact test. The SAPS II ranges from 0 to 163, with higher scores indicating greater severity of illness. The ABSI ranges from 0 to 18, with higher scores indicating a greater probability of death after the burn injury. Normal values of AST, ALT, ALP, GGT, and total bilirubin are 35 U/L, 33 U/L, 104 U/L, 40 U/L and 17 μmol/L, respectively. ABSI, abbreviated burn severity index; AKI, acute kidney injury; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ARDS, acute respiratory distress syndrome; BAC, burn-associated cholestasis; BICU, burn intensive care unit; BMI, body mass index; BSA, body surface area; GGT, gamma-glutamyl-transferase; IQR, interquartile range; RBCs, red blood cells; RRT, renal replacement therapy; SAPS II, simplified acute physiology score II; ULN, upper limit of normal.

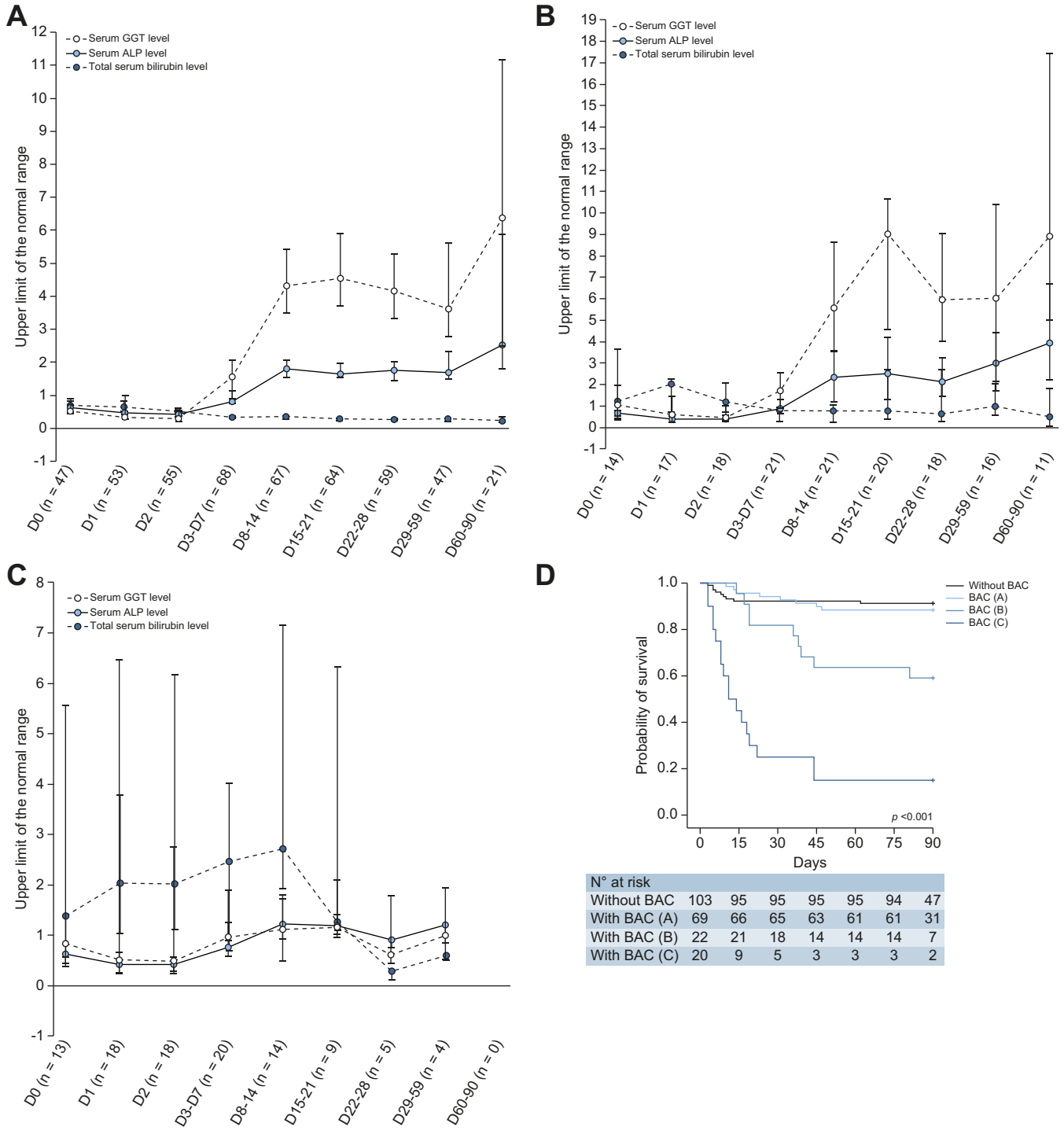

Fig. 3. Liver test evaluations according to BAC subtype and outcomes. (A) Median (95% CI) variations in liver function test results during the burn unit stay (with number at risk) among patients with BAC A (n = 69). (B) Median (95% CI) variations in liver function test results during the burn unit stay (with number at risk) among patients with BAC B (n = 21). (C) Median (interquartile range) variation variations in liver function test results during the burn unit stay (with number at risk) among patients with BAC C patients (n = 20). (D) Probability of survival among severely burned patients with subtypes of BAC (N = 214). The risk (panel D) was computed with the log-rank test. BAC subtypes were defined as follows: BAC (A): ALP level ≥1.5x the ULN and GGT level ≥1.5x the ULN and total bilirubin level <2x the ULN; BAC (B): ALP level ≥1.5x the ULN and GGT level ≥1.5x the ULN and total bilirubin level ≥2x the ULN; BAC (C): ALP level <1.5x the ULN or GGT level <1.5x the ULN and total bilirubin level ≥2x the ULN. ALP, alkaline phosphatase; BAC, burn-associated cholestasis; GGT, gamma-glutamyltransferase; ULN, upper limit of normal.

higher burn BSA, higher severity of illness, more pronounced hepatic injury, and higher peak aminotransferase levels, AST/ALT ratios and total bilirubin levels than patients without BAC-associated sclerosing cholangitis (Table S8). At the end of follow-up, which was 20 (3.5–35) months after discharge, patients with secondary cholangitis had ALP and GGT levels of 5.8x (1.7–15) and 11x (4.5–22) the ULN, respectively. Follow-up MRIs showed indirect signs of biliary cirrhosis (Fig. 4E and

Table 2. Multivariate Cox regression models for predicting the 90-day mortality of patients with severe burn injuries (n = 214).

Characteristic	Adjusted hazard ratio (95% CI)	*p* value
Time-dependent Cox model		
Onset of BAC	2.28 (1.06–4.92)	0.036
Age (years)	1.04 (1.02–1.06)	<0.001
ABSI severity score	1.18 (1.03–1.53)	0.017
AKI	9.91 (2.29–42.89)	0.002
ARDS	4.16 (1.78–9.71)	0.001
Septic shock	1.61 (0.60–4.32)	0.345
Cox regression model		
BAC subtypes		<0.001
BAC (A)	0.39 (0.15–1.05)	0.062
BAC (B)	0.27 (0.10–0.77)	0.015
BAC (C)	4.51 (1.87–10.87)	0.001
Age (years)	1.06 (1.03–1.08)	<0.001
ABSI severity score	1.33 (1.16–1.52)	<0.001
AKI	11.99 (2.98–51.45)	0.001
ARDS	9.06 (3.82– 21.48)	<0.001

Adjusted hazard ratios were computed with multivariate Cox models in which BAC was entered either as a time-dependent variable (upper panel) or as a time-independent variable in the backward cox regression model (lower panel). The SAPS II ranges from 0 to 163, with higher scores indicating greater severity of illness. The ABSI ranges from 0 to 18, with higher scores indicating a greater probability of death after the burn injury. BAC was defined as an increase in the levels of ALP ≥1.5x the ULN and GGT ≥1.5x the ULN and/or an increase in total bilirubin levels ≥2x the ULN. The BAC subtypes were defined as follows: BAC (A): ALP level ≥1.5x the ULN and GGT level ≥1.5x the ULN and total bilirubin level <2x the ULN; BAC (B): ALP level ≥1.5x the ULN and GGT level ≥1.5x the ULN and total bilirubin level ≥2x the ULN; BAC (C): ALP level <1.5x the ULN or GGT level <1.5x the ULN and total bilirubin level ≥2x the ULN.
ABSI, abbreviated burn severity index; AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; BAC, burn-associated cholestasis.

4F) in 4 patients 18 months after the burn injury. One patient had a liver biopsy, which confirmed biliary cirrhosis. One patient with BAC and persistent cholestasis died just after being discharged from the BICU with sepsis.

Discussion

Our results demonstrate that intrahepatic cholestasis occurs frequently after severe burn injuries: in this large, retrospective, cohort study, BAC was found in more than half of patients. The incidence of cholestasis in severely burned patients is considerably higher than in patients who were not treated in the BICU,[21] which may suggest that the liver is specifically targeted after burn injuries. Although BAC doubled the risk of 90-day mortality, the prognosis of patients with BAC differed between the 3 BAC subtypes that were retrospectively defined according to the patterns of elevated enzyme levels in the post-burn injury liver function tests; patients with hyperbilirubinemia without elevated GGT and ALP levels had poor outcomes. Conversely, BAC was strongly associated with the severity of illness, bacteraemia, sepsis and organ failure. Patients with BAC were at risk of secondary sclerosing cholangitis, which calls for long-term follow-up of patients with BAC.[11]

Our observations are consistent with those of a previous study[22] that reported the prevalence of liver function test abnormalities to be 58% in a cohort of 81 patients with severe burn injuries. However, the liver function tests were only performed the first 7 days after admission, and the associations with the outcomes were not reported. We modelled all liver function tests and outcomes and proposed a classification system for BAC based on the pattern of cholestasis after burn injury and its relationship with the outcome. The occurrence of hyperbilirubinemia with or without a concomitant increase in the levels of GGT ≥3x the ULN and ALP ≥1.5x the ULN segregated the patients into 3 BAC subtypes: BAC with elevated bilirubin levels (BAC (B) and (C)) occurred early and was associated with mortality (41% and 85% respectively), and BAC without elevated bilirubin levels (BAC (A)) developed later during the BICU stay and was associated with better outcomes (mortality rate was close to the mortality rate of patients without BAC [12% and 9%, respectively]). Our BAC classification is consistent with several previously reported pathophysiological observations; the main observation was that bilirubin levels and elevated GGT/ALP levels are associated with different outcomes. Bilirubin levels are associated with a high risk of death in hospitalized patients and are an item of the well-validated 'sequential organ failure assessment (SOFA) score'.[13] In contrast, elevated GGT/ALP levels are protective: ALP was shown to limit the inflammatory response to Gram-negative bacteria.[23] Rats injected with *E. coli* had increased mortality when ALP was inhibited.[24] Several studies have shown that ALP has a protective effect on renal function during sepsis.[25–27] In a recent randomized trial,[18] human recombinant ALP supplementation was associated with lower 28-day and 90-day mortality in septic patients. Many data also suggest a protective role of GGT. An early increase in GGT levels was associated with a good long-term outcome in 2 studies after liver transplantation.[16,28] Moreover, experimental data showed that an increase in the GGT level is associated with liver regeneration.[29,30] GGT catalyses the transfer of the gamma glutamyl moiety of glutathione that allows for cysteine to enter into cells and restores the stock of intracellular glutathione,[31,32] which is one of the frontline non-enzymatic intracellular antioxidants.[33] An early increase in the GGT level after a burn injury may reflect the ability of the host to initiate an appropriate protective systemic response.

In this cohort, the severity of the burn injury was an independent risk factor for BAC. Two mechanisms could explain the direct connection between burn injuries and cholestasis. First, cholestasis may be associated with hypoxic hepatitis.[34] Severe burn injuries are associated with hypovolemic shock on the first day following the burn injury.[14] This hypothesis is strengthened by the fact that patients with BAC (B) and BAC (C) had higher peak AST and ALT values with higher AST/ALT ratios than patients with BAC (A). Moreover, the use of norepinephrine infusions during the initial resuscitation period was the second independent risk factor for BAC, and

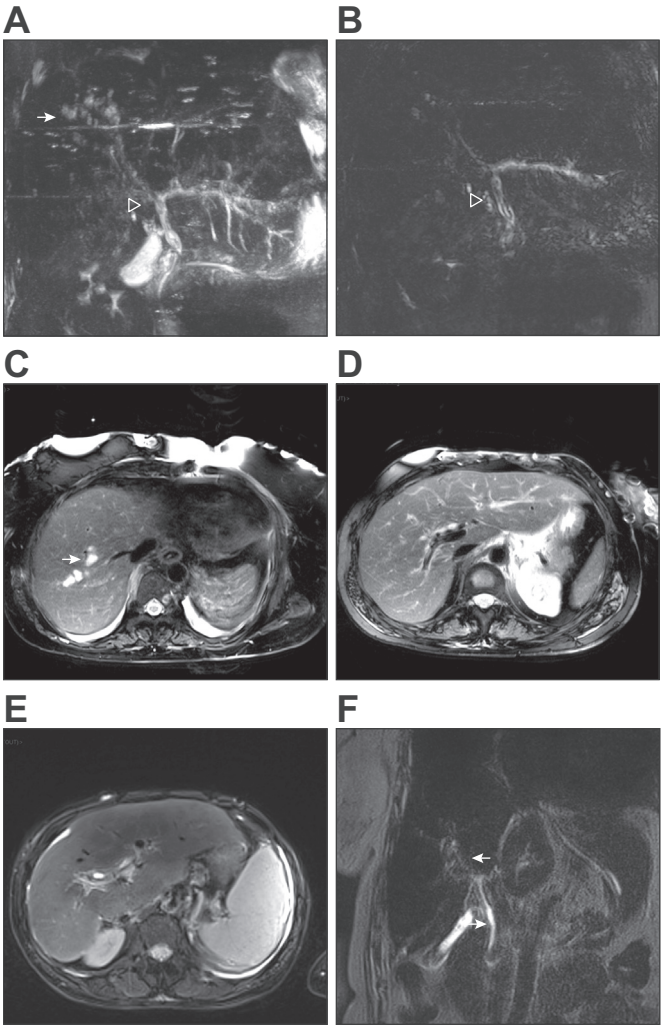

Fig. 4. Magnetic resonance imaging of cholangiopancreatography of sclerosing cholangitis. (A-D) Imaging acquired during the intensive care unit stay. Intra- and extrahepatic bile duct dilatation with casts in the common bile duct (arrowheads in A and B), bilomas (arrows in A and C), and no dysmorphic changes in the liver (D). There were major dysmorphic change in the liver (E) at 18 months. (F) Control magnetic resonance cholangiopancreatography at 18 months post-burn injury with severe intra- and extrahepatic duct strictures (arrows).

norepinephrine was used more frequently in patients with BAC (B) and (C). An infusion (even at a low dose) of norepinephrine reduces the hepatic arterial blood flow and induces cholestasis in the isolated perfused rat liver.[35] The impaired hepatic arterial blood flow compromises the integrity of the biliary epithelium because of its specific vascular anatomy, which receives blood exclusively from the hepatic arterial branches that are known as the peribiliary vascular plexus.[36] The second mechanism by which burn injuries may directly lead to BAC is gut ischaemia and its subsequent inflammatory response.[37] Interleukin-6 and tumour necrosis-alpha, which are increased in the early phase of a severe burn injury,[38] have been associated with hyperbilirubinemia[39] and organ dysfunction.[40]

Bacteraemia was also strongly and consistently associated with cholestasis. Cytokines associated with sepsis, including interleukin-6 and tumour necrosis-alpha, can reduce the hepatocyte canalicular membrane expression of biliary transporters[41,42] and the biliary secretion of conjugated bilirubin. Burn-associated liver injuries could also promote bacteraemia by themselves. Splanchnic ischaemia-reperfusion injury increases gut microbiota translocation. As cholestasis impairs the ability of Kupffer cells to clear bacteria[43] by overexpressing interleukin-10,[44] BAC could directly promote gut-derived bacteraemia. Ischaemia of the biliary plexus and cholangitis could also promote bacteraemia. Burn injuries could therefore trigger a vicious cycle with liver injuries promoting sepsis, and sepsis promoting cholestasis. The occurrence of BAC should therefore raise awareness and warn physicians of the risk of sepsis and/or an ongoing episode of sepsis.

Lastly, diabetes mellitus and cirrhosis were associated with BAC and BAC (C), respectively. The reason for such an association may be due to underlying liver fibrosis that preferentially leads to hepatic ischaemia and hyperglycaemia, which then preferentially lead to cholestasis.[45] Parenteral nutrition may also contribute to BAC development as it encourages lipid infiltration of the liver, which is common after a severe burn injury.[46]

Our study has limitations. First, this was a retrospective and observational study, which prevents us from determining any causal relationships between liver response and survival or the occurrence of complications. Although this study is the largest published series on cholestasis in ICU patients, this was a single centre study from a large expert university centre, which may limit the generalization of the results.

Finally, the definitions used to define BAC may appear arbitrary but were based on clinico-physiological background parameters. Furthermore, using well-established cut-offs allows the immediate translation of the current definition to current practice, and physicians are aware of these cut-offs. We acknowledge the potential overlap between our BAC subtypes.

Although the role of medication was not fully explored, burn care is protocolized in our centre, which limits the impact of medications on the occurrence of cholestasis. Furthermore, the severity of illness limits the influences of drugs in this context. Finally, the long-term outcome of cholestasis merits further investigation.

Conclusion

BAC is common after severe burn injuries. While hyperbilirubinemia occurs early and is associated with a high mortality rate, isolated elevations in ALP and GGT levels occur after the first week and are strongly associated with episodes of bacteraemia but do not appear to be associated with short-term mortality. However, cholestasis may be associated with long-term cholangiopathy. The long-term outcome of BAC remains to be further explored.

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Conflict of interest

This study was not funded by a sponsor. Dr. Legrand reports personal fees from Baxter, personal fees from Fresenius, grants from French ministry of Health, non-financial support from Shingotec, personal fees from Novartis, outside the submitted

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conception and design: VM, ML, CdT. Data acquisition: NM, CdT, TV, FD, MC, MB, AF, AC, MJ, HO, AF, PS, AMZ, EdK. Analysis and interpretation of the data: VM, ML, CdT. Drafting of the article: CdT, FD, SS, VM, ML. Critical revision of the article for important intellectual content: VM, ML, RM, AM, PS. Final approval of the article: all authors.

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Supplementary data

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Author names in bold designate shared co-first authorship

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