Serum Bile Acid Concentrations, Histopathological Features, and Short-, and Long-term Survival in Horses with Hepatic Disease

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Abstract

Background: Serum bile acid concentrations (SBA) and a histopathological biopsy score [Equine Vet J 35 (2003) 534] are used prognostically in equine hepatic disease.

Hypothesis: Histopathologic features and scores, but not SBA, differ between survivors and nonsurvivors and correlate with histopathologic evidence of hepatic inflammation and fibrosis.

Animals: Retrospective study. Records (1999–2011) of horses with hepatic disease diagnosed by biopsy and with concurrent measurements of SBA.

Methods: Retrospective cohort study. Biopsies were examined for inflammatory cell infiltration including type and distribution, fibrosis, irreversible cytopathology affecting hepatocytes, hemosiderin, or other pigment deposition and bile duct proliferation. SBA, histopathological findings and a histological score [Equine Vet J 35 (2003) 534] were compared between short- (survival to discharge) and long-term (>6 months) survivors and correlations between SBA and histopathological findings investigated.

Results: Of 81 cases 90% survived short-term and 83% long-term. Short-term and long-term nonsurvival were associated with SBA (P = .009; P = .006), overall (P = .001; P = .002) and parenchymal (short-term only; P = .01) inflammation, portal and bridging fibrosis (all P < .001), apoptosis or single cell necrosis (P < .001; P = .008), hemosiderin deposition in hepatocytes (P = .011; P = .028), biliary (both P < .001), vascular (P = .003; P = .045) and endothelial (P < .001; P = .02) hyperplasia, nucelar changes (P = .004; P = .001) and the histopathological score (both P < .001). SBA were significantly and positively correlated with overall (P = .001), parenchymal (P < .001) and portal (P = .004) inflammation and portal (P = .036) and bridging (P = .002) fibrosis.

Conclusions and Clinical Importance: SBA, histopathological findings and scores differ between survivors and nonsurvivors. SBA concentrations are associated with inflammation and fibrosis suggesting interference with hepatic function. A histopathological score >2 and, less so, SBA >20 µmol/L are specific but not sensitive indicators of nonsurvival.

Key words: Hepathopathy; Hepatic failure; Hepatitis; Liver biopsy.

Abbreviations:

SBA serum bile acid concentrations

Currently, histopathologic examination of liver tissue is regarded by many clinicians as the most sensitive method of diagnosing hepatic disease, and histopathologic findings from biopsied tissue in people correlate well with subsequent gross postmortem findings. Histopathology is regarded as the best indicator of prognosis in hepatic disease in horses and a scoring system has been developed for this purpose. However, this scoring system has not been evaluated in a different case population. In the absence of a liver biopsy or for continuous monitoring purposes, many clinicians anecdotally use serum bile acid concentrations (SBA) as a surrogate prognostic indicator and concentrations exceeding 20 µmol/L are predictive of nonsurvival.

However, our clinical impression indicated that this might not be uniformly applicable to all equine populations with hepatic disease. The prognostic value of SBA relies on the fact that the detected loss of hepatic function is permanent or progressive or both. It is conceivable that reversible, such as inflammatory infiltration or reversible hepatocyte damage, could temporally interfere with hepatic function, leading to an increase in SBA. With appropriate treatment or time, the condition might resolve which would make SBA an unreliable prognostic indicator. Furthermore, a large number of horses in the early stages of liver disease have SBA within the reference ranges and gaining additional prognostic information from histopathologic features would be useful. To date, few studies have investigated the relationship between SBA and individual histopathologic features and short- and long-term survival. As SBA are considered to be indicators of liver function, a close association between SBA and histopathological findings would be expected, particularly between histological findings such as inflammation and fibrosis, that could interfere with normal function.

The aim of the study was to determine whether SBA and histologic variables including a histological score were associated with short- (survival to discharge) and long-term (>6 months after discharge) survival; differences between biochemical and hematological variables...
in short- and long-term survivors and non-survivors were also evaluated. The study further assessed whether SBA concentrations in horses with liver disease correlated with histologic features. It was hypothesized that histopathologic features and score, but not SBA, differed between survivors and non-survivors and that SBA concentrations correlated with histopathologic evidence of inflammation and fibrosis.

Materials and Methods

The study was carried out in accordance with the ethical guidelines of the participating institutions. In a retrospective study, all horses presenting to two equine referral hospitals that had a liver biopsy and concurrent (±3 days) SBA measurement performed between the years 1999 and 2011 were eligible for inclusion into the study. The animal’s date of birth, age at biopsy, gender and breed, SBA and, where available, other clinicopathological data were recorded.

 Archived formalin-fixed paraffin wax embedded trucut liver biopsy samples were recut and 4 μm sections stained with haematoxylin & eosin and van Gieson staining. To highlight fibrosis, deparaffinised sections were stained for 7 minutes with Van Gieson stain (picric acid and aqueous acid fuchsin, Leica Biosystems, Leica Microsystems (UK), Ltd, Milton Keynes, Buckinghamshire, UK), dehydrated through alcohol, cleared in xylene and mounted with DPX. All samples were reviewed and graded by two pathologists (MJF and AKF) blinded to the outcome of the case. Only specimens with more than 6 lobules were scored. The portal tracts and hepatic parenchyma were assessed for bile duct proliferation, fibrosis, irreversible cytology affecting hepatocytes, hemosiderin or other pigment deposition and inflammatory cell infiltration as detailed below.

The total length of the biopsies or where more than one slice was available, the sum of the lengths was documented. Portal numbers were counted and the degree of inflammatory infiltration overall and separately within portals and parenchyma was scored as absent, mild, moderate or severe. Inflammatory infiltrates were graded as mild if up to 5 leucocytes were present within a portal tract or lobule, moderate if 5–20 leucocytes were seen and severe if more than 20 leucocytes were found.

Cell types within portal areas were counted and described based on the following protocol: 0 = no inflammatory cells present, 1 = mononuclear cells present alone, 2 = neutrophilic with or without mononuclear cell infiltrates and 3 = hemosiderophages alone, or in combination with any other cell types. Cell types within the parenchyma were also identified and described: 0 = no inflammation, 1 = neutrophilic infiltration in the parenchyma and also affecting portal areas, 2 = neutrophilic infiltration without portal infiltrates and 3 = other cellular infiltrate.

Fibrosis was assessed and classified as absent, mild, moderate or severe. Fibrosis was defined as mild where immature or mature fibrous tissue expanded a portal tract up to twice the normal size, moderate where a tract was 3 times the normal size and severe where a tract was greater than or equal to 4 times the normal size (often seen as bridging fibrosis across adjacent lobules). Bridging fibrosis was also further graded as absent, mild (delicate fibrosis confined to portal-portal regions), moderate (extensive fibrosis but still predominantly confined to portal-portal regions) or severe (extensive fibrosis, extending across the portal plate, with disruption of the normal lobular pattern).

Apoptosis or single cell necrosis or both were graded as absent, mild (less than 2 cell per lobule), moderate (2–5 cells per lobule) or severe (>5 cells per lobule, often also including some individual cell necrosis).

Distribution of hemosiderin was assessed within 3 regions: within Kupffer cells, portal areas and hepatocytes. Presence of hemosiderin was recorded as absent, mild (fewer than 25% of cells affected), moderate (25–50% of cells affected) and severe (>50% of cells affected).

Bile duct proliferation, vascular hyperplasia and hyperplasia of the sinusoidal endothelium (endothelial hyperplasia) were graded according to the number of biliary branches/number of blood vessels/hyperplastic sections in portal tracts sectioned in a typical portal triad as absent or mild containing 2–3, moderate containing 4–6 and severe containing greater than 7 biliary branches/vessels/sections.

Bile stasis (canalicular and ductal) was assessed as absent or present.

Cytoplasmic swelling was also assessed and scored as absent, mild (approximately 1.5× normal diameter), moderate (approximately 2× normal diameter) or severe (≥3× normal diameter). The swelling distribution was then further classified as absent, affecting hepatocytes closest to arterial and portal inflow, within the transitional zone, periacinar (comprising the hepatocytes nearest to the outflow; terminal hepatic venule), or combinations of the above.

Changes to hepatocyte nuclei (nucleic changes) were scored as absent (no significant anisokaryosis), mild (mild anisokaryosis without megacaryocytosis), moderate (mild anisokaryosis ± occasional megacaryocytosis) and severe (frequent megacaryocytosis >1 per lobule).

A histological score was assigned.

Records were reviewed to determine if animals were known to have died, either before discharge or subsequently. Owners or the referring veterinary surgeons of horses that were not known to be dead were contacted at least 6 months after discharge to determine survival outcome. Where applicable, the date and cause of death was ascertained. Survival was categorised as short- (survival to discharge) and long-term survival (survival to at least 6 months after discharge).

Statistical analysis

Continuous data are summarized as mean ± standard deviation (normally distributed), median (range) (not normally distributed) and categorical data as number and percentage. Normality of the data was assessed using a Shapiro-Wilk test. Differences in SBA, histological features and scores and biochemical and hematologic variables between short- and long-term survivors and non-survivors were explored using a Student’s t-test (normally distributed continuous data) or Mann-Whitney U-test (not normally distributed continuous data) and chi-square or Fisher’s exact test (categorical data), respectively. The correlation between log transformed SBA and histological features was explored using univariate analysis of variance. Receiver-operator curves were generated to determine optimal cut-off points for SBA as indicator for short- and long-term non-survival and histopathology scores as indicators for short- and long-term non-survival. Sensitivity and specificity were reported. In addition, the previously suggested cut-off point of >20 μmol/L was explored in the same manner. The area under the curve (AUC) was also determined. All statistical analyses were performed using a commercially available software program; significance was set at P ≤ .05.

Results

Eighty-one horses fulfilled the inclusion criteria for the study, 36 from hospital 1 and 45 from hospital 2; 24 horses (30%) were female and 57 (70%) were male with a mean age of 11 ± 5.4 years. Two horses from hospital 1 had biopsies performed before June 2001 and might have been included in an earlier study. Eight
(10%) horses died or were euthanized before discharge from the hospital whilst 6 (7%) animals were euthanized or died within 6 months of discharge, all for reasons pertaining directly to liver disease. Sixty-seven animals (83%) survived long-term and 14 (17%) did not.

The mean biopsy length was 18.4 ± 7.8 mm and was not different between short-term survivors and nonsurvivors (18.5 ± 7.9 mm versus 16.8 ± 8.2 mm; P = .58) and long-term survivors and nonsurvivors (18.2 ± 7.8 mm versus 18.9 ± 8.6 mm; P = .76). SBA, overall and parenchymal (short-term survival only) inflammation, portal and bridging fibrosis, apoptosis or single cell necrosis, or both hemosiderin deposition in hepatocytes, biliary and vascular hyperplasia, endothelial hyperplasia, nucleic changes, and the histopathology score were significantly different between survivors and nonsurvivors (Tables 1–3). Comparison of biochemical and hematologic variables between short- and long-term survivors and nonsurvivors are shown in Table 1.

SBA was significantly and positively correlated with overall, parenchymal and portal inflammation, portal and bridging fibrosis, hemosiderin deposition in Kupffer cells, nucleic changes and the histological score (Table 4). The AUC for SBA was 0.78 (95% confidence interval [CI]: 0.66–0.91; P = .009) for short-term survival and 0.8 (95% CI: 0.6–1.0; P = .005) for long-term survival. The AUC for the histological score was 0.74 (95% CI: 0.56–0.89; P = .006) as indicator for short-term survival and 0.85 (95% CI: 0.7–0.99; P < .001) as indicator for long-term nonsurvival. The optimal cut off points for short- and long-term nonsurvival were SBA ≥17 μmol/L and a histology score > 3 and ≥16 μmol/L and a histological score ≥ 3, respectively. Sensitivity and specificity are reported in Table 5.

No long-term survivor had a score > 3. However, 29% (n = 4) of horses that did not survive > 6 months had a score of 0 or 1 and SBA concentrations of 2 of these (14%) were also within the reference range (<12.8 μmol/L).

**Discussion**

In agreement with other studies SBA concentrations were higher in short- and long-term nonsurvivors than short- and long-term survivors.5,7 However, the clinical impression that high SBA were not necessarily associated with nonsurvival in the examined population was supported by the relatively low specificity of two cut-off points (one determined in the study and the previously reported cut-off of ≥20 μmol/L).

The diagnostic value of SBA in people is undisputed as an increase in the serum is a highly specific for hepatic disease, although only moderately sensitive. In cases of liver cirrhosis in people SBA have long-term prognostic value8,9 while in cases of acute hepatitis, clinical improvement is often paralleled by decreasing SBA, corresponding with normalizing hepatic function.10,11 In these instances, one time measurements of SBA would not be useful prognostic indicators.

In horses, classically described hepatic diseases such as serum hepatitis, cholangiohepatitis, neoplasia, *Clostridium piliformis* infection, and pyrrolizidine alkaloid toxicity are all associated with severe or irreversible liver damage, or both, and a high case fatality, often ranging from 50 to 100%.7,12,13 In many of these cases, loss of hepatic function is expected to be permanent or progressive, or both, and SBA are likely to be indicative of extend of the irreparable damage and therefore also on the prognosis. In those cases, a close proportion between SBA, presence and severity of hepatic damage and a negative outcome would be expected. Similar to the findings in people, SBA would be expected to correlate with irreversible hepatic changes. In this study, a correlation between SBA and portal and bridging fibrosis as well as nucleic changes, which are considered to be irreversible, was demonstrated and those histological features were also more commonly observed in nonsurvivors.

However, SBA returned to normal limits in horses that survived hepatic necrosis which presumably corresponded to improved hepatic function and regeneration ensued.14 The horses examined here experienced predominantly mild-to-moderate hepatic disease of unknown etiology and in these cases SBA might be more indicative of a temporary and potentially reversible compromise of hepatic function. This assumption is supported by the low specificity of SBA and > 20 μmol/L for normal and a high specificity of significant correlation between SBA and potentially reversible histological findings such as inflammation and hemosiderin accumulation in Kupffer cells. However, overall and parenchymal (short-term survival only), but not portal, inflammation was also more common in nonsurvivors and although inflammation may be reversible it could equally be progressive. Enhanced prognostic information could therefore probably be gained from serial SBA monitoring.14

The histological score performed well as a specific albeit only moderately sensitive indicator of short- and long-term nonsurvival and the relatively high specificity of the histological score provided the best prognostic information for long-term survival and was numerically superior to SBA for short- and long-term survival. A limitation of the study is that the score was developed in the same geographic location (South-East England) in which this study was performed and it is likely that the nature of hepatic disease was very similar in the horses used to develop the score to the horses investigated here. Results might not be directly transferrable to areas with very different disease types and re-evaluation of the scoring system is therefore advisable before it is used for diagnostic purposes in different areas. Furthermore, all horses with hepatic disease were included together, irrespective of the underlying etiology and results might be different for certain subpopulations. The scoring system was designed for diffuse, non-neoplastic hepatopathies and most cases described here would have fallen into this category.5 Lastly, outcome could have been influenced by the initiation of adequate treatment, or lack thereof. All nonsurvivors in the study...
Table 1. Comparison of biochemical and haematological variables between short- (survival to discharge) and long-term (survival >6 months after discharge) survivors and nonsurvivors.

| Variable                  | Short-term Survivor | Short-term Nonsurvivor | P-value | Long-term Survivor | Long-term Nonsurvivor<sup>a</sup> | P-value |
|---------------------------|---------------------|------------------------|---------|--------------------|-----------------------------------|---------|
| Serum bile acids (µmol/L) | 28.1 (11.9–42.8)    | 12.3 (1.5–82.5)        | .009    | 28.1 (6.3–45.2)    |                                   | .006    |
| n = 73                    | n = 63              | n = 67                 |         |                    |                                   |         |
| Total plasma protein (g/dL)| 6.5 ± 0.6           | 6.5 ± 0.58             | .001    | 6.9 ± 1.0          |                                   | .94     |
| n = 68                    | n = 62              | n = 62                 |         |                    |                                   |         |
| Albumin (g/dL)            | 3.5 ± 0.41          | 3.5 ± 0.38             | .003    | 2.9 ± 0.48*        |                                   | <.001   |
| n = 69                    | n = 63              | n = 63                 |         |                    |                                   |         |
| Globulin concentration (g/dL)| 3.0 (1.8–4.4)    | 4.2 (4.0–7.2)          | <.001   | 3.0 (1.8–4.1)      | 4.0 (2.1–7.2)                     | .001    |
| n = 68                    | n = 67              | n = 67                 |         |                    |                                   |         |
| Fibrogen (mg/dL)          | 280 (100–950)       | 270 (100–510)          | .025    | 590* (100–950)     |                                   | .019    |
| n = 57                    | n = 55              | n = 50                 |         |                    |                                   |         |
| AST (IU/L)                | 530 (227–2520)      | 547 (227–2520)         | .26     | 436 (159–762)      |                                   | .091    |
| n = 72                    | n = 66              | n = 66                 |         |                    |                                   |         |
| Creatine kinase (IU/L)    | 300 (86–2180)       | 306 (86–2180)          | .43     | 277 (179–2889)     |                                   | .84     |
| n = 64                    | n = 58              | n = 58                 |         |                    |                                   |         |
| LDH (IU/L)                | 730 ± 305           | 641 (318–1614)         | .13     | 279* (171–831)     |                                   | .041    |
| n = 30                    | n = 29              | n = 4                  |         |                    |                                   |         |
| Sorbitol dehydrogenase (IU/L)| 16 (1.9–209)   | 17 (1.9–209)           | .051    | 22 (8.5–328)       |                                   | .59     |
| n = 36                    | n = 31              | n = 8                  |         |                    |                                   |         |
| Gamma-glutamyltransferase (IU/L)| 130 (14–1587) | 136 (14–1587)          | .56     | 90 (42–300)        |                                   | .24     |
| n = 73                    | n = 67              | n = 9                  |         |                    |                                   |         |
| GLDH (IU/L)               | 7.9 (1.2–5704)      | 7.2 (1.2–5704)         | .05     | 12.7 (9.2–15.3)    |                                   | .68     |
| n = 64                    | n = 30              | n = 3                  |         |                    |                                   |         |
| Urea (mg/dL)              | 14.3 (6.4–22.4)     | 14.6 (9.8–22.4)        | .32     | 13.7 (6.4–56.9)    |                                   | .51     |
| n = 49                    | n = 45              | n = 9                  |         |                    |                                   |         |
| Creatinine (mg/dL)        | 1.3 (0.9–2.1)       | 1.0 (0.9–2.1)          | .01     | 1.2 (1.1–4.4)      |                                   | .1      |
| n = 49                    | n = 45              | n = 11                 |         |                    |                                   |         |
| Serum amyloid A (mg/L)    | 0 (0–249)           | 0 (0–67,3)             | <.001   | 147* (0.6–990)     |                                   | <.001   |
| n = 38                    | n = 36              | n = 6                  |         |                    |                                   |         |
| Total bilirubin (mg/dL)   | 1.5 (0.4–12.3)      | 1.4 (0.4–12.3)         | .023    | 2.5* (0.9–6.1)     |                                   | .027    |
| n = 57                    | n = 51              | n = 9                  |         |                    |                                   |         |
| Direct bilirubin (mg/dL)  | 0.25 ± 0.09         | 0.2 (0.1–0.4)          | .4      | 0.2 and 0.4        |                                   | .4      |
| n = 40                    | n = 39              | n = 2                  |         |                    |                                   |         |
| Indirect bilirubin (mg/dL)| 1.0 (0.2–6.8)       | 1.0 (0.2–6.8)          | .23     | 1.5 and 5.9        |                                   | .23     |
| n = 20                    | n = 19              | n = 2                  |         |                    |                                   |         |
| Triglycerides (mg/dL)     | 41 (15–743)         | 41 (15–743)            | .15     | 1496 (15–3186)     |                                   | .15     |
| n = 8                     | n = 8               | n = 3                  |         |                    |                                   |         |
| Red blood cell count (×10<sup>6</sup>/µL)| 7.9 (4.7–11.8) | 8.0 (4.7–11.8)         | .097    | 8.5 (5.2–17.8)     |                                   | .45     |
| n = 31                    | n = 29              | n = 5                  |         |                    |                                   |         |
| Hemoglobin (g/dL)         | 13.2 (8.9–17.2)     | 13.3 (9.9–17.2)        | .64     | 8.9 (7.6–16.4)     |                                   | .26     |
| n = 31                    | n = 30              | n = 5                  |         |                    |                                   |         |
| Leukocyte count (×10<sup>3</sup>/µL)| 7.5 (3.3–18.1)   | 7.3 (3.3–18.1)         | .52     | 8.1 (4.2–16.4)     |                                   | .077    |
| n = 31                    | n = 30              | n = 5                  |         |                    |                                   |         |
| Neutrophil count (×10<sup>3</sup>/µL)| 4.2 (1.4–14.9)   | 4.1 (1.4–14.8)         | .32     | 7.0* (1.2–13.3)    |                                   | .042    |
| n = 59                    | n = 53              | n = 10                 |         |                    |                                   |         |
| Lymphocyte count (×10<sup>3</sup>/µL)| 2.5 ± 0.9      | 2.5 ± 0.9              | .71     | 2.7 (1.1–4.6)      |                                   | .55     |
| n = 59                    | n = 53              | n = 10                 |         |                    |                                   |         |

<sup>a</sup>Long-term nonsurvivors include all short- and long-term nonsurvivors.

*P ≤ .05.

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; GLDH, glutamate dehydrogenase; statistical significance was set at P ≤ .05.

were euthanized because of progression of hepatic disease, despite administration of treatment, and no animal was euthanized because of financial limitations.

In the addition to the histological features evaluated in the previous study, other histologic aspects of hepatic disease were included that, to the authors' knowledge, have not been investigated previously. Endothelial hyperplasia, a feature in people referred to as capillarization of the sinusoidal endothelium, is defined as the loss of the characteristic fenestrated phenotype of the endothelial cells with formation of an organized basement membrane. Capillarization precedes fibrosis in people and experimental animals and it has been suggested that capillarization might even initiates the
Interestingly, endothelial hyperplasia was significantly associated with short- and long-term survival in this study but further investigations are required to investigate the importance and implications of this histological finding.

Similar to other studies, significant differences in haematological and biochemical data between survivors and nonsurvivors were established. As previously reported, low albumin and high globulin concentrations were associated with short- and long-term nonsurvival. Low albumin concentrations are an infrequent finding in hepatic disease in horses. The presence of hypoalbuminemia in combination with hypoglobulinemia might therefore be particularly relevant when trying to establish a prognosis. The finding of lower LDH activities in nonsurvivors was unexpected and is difficult to explain. LDH is not a liver-specific enzyme and it is possible that muscle damage...
in survivors in association with the low number of nonsurvivors in which the test was performed has contributed to the finding of this counterintuitive statistical difference although differences in creatine kinase (CK) might have been expected if muscle damage was involved. Plasma fibrinogen concentrations are higher in nonsurviving horses with hepatic disease and it is possible that long-standing inflammatory processes in the liver ultimately lead to liver failure and death.5 No specific inflammatory cell type was associated with nonsurvival. While parenchymal and portal inflammation correlate with SBA and therefore might negatively influence liver function only parenchymal inflammation was associated with short-term survival. This could suggest that parenchymal inflammation is more likely to contribute to or trigger events that lead to hepatic failure and nonsurvival. Further large scale investigation into the different distributions and also types of inflammatory infiltrates are clearly needed.

In summary, SBA, some histopathological findings and histopathological scores differed between survivors and nonsurvivors. SBA concentrations are associated with inflammation and fibrosis suggesting that both interfere with hepatic function. A histopathological score >2 and, to a lesser degree, SBA >20 μmol/L are specific but not sensitive indicators of nonsurvival. For the population examined the histological score offered the best specificity for long-term survival.

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**Table 3.** Comparison of histological score as described by Durham et al. (2003) between short- (survival to discharge; A) and long-term (survival >6 months after discharge; B) survivors and nonsurvivors.

| A | Short Term Survivor (n = 73) | Short-term Nonsurvivor (n = 8) | P-value |
|---|-----------------------------|-------------------------------|--------|
| Histological score | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 4 | 8 | 14 |
| | 52 (71%) | 12 (16%) | 2 (3%) | 5 (7%) | 1 (1%) | 1 (1%) | 2 (25%) | 1 (13%) | 3 (38%) | 1 (13%) | 1 (13%) | <.001 |

| B | Long-term Survivor (n = 67) | Long-term Nonsurvivor (All Nonsurvivors; n = 14) | P-value |
|---|-----------------------------|-------------------------------|--------|
| Histological score | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 4 | 5 | 8 | 14 |
| | 51 (78%) | 12 (18%) | 1 (2%) | 3 (5%) | 3 (21%) | 1 (7%) | 1 (7%) | 2 (14%) | 4 (29%) | 1 (7%) | 1 (7%) | <.001 |

*Long-term nonsurvivors include all short- and long-term nonsurvivors.

*P ≤ .05; statistical significance was set at P ≤ .05.

**Table 4.** Correlation between log transformed serum bile acid concentrations and histological features and scores.

|                      | Regression Coefficient ± SEM | P-value |
|----------------------|-----------------------------|---------|
| Inflammation*        | 0.18 ± 0.051                | .001    |
| Portal inflammation* | 0.141 ± 0.047               | .004    |
| Parenchymal inflammation* | 0.155 ± 0.038 | <.001   |
| Portal fibrosis      | 0.095 ± 0.045               | .036    |
| Bridging fibrosis*   | 0.141 ± 0.044               | .002    |
| Apoptosis and/or single cell necrosis | 0.085 ± 0.051 | .099 |
| Hemosiderin (portal) | 0.083 ± 0.042               | .051    |
| Hemosiderin (Kupffer cells)* | 0.104 ± 0.046 | .026 |
| Hemosiderin (hepatocytes) | 0.053 ± 0.05    | .291    |
| Biliary hyperplasia  | 0.094 ± 0.049               | .056    |
| Vascular hyperplasia | 0.056 ± 0.06               | .347    |
| Bile stasis          | 0.078 ± 0.306               | .8      |
| Endothelial hyperplasia | 0.068 ± 0.07 | .33    |
| Cytoplasmatic swelling | -0.01 ± 0.049          | .85     |
| Distribution of cytopl swell | 0.004 ± 0.011 | .69 |
| Nuclei*              | 0.199 ± 0.055               | .001    |
| Histological score*  | 0.05 ± 0.015                | .002    |

*P ≤ .05.

Distribution of cytopl. swell: distribution of cytoplasmatic swelling; SEM: standard error of the mean; statistical significance was set at P ≤ .05.

**Table 5.** Sensitivity and specificity of different cut-off points for serum bile acid concentrations (SBA) and histological score as indicators of nonsurvival.

| Short-term Nonsurvivors | Sensitivity (%) | Specificity (%) |
|-------------------------|-----------------|-----------------|
| SBA ≥17 μmol/L          | 75              | 70              |
| SBA ≥20 μmol/L          | 62.5            | 78.1            |
| Histological score >2   | 62.5            | 90.4            |
| Histological score >3   | 62.5            | 97.3            |

| Long-term nonsurvivors (all nonsurvivors*) | Sensitivity (%) | Specificity (%) |
|-------------------------------------------|-----------------|-----------------|
| SBA ≥16 μmol/L                           | 78.6            | 65.7            |
| SBA ≥20 μmol/L                           | 57.1            | 80.6            |
| Histological score >2                    | 64.3            | 95.5            |
| Histological score >3                    | 50              | 100             |

*Long-term nonsurvivors include all short- and long-term nonsurvivors.

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**Footnote**

IBM SPSS statistics 19, P.O. Box 41, North Harbour Portsmouth, Hampshire PO6 3AU, UK

**Acknowledgments**

Conflict of Interest Declaration: Authors disclose no conflict of interest.
**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

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