The use of liver biopsies and blood values in hepatic disease in horses

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Abstract

Reasons for performing study: Find out if the scoring system made by Durham et al.[1] is useful for the situation in the department of equine species (DES) of the faculty of veterinary medicine at the University Utrecht. The diagnostic value of specific hepatic blood values is not compared with the scoring table as well.

Hypothesis: The method of Durham et al.[1] is easy to use and gives comparable results with the situation at the DES. Blood results are not of great prognostic value.

Methods: liver biopsies of 33 horses were examined at the same way as done by Durham et al.[1] results are noted and compared with the results of Durham et al.[1] Al histology is noted; fibrosis, irreversible and reversible cytopathology, inflammatory infiltration, haemosiderin and cupper accumulation, biliary hyperplasia and cholestasis. In the scoring system minimum was 0 points and maximum was 14 points. A new scoring system was made for the situation in the DES. Were other point categories were changed to the situation of DES and cholestasis and reversible cytopathology were included. Now minimum was 0 points and maximum was 17 points. Morphological diagnosis were also compared with the average score. Average blood values of GGT, AST, LDH and ALP are compared with the scoring system of Durham et al.[1] and with the survival rate to see the predictive value of blood work done in horses suspected of hepatic disease.

Results: 30 horses were included in this study, 17 (55.67%) did survive. Results were not comparable with Durham et al.[1] big differences were found in inflammatory infiltration, haemosiderin accumulation and biliary hyperplasia. Those categories are changed in a new developed scoring table. Durham et al. did not find cholestasis and reversible cytopathology was not included in their table as well. In this study a high mortality rate of those categories was found so those were included in the new developed scoring table for the situation at the DES. Morphological diagnosis was compared with the average score for survivors and non survivors but no significant difference was seen. A difference is seen between GGT and ALP for different scoring groups but significance difference was found. Also no significant difference was seen between the average blood values of GGT, ALP, AST and LDH between survivors and non survivors.

Conclusions: The scoring system of Durham et al. was easy to apply. Results were not comparable with those of Durham et al so a new scoring system for the situation in DES is made. A bigger population is necessary to see if there is any significant difference and to improve the scoring table. To find any possible significant difference in blood values the research should be standardized.

Introduction

Liver disease is often not recognized in horses. The liver has a great reserve capacity, which means that liver disease often occurs sub-clinically. When 50-80% of the liver is damaged the disease can still be sub-clinical[2, 3]. Clinical signs of liver disease include weight loss, icterus, neurological signs, hypoglycemia, photosensibility and blood coagulation problems. A lot of these symptoms are not-specific for liver disease and also occur with a lot of other diseases as well, although clinical signs do

have some prognostic value[2-5]. Horses suffering from liver disease with a combination of symptoms have a 38 times higher mortality rate than horses without clinical signs[4]. Ponies have a lower survival rate than horses. A diagnosis and a treatment plan cannot be based solely on clinical sings[4].

Blood examination is a good method to identify liver damage[4]. When clinical signs lead to the suspicion of a liver problem, liver and bile duct enzymes are good indicators of liver disease[2]. The exact diagnosis and prognosis are mostly not identifiable with blood examination. The only enzymes that will give an idea of the prognosis are γglutamyltransferase (GGT) and Alkaline phosphatase (ALP)[2-7].

Ultrasound of the liver is another possibility for non-invasive liver diagnostics. Ultrasound can be used to identify abnormalities. Only a small part of the liver is visible with the ultrasound. The other problem is that if abnormalities are visible a diagnosis is not always visible. No research has been published on the clinical use of ultrasound to predict the prognosis of liver disease[4].

Liver biopsy is the gold standard for the ante mortem diagnosis of liver disease[1, 3, 6]. It is the most sensitive and specific diagnostic method. When a representative part of the affected liver is biopsied a specific diagnosis can be made[1]. When a diagnosis has been made it may be possible to choose a specific therapy. Not much research has been performed on the prognostic value of liver biopsy. Durham et al. made a start and found that fibrosis was the most useful parameter in biopsies for the prognosis. A high score for fibrosis was associated with a lower survival chance. They also found bile duct hyperplasia to be a useful parameter. A scoring system was developed by them to estimate the prognosis for an individual patient[1].

The aim of this research was to determine if liver biopsies taken at the department of equine sciences (DES) at Utrecht University provide an indication of the prognosis and diagnosis for horses suspected of liver disease. In addition the usability of Durham's scoring system was investigated. The results of blood examination were also compared with the scoring system and outcome.

Materials and methods

Patient records of all patients which had undergone a liver biopsy because of suspected liver disease in the last 5 years (January 2008 until May 2013) were reviewed. This included liver biopsies obtained at necropsy from horses which suffered liver disease. The horses were presented to the DES between January 2008 and May 2013. The only criterion for inclusion in this study was a liver biopsy.

In all horses blood examination was performed and results were recorded (attachment 1). The same tests were not performed in all horses. Blood values were recorded to determine the prognostic value and to correlate the biopsy score and the level of some commonly used blood values.

The biopsies were taken using a biopsy needle under ultrasound guidance. The biopsy site was chosen where pathology was found by ultrasound (if applicable), although a site needs to be chosen away from hepatic vessels. The right side is easier to biopsy because of the greater mass of liver which is available. Before a biopsy was taken the horse was sedated with an α2-agonist with or without butorphenol (depending on clinician preference), the biopsy site was clipped and disinfected. The skin was infiltrated with lidocaine (5 ml) down to the parietal peritoneum. The ultrasound probe was placed inside a sterile glove to protect it from the alcohol and make it sterile. A stab incision was made through the skin. The biopsy needle was checked to ensure that it was firing correctly. Then the needle could be advanced, fired and retracted, all under ultrasound guidance. The samples were removed with a sterile needle and placed in a 4 % formaldehyde solution for histopathology. When a good sample had been acquired the biopsy site was cleaned and stapled or

sutured. No antibiotics or anti-inflammatory drugs are indicated after this procedure. The horses were withheld from food and water until sedation had worn off[8].

The necropsy material was taken from a representative part of the affected liver.

All owners were called to ask how the horse was doing after the biopsy. Owners were asked how the horse had responded to treatment, if the horse was still alive and, if not, how long the horse had survived following biopsy. The owners were also asked for the reason of death, if known, and if it was related to the liver problems.

The biopsies were scored using the system introduced by Durham et al. using the same criteria[1] (table 1). In each biopsy specimen 5-12 representative lobules were examined. The biopsy was first screened to see how many intact lobules were present. Most biopsies contained more than 25 intact lobules but three of them had only intact 5 lobules that could be used. Each lobule was examined to assess the portal tracts and hepatic parenchyma. In the portal tracts inflammatory cell infiltration, bile duct proliferation, vascular changes and fibrosis were evaluated. The hepatic parenchyma was examined for reversible and irreversible cytopathology, haemosiderin and copper deposition, cholestasis and inflammatory cell infiltration.

The scoring was done by a veterinary student (Michael Woltheus), who was taught by a veterinary pathologist (Guy Grinwis). A morphological diagnosis was determined by an experienced pathologist.

Table 1. An overview of scoring classification

	Mild	Moderate	Severe
Fibrosis	2 times increased	3	≥4
irreversible cytopathology	< 25 %	25-50%	> 50%
reversible cytopathology	< 25 %	25-50%	> 50%
inflammatory infiltration	≤ 5 cells	≤ 20	> 20
haemosiderin accumulation	< 25 %	25-50 %	> 50%
biliary hyperplasie	2 tot 3	4 to 6	≥ 7
copper accumulation	< 25 %	25-50%	> 50%
Cholestasis	< 25%	25-50%	> 50%

The extent of fibrosis was determined by the enlargement of the bile duct due to fibrin. When this was enlarged to two times the normal size the fibrosis was called mild, moderate when it was three times enlarged and severe if enlarged by four or more times. When fibrosis was severe there might be bridging fibrosis, which means that lobules are connected by fibrin. Necrosis, megalocytosis and amyloidosis are examples of irreversible cytopathology. Megalocytes are hepatocytes with enlarged nuclei and increased cytoplasmic volume[5, 9]. They may be many times the size of normal hepatocytes. The megalocytes are induced by pyrrolizidine alkaloids, which prevent cell division but not DNA synthesis. The DNA synthesis is stimulated because the cells want to replace those cells that have undergone necrosis. Megalocytosis is also seen with other toxins such as aflatoxins produced by fungi[9]. Amyloidosis is protein accumulation which consists of β-plated sheets of non-branched vessels. On histology it will look like clear eosinophilic amorphous deposition[9]. The grade of irreversible cytopathology was also defined as absent, mild, moderate and severe, where mild means that < 25 % of a representative lobule was affected, moderate 25-50% and severe where more than 50% of the lobule was affected. The irreversible cytopathology was classified in the same way as the reversible cytopathology. As signs of reversible cytopathology hydropic degeneration, cloudy swelling

and cytoplasmic granularity of hepatocytes were evaluated. Hydropic degeneration is cell swelling, it is the most frequent form of reversible cytopathology. The cell swells due to more water in the cell because of a lack of normal homeostasis. There is also a degeneration of the cell organelles, leading to cloudy swelling and cytoplasmic granularity[9]. The inflammatory infiltration was graded based on the number of leucocytes in the portal tract. It was mild in cases with less than 5 cells, moderate at less than 20 cells and severe at more than 20 cells. When it is severe there are mostly also cells in the hepatic parenchyma. The haemosiderosis was scored in the same way as the reversible cytopathology. Haemosiderosis is the accumulation of iron in the hepatocytes and is seen as dark spots in the cells in an H.E. histology preparation. In some cases, in the samples from horses suspected of suffering from too much copper, a copper stain preparation was made. The scoring was the same as in the haemosiderosis. Bile duct proliferation was considered to be present when the bile duct had divided into multiple branches. It was noted as mild when there were 2-3 branches, moderate at 4-6 branches and severe at 7 or more. Cholestasis was again graded as mild at < 25%, moderate 25-50% and severe at > 50%[1].

Durham et al. did not score the cholestasis and copper accumulation because it was not found in their study[1].

In this study the possible relationship between histological abnormalities and the prognosis was examined. Copper was only possible to see in some biopsies, because it needed a special stain.

Durham et al. developed a scoring system[1] (table 2) based on their research of 73 horses. The scores are based on survival rate in Durham's research. A high mortality rate received 4 points and 0 points are changes with a normal survival rate[1]. That scoring system was used for this research to compare the patients of the department of Equine science at Utrecht with the situation in the UK.

Table 2. scoring system of Durham et al.[1] (minimum score = 0. maximum = 14)

	Severity			
Variable	Absent	Mild	Moderate	Severe
Fibrosis	0	0	2	4
Irreversible cytopathology	0	1	2	2
Inflammatory infiltrate	0	0	1	2
Haemosiderin accumulation	0	0	0	2
Biliary hyperplasia	0	0	2	4

Based on our findings in the present study a slight adaptation of the grading system was developed (table 3). The same criteria are tried to use to make this scoring table. The population at the DES had a high mortality rate in reversible cytopathology and cholestasis. By that those are included in the scoring table.

For comparison the average score between survivors and non survivors the two-sample t-test was used. This test was also used for the new scoring system and to compare the blood values of survivors and non-survivors. For comparing the blood values of GGT and ALP in different scoring groups the paired sample t-test was used. To compare the difference in survival rate between each score category the chi-squared test was used[10].

Table 3. scoring system made for the DES (minimum score = 0, maximum = 17)

	Severity			
Variable	Absent	Mild	Moderate	Severe
Fibrosis	0	0	2	2
Reversible cytopathology	0	0	0	4
Irreversible cytopathology	0	1	2	2
Inflammatory infiltrate	0	0	0	1
Haemosiderin accumulation	0	0	1	2
Biliary hyperplasia	0	0	1	4
Cholestasis	0	2	2	2

Results

All owners were called. Of the 32 cases 30 owners were available for information. Of the total of 30 horses with suspected hepatopathy, 17 (55,67 %) survived for at least 6 months following biopsy.

Fibrosis was found in many (n= 24) cases (table 4). Only horses with moderate fibrosis had a higher (80%) mortality rate. In the other categories mortality varied between 33 and 38%, with severe fibrosis having the same mortality rate as no fibrosis. This suggests that there is no increased risk of non-survival with more fibrosis in this population of horses, although case numbers are small.

Irreversible cytopathology was not found in 21 of the cases. Absent and mild irreversible cytopathology had the same mortality rate (62%). Moderate irreversible cytopathology was found in only one case and that horse did not survive. There were no cases with severe irreversible cytopathology in this study/population of horses.

Reversible cytopathology was associated with a non-survival percentage of 42%. Mild and moderate reversible cytopathology had a lower mortality rate. Severe reversible cytopathology was found in 3 cases, none of which survived.

Inflammatory infiltration was found in all cases. More inflammatory cells were associated with a higher risk of non-survival. Most cases (n=20) had moderate inflammatory infiltration (5- 20 cells). Moderate inflammatory infiltration had the same survival rate as the overall survival rate. Severe inflammatory infiltration had a mortality rate of 100%, but included only one case.

Haemosiderin accumulation was found to be mild in almost half of the cases, with a survival percentage of 57%. The absence of haemosiderin was associated with a higher survival rate, 64%, which was not a poorer survival rate than average. With moderate haemosiderin accumulation the survival rate was 40%. In this study no cases of severe haemosiderin accumulation were found.

Mild and moderate biliary hyperplasia were associated, in this research, with a lower risk of death than when hyperplasia was absent. One case with severe biliary hyperplasia did not survive.

Cholestasis was not scored in the system used by Durham et al because they did not find it[1]. In the present study it was found in 2 cases. Both of those cases did not survive. It was only classified as mild. The absence of cholestasis was associated with a higher survival rate.

Copper accumulation was found in only one case. This case was suspected of having a large amount of copper and a special stain was performed. Most cases did not have the copper stain and they were all categorized as absent.

Table 4. Numbers and percentages of survivors and non-survivors in each histopathological category

		Survivors		Non survivors	
Variable	**Category**	**n**	**%**	**n**	**%**
Fibrosis	Absent	4	67	2	33
	Mild	8	62	5	38
	Moderate	1	20	4	80
	Severe	4	67	2	33
Irreversible cytopathology	Absent	13	62	8	38
	Mild	4	50	4	50
	Moderate	0	0	1	100
	Severe	0	-	0	-
Reversible cytopathology	Absent	11	58	8	42
	Mild	4	80	1	20
	Moderate	2	67	1	33
	Severe	0	0	3	100
Inflammatory infiltration	Absent	0	-	0	-
	Mild	6	67	3	33
	Moderate	11	55	9	45
	Severe	0	0	1	100
Haemosiderin accumulation	Absent	7	64	4	36
	Mild	8	57	6	43
	Moderate	2	40	3	60
	Severe	0	-	0	-
Biliary hyperplasia	Absent	4	50	4	50
	Mild	8	53	7	47
	Moderate	5	83	1	17
	Severe	0	0	1	100
Cholestasis	Absent	17	61	11	39
	Mild	0	0	2	100
	Moderate	0	-	0	-
	Severe	0	-	0	-
Copper accumulation	Absent	16	55	13	45
	Mild	0	-	0	-
	Moderate	0	-	0	-
	Severe	1	100	0	0

All cases where scored according to the table of Durham et al[4]. Almost half of the cases had a biopsy score between 2 and 6. The survival rate decreased with an increased biopsy score but there was no statistically significant difference between the groups of 2 – 6 and 7-14. An overview was given in table 5.

Table 5. numbers and percentages of survivors and non-survivors in each scoring category

	Survivors		Non-survivors		Total
Biopsy score	N	%	N	%	N
0	2	67	1	33	3
1	8	80	2	20	10
2 - 6	6	43	8	57	14
7 - 14	1	33	2	67	3
	17		13		30

A morphological diagnosis was assigned by the pathology department of the faculty of veterinary medicine of Utrecht University. All horses had a morphological diagnosis without using the scoring system. These diagnoses were compared to the average score of the scoring system (table 6). The biggest difference between survival rate and biopsy score was in the pyrrolizidine alkaloid (PA) toxicity group, although only 2 horses were given those diagnosis. With the other diagnoses a higher score was found for survivors than for non-survivors. This shows that there is no significant correlation between the scoring system used and the morphological diagnosis.

Table 6. Numbers and average score of survivors and non-survivors of each morphological diagnosis

Diagnosis	# survivors	Average score	# non-survivors	Average score
Aspecific reactive hepatitis	7	1,83	2	1,5
Chronic hepatitis/ cirrhosis	2	7	3	5,67
Hemochromatosis	2	6	0	0
Hyperlipemia	0	0	3	1,67
Pyrrolizidine alkaloid toxicity	1	1	1	7
Cholangiohepatitis	0	0	2	1
Parasitic hepatitis	1	1	0	0
Isoerythrolysis neonatal	0	0	1	4
No histological abnormalities	4	1	1	1

In most horses blood examination was performed. The results of γ-Glutamyltransferase (GGT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) are compared to the biopsy score. The same blood examinations were not performed in all horses. Table 7 shows the average of each blood value in U/L. The blood values for AST and LDH did not correlate with the biopsy score. The values for GGT and ALP showed an increase at a higher biopsy score but was not significant (Attachment 2). Although GGT was increased at score 0, this was the case for only one patient. The correlation is shown in figures 1 and 2.

Table 7. Average blood enzyme concentrations vs. biopsy score

	Average value U/L			
Biopsy score categorie	0	1	2 - 6	7 – 14
GGT	147	183,11	317,81	469,33
AST	1167	401,5	352,3	410
LDH	640	448,67	695,3	365
ALP	1200	348,67	685,6	1069,5

Figure 1. Average blood enzyme concentrations vs. biopsy score

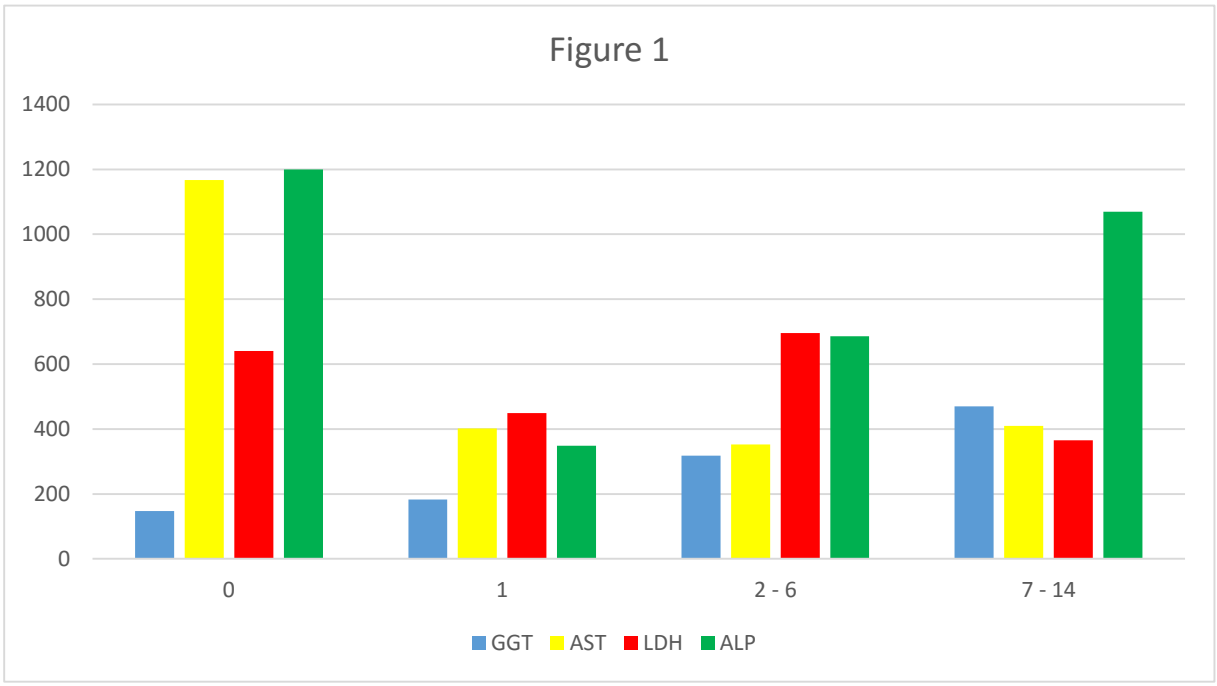


Figure 2. Average blood enzyme concentrations vs. biopsy score of GGT and ALP n = number of cases included

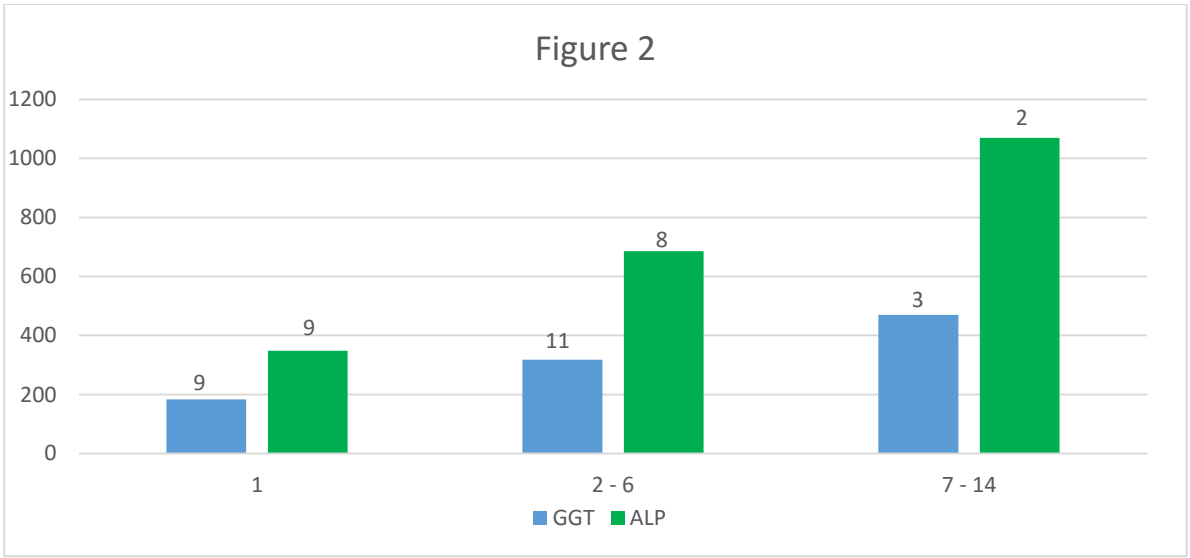
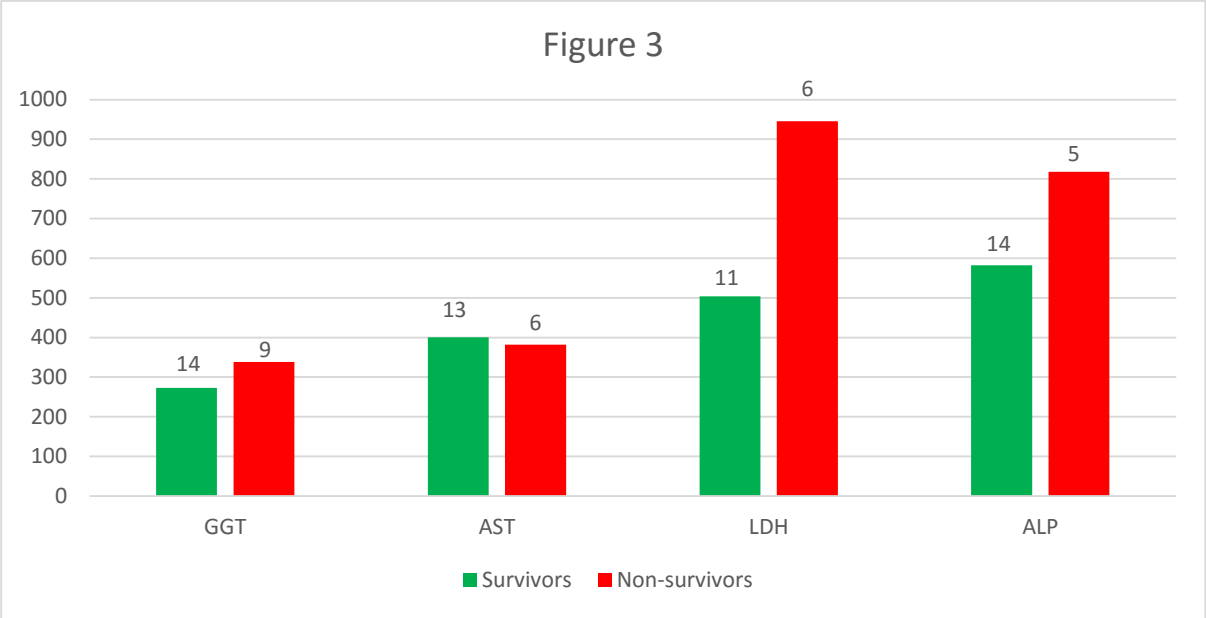


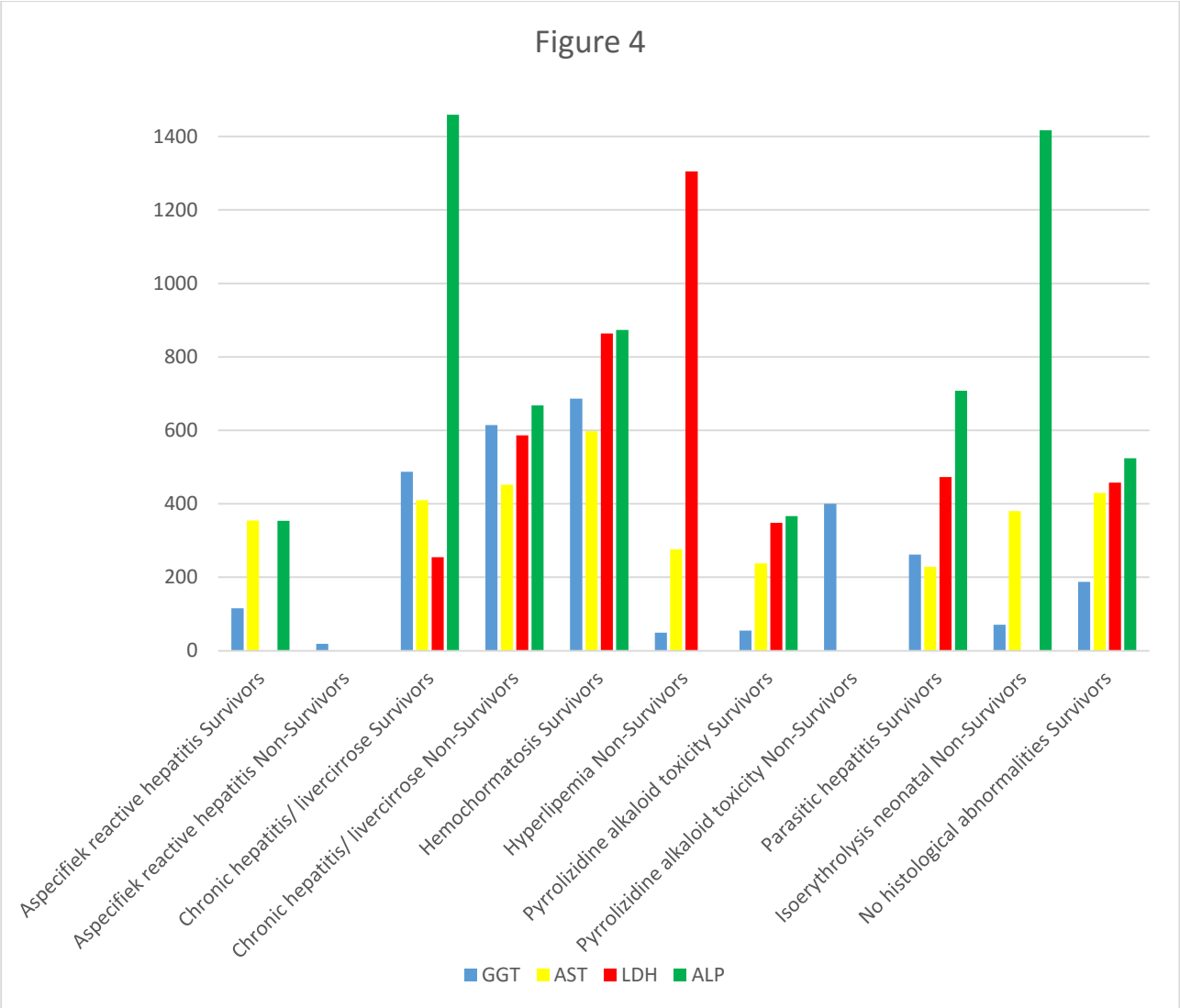
Figure 3. Average blood enzyme concentrations for survivors and non-survivors, n = number of cases included



The average blood value of GGT, AST, LDH and ALP was compared for survivors and non-survivors. In attachment 1 blood values are shown. The biggest difference was seen at LDH values (figure 3), but was not significant with a P value of 0.064 (attachment 3). GGT and ALP are the only blood values found in other studies with a correlation to survival in horses suffering from liver disease[1-3]. In this population a small difference was seen in GGT and ALP levels in survivors and non-survivors. Neither difference was statistically significant with P values of 0.294 for GGT and 0.416 for ALP (attachment 3).

Average blood concentrations are compared for each morphological diagnosis and survivors vs non-survivors (figure 4). No significant difference was found between survivors and non-survivors in any group, for any morphological diagnosis. Most of the groups consisted of only a few patients and not always all blood work was done. It's also hard to say something about the difference between each morphological diagnosis. The number of patients in each morpholological diagnosis with all blood work done are too low to detect a significant difference.

Figure 4. Average blood enzyme concentrations for each morphological diagnosis in survivors and non-survivors



Discussion

Liver biopsy is considered the gold standard to establish a morphological diagnosis[2, 4, 7]. In this study a morphological diagnosis was made in 25 of the 30 cases. The five cases without a diagnosis had no histological abnormalities. This means that in 83.3% of the cases a morphological diagnosis was reached. The horses with no diagnosis had an average score of 1 and only one of them did not survive. Because no histological abnormalities were found in those cases it means that the biopsy was of added value. This study shows that liver biopsy is of great value to establish a morphological diagnosis which is necessary to decide a good treatment.

A high non-survival rate was found in this study in comparison with the study of Durham et al[4]. They had a survival rate of 75% and in this study the survival rate was 55.7%. The big difference may be the patient selection. Durham et al. used only horses with an ante-mortem liver biopsy, in this study use was also made of necropsy material of 10 horses. Survival rate of horses with an ante-mortem liver biopsy was 85%.

In this study only 3 horses were found to have biopsy score of 0 and also only 3 with a score between 7 and 14. The cases with a biopsy score 0 had a survival rate of 66.7%, one horse did not survive. This horse had no biopsy but a necropsy sample. This horse had suffered low foot pain and had had a high

blood GGT on several occasions. It might be possible that the horse would have survived if there had been no history of lameness. Durham et al. correlates the survival rate of biopsy scores with those of score 0[4], in this study the number of cases with score 0 is too low. Only ten percent of cases had score 0. A difference between score 1 and score 2-6 was seen, they respectively had a survival rate of 80% and 43%. No significant difference (P is 0.85) was found between the average score in survivors versus non-survivors (attachment 4). Also, no correlation was seen between the scoring categories and survival (P is 0.47) (attachment 5). The average score and the survival rate is also not statistical correlated (P is 0.21) (attachment 6) More cases might be necessary to detect a possible difference in average score between the survivors and non-survivors. It might also not be good to take necropsy material and include only horses that have been treated. Also, it is not always easy/possible to determine if the cause of death (or reason for euthanasia was related to liver disease).

The scoring system developed by Durham et al. was simple to use. They made a clear classification with common abnormalities which were easy to identify[4]. Although the scoring system was not really representative for the cases used in this study, because big differences are seen. An slight variation of this scoring system, useable for the population in the DES, is presented in this paper (table 3).

In this study 3 cases were found with severe reversible cytopathology and none of those cases survived. This classification (severe reversible cytopathology) should have 4 points and moderate reversible cytopathology 0. There was no difference between survival rate in moderate reversible cytopathology and the average survival rate. Severe reversible cytopathology had a non-survival rate of 100% and this is serious enough to assign 4 points. Durham et al. did not find a difference in survival rate with reversible cytopathology and therefore they did not use it for their scoring system[4]. In the present study a non-survival rate of 100 % for severe and of 33% for moderate reversible cytopathology was found. In 10% of the cases a severe reversible cytopathology was found. That is not a large amount of cases but may be enough to use in our system.

Cholestasis was not found in the study by Durham et al. but they had looked for it in the biopsies[4]. In the present study 2 biopsies were found with cholestasis. This represents only 6.7% of the cases although neither horse survived. They both only had mild cholestasis, and this might indicate that only a small amount of cholestasis is associated with a poor prognosis. Therefore 2 points were given for moderate cholestasis. No cases of mild or severe cholestasis were found, therefore they received a score of 2 as well. If a greater number of horses with cholestasis had been included in the study more evidence regarding the value of including cholestasis in our system would have been provided.

A mild or moderate degree of hemosiderin accumulation was found in 60% of cases, with moderate haemosiderin accumulation seen in 16.7%. A score difference in haemosiderin accumulation was found as well. In the study by Durham et al. moderate haemosiderin accumulation was assigned a score of 0 and had a low mortality rate of 20%. In this study the group of non survivors was given 1 point because of a slightly higher non survival rate as in the average survival rate.

Inflammatory infiltration was moderate in most cases and was not associated with a higher non-survival rate than average. This is the reason that 0 points are given for moderate inflammatory infiltration. Only one case had a severe score, but because of the small number of cases and the fact that severe inflammatory infiltration did not have a big effect on survival rate a score of 1 was given.

Moderate biliary hyperplasia had a low non-survival rate, which was lower than in cases with mild biliary hyperplasia. Therefore the scoring received only 1 point. Mild biliairy hyperplasia had more survivors than when biliary hyperplasia was absent. Therefore, 0 points were assigned for mild biliary hyperplasia. Moderate has a non-survival rate of 100% with only 1 case, 4 points were given, as was the case in the scoring system of Durham et al[4].

Table 8. numbers and percentages of survivors and non-survivors in each scoring category in the new developed scoring table for the DES

	Survivors		non-suvivors		Total
Biopsy score	N	%	N	%	N
0	8	80	2	20	10
1	4	67	2	33	6
2 – 6	5	36	9	64	14
7 – 17	0	-	0	-	0
	17		13		30

Hepatic fibrosis is normally seen as the most useful prognostic indicator for prognosis[4]. In this study moderate fibrosis had a higher non-survival rate than severe fibrosis, therefore both categories were given 2 points. In Durham et al. no horse survived with severe fibrosis, while in the present study 4 horses with severe fibrosis were still alive 6 months after biopsy. This is an unexpected finding, the explanation of this finding may be found in the treatment of those horses or in the (lack of) experience of the researcher in our study, although bridging fibrosis is easy to recognize under the microscope.

No significant difference in the average biopsy score was found between survivors and non-survivors using the new scoring system, although there was a trend towards lower survival with increasing biopsy score category (P=0.069)(attachment 7). A bigger difference in average biopsy score between survivors and non-survivors as seen using the new scoring system compared to that described by Durham et al. but this difference was not statistically significant. More horses are likely to be necessary to determine if a real difference in survival exists between different biopsy score categories using the new scoring system.

Blood concentrations of GGT and ALP increased with biopsy score category. This may be due to a higher biopsy score having more liver damage[7]. Liver enzyme concentrations will rise in blood with more liver damage. Such a correlation was not found for LDH and AST. This is unexpected but could be due to the release of these enzymes into the blood without or preceding cellular damage which would lead to microscopically visible alterations (and thus increased biopsy score). It could also be due (in part) to the fact that not all blood enzymes were measured for all horses and that blood was not always sampled at the same time before the liver biopsy was taken.

Blood concentrations and survival rate were also compared. The biggest difference was seen for LDH. GGT and ALP showed an increase in blood enzyme concentrations. Although no significant difference is seen. In AST the concentrations were higher in survivors. For a better understanding of the significance of these biochemical parameters it would be a good idea to standardise the blood sampling. The same parameters should be determined, at the same time in relation to the biopsy, in all animals undergoing a liver biopsy.

Most categories only included a small number of horses. Some biopsy score categories only contained 3 horses and the same blood examination was not performed in all horses. These small numbers make it difficult to find real (statistically significant) differences between categories

Conclusion

In this study the scoring system of Durham et al. was applied to a patient population at the department of equine science at Utrecht University in the Netherlands and the ease of use of the grading system was assessed. Blood examination values of various (liver) enzymes were also compared with the survival rate. All horses which underwent a liver biopsy between January 2008 and may 2013 were included in this study.

Liver biopsy was found to a useful diagnostic modality, with most horses having a morphological diagnosis after biopsy. The scoring system described by Durham et al was easy to apply, as clear definitions of the categories were provided.

In the present study several histopathological findings not reported by Durham et al were encountered and a new scoring system (for the situation at the DES) was developed. To further investigate the validity of the new scoring system more horses should be studied. With more horses a new comparison between the scoring system according to Durham and the new scoring system could be made. It might also be necessary to futher adapt the scoring system.

Blood examination of liver enzymes were also compared with survival rate and again no significant differences were found. For this aspect also more horses are necessary and the same blood tests need to be performed in all horses, at the same time (in relation to biopsy). Liver enzyme values were also compared with the morphological diagnosis but there were too many diagnoses, with only a few cases in each category, to find any significant difference.

In the present study the liver biopsy scoring system described by Durham et al. was applied to a population of horses suspected of liver disease at the DES, but did not prove to be of prognostic value in these horses. More research, including larger numbers of cases, needs to be performed to develop a scoring system capable of providing accurate prognostic information following liver biopsy in the horse.

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Attachment 1 GGT	AST	LDH	AF	bilirubine	bil direct	galzuren	ammoniak	ht	leuco's	TE	spectrum (albumine)	PT (s)	APTT (s)
<20 U/L	125-275 U/L	150-240 U/L	140-300 U/l	<35 µmol/L		µmol/L	<30 µmol/L	0,32-0,42	7,0-10,0	55-75 g/L	26-37 g/L		
147	1167	640	1200	134,6		97	10	0,37	27,8	59	31		
400							220	0,55	11,1			verlengd	
												10,5	46.9
44	302	879		43,1	7,2		61	0,5	9.2	63	27		
54	251	614		24,2	3,2			0,42	9,9	66	26		
785	655	1133	1372	28,1	4,8	40	15						
19				61,9	2,6			0,35	3,6				
769	410	255	1459	78,8	31,2	140	50	0,37	10,4	72	31	13,6	55,8
		2422						0,37	2,1	74			
55	238	348	367	44,2	3,6	7		0,37	15,5	63	33	12,3	78,8
			589			12	<7					13,4	130
239		475	680	13,1	3	6						12,5	59,9
145	221		389	12,9	3,3	7	13	0,41	9,8	63	35	13,1	52
												12,3	80,6
												12,4	65,6
35	185	414	241	19,6	2,3	6		0,41	11,1	63	36		
205				28,7	3,5	5		0,35	10,7	66	31	11,8	> 300,0
27	179	359	332	24,2	2,6	5		0,33	7,8	61	33	13,4	108
316	483		386	21	2,3	13	9	0,38	6,6	60	35	12,6	57,8
127	393	335	192	12,1	2,6	9		0,33	2,8	72	33	13	54
										75	37	13,4	144
262	228	473	708	56,3	6,6	27	11	0,31	6,3	68	36	14,7	81,4
398	570		207									13,5	> 300
295	926	704	126	11,3	3	10	< 7	0,31	7,2	70	38		

779	596	570	785	23,4	4,2	60	247	0,4	9,8	80	35	14,1	> 300
1152	442		553	83,9	8,3	83		0,4	10,1	75	31	13,9	57,1
141	189	418	322				9	0,34	6,2	61	36	13,9	51,9
138	235	574	394	17,1	3,3	4		0,29	10,8	69	34	12,6	> 300
587	540	594	374	10,9	2,6	49	17	0,37	25,6	89	25		
								0,37	5	36	21		
71	380		1417	231,1	104,4		47	0,24	29,6	56	33		
288	318	714	654				136	0,32	12,5	68	27		

Attachment 2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	GGT categorie 1	183,1111	9	138,19501	46,06500
	GGT categorie 2-6	327,7778	9	391,16166	130,38722

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	GGT1 & GGT 2-6	9	-,248	,520

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	GGT 1 – GGT 2-6	-144,66667	446,01037	148,67012	-487,50059	198,16725	-,973	8	,359

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ALPcategorie 1	352,0000	8	205,04634	72,49483
	ALP categorie 2-6	685,6250	8	450,36523	159,22815

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	ALP 1 & ALP 2-6	8	-,292	,484

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	ALP 1 – ALP 2-6	-333,62500	546,54681	193,23348	-790,54956	123,29956	-1,727	7	,128

Attachment 3

Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
LDH	Survivors	11	503,9091	240,73157	72,58330
	Non Survors	6	945,6667	736,25123	300,57330

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LDH	Equal variances assumed	4,004	,064	-1,859	15	,083	-441,75758	237,68111	-948,36287	64,84771
	Equal variances not assumed			-1,429	5,591	,207	-441,75758	309,21295	-1212,00904	328,49389

Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
GGT	Survivors	14	272,9286	260,33040	69,57623
	Non survivors	9	338,4444	389,44868	129,81623

Independent Samples Test

	Levene's Test for Equality of Variances	t-test for Equality of Means

		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GGT	Equal variances assumed	1,161	,294	-,486	21	,632	-65,51587	134,92689	-346,11171	215,07996
	Equal variances not assumed			-,445	12,616	,664	-65,51587	147,28579	-384,69596	253,66422

Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
ALP	Survivors	14	581,8571	437,85014	117,02037
	Non survivors	5	817,8000	344,98362	154,28137

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
ALP	Equal variances assumed	,695	,416	-1,084	17	,294	-235,94286	217,70014	-695,25001	223,36430
	Equal variances not assumed			-1,218	9,009	,254	-235,94286	193,64015	-673,92161	202,03589

Attachment 4

Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
Average score	Surviors	17	2,5294	2,50294	,60705
	Non survivors	13	3,0000	2,51661	,69798

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Average score	Equal variances assumed	,039	,845	-,509	28	,615	-,47059	,92434	-2,36401	1,42284
	Equal variances not assumed			-,509	25,904	,615	-,47059	,92504	-2,37237	1,43119

Attachment 5

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Category * Survivor	30	100,0%	0	0,0%	30	100,0%

Category * Survivor Crosstabulation

Count

		Survivor		
		Yes	No	Total
Category	0	2	1	3
	1	8	3	11
	2-6	6	7	13
	7-14	1	2	3
Total		17	13	30

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,528[a]	3	,470
Likelihood Ratio	2,580	3	,461
N of Valid Cases	30		

a. 5 cells (62,5%) have expected count less than 5. The minimum expected count is 1,30.

Attachment 6

Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
Average score	Survivors	17	1,2353	1,52190	,36911
	Non survivors	13	3,0000	2,04124	,56614

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Average score	Equal variances assumed	1,626	,213	-2,716	28	,011	-1,76471	,64967	-3,09549	-,43392
	Equal variances not assumed			-2,611	21,462	,016	-1,76471	,67584	-3,16835	-,36106

Attachment 7

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Categorie * Survivor	30	100,0%	0	0,0%	30	100,0%

Categorie * Survivor Crosstabulation

Count

		Survivor		
		No	Yes	Total
Categorie	0	2	9	11
	1	2	3	5
	2-6	9	5	14
Total		13	17	30

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5,359[a]	2	,069
Likelihood Ratio	5,644	2	,060
N of Valid Cases	30		

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 2,17.