TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2011; 35(1): 51-58

doi:10.3906/vet-0912-20

Haematology of downer dairy cows with fatty liver

Emmanouil KALAITZAKIS[1], Nikolaos PANOUSIS[1,*], Nikolaos ROUBIES[2],
Eleni KALDRYMIDOU[3], Harilaos KARATZIAS[1]

[1]Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24 Thessaloniki - GREECE

[2]Laboratory of Clinical Diagnosis and Clinical Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24 Thessaloniki - GREECE

[3]Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24 Thessaloniki - GREECE

Received: 11.12.2009

Abstract: This study examined downer dairy cows with concurrent fatty liver of different degrees and evaluated the haematological values, the relationship and joint occurrence of fatty liver severity and haematology and compared the haematological values in the downer animals that finally died or cured. Blood and liver biopsy samples were obtained from 36 Holstein downer cows shortly after they became recumbent and before they were treated. Blood analysis included determination of haematocrit, haemoglobin, white blood, granular cell and lymphocytes count, and platelet number. Liver tissue was examined histologically and was classified according to severity of fatty liver. The majority of downer cows had fatty liver. Concerning outcome, 9 of the 36 cows were finally cured and 27 were confirmed dead by the deadline decided for cases' follow up, which was the 7th day-in-milk. There was a significant association between clinical outcome (cured or died) and severity of fatty liver, while none of the haematological parameters showed any significant difference between the cured vs. animals that died. The haematology of downer cows with concurrent fatty liver did not offer sound help in patient evaluation. There was no strong correlation between blood parameters and liver triglyceride content or other liver diagnostic parameters. Neither haematological parameter showed any significant difference between animals which finally either died or were cured.

Key words: Haematology, downer cows, fatty liver

Introduction

Downer cow syndrome is a major concern in dairy farms worldwide. It occurs mainly in the early postparturient period and is usually caused by multiple disease processes. The most common cause of downer cow syndrome is hypocalcaemia (milk fever) (1) but it has also been reported to be caused by injuries, muscle damage, macromineral deficiencies, toxic mastitis, or metritis (2). A commonly used definition for downer cow syndrome is recumbent cases of milk fever that fail to rise following treatment with calcium (2). Fatty liver may also contribute to cows becoming downers (3,4). Almost all high producing dairy cows are in negative energy balance in early lactation because energy

* E-mail: panousis@vet.auth.gr

requirements exceed feed consumption capacity (5). The liver plays a central role in metabolism and dairy cows are generally prone to liver disease (6). A high proportion of dairy cows experience fatty liver before and after parturition (7).

Liver status affects blood synthesis in many ways and fatty liver disease causes changes in haematology of the cows (3,8). Moreover, fatty liver can cause disturbances in blood clotting and immune response (9). Reports about the haematology in fatty liver cows have been published earlier (10).

Recumbency status of downer cows is a stressful condition that affects the health and prognosis of the animals in many ways. When a downer cow is suffering concurrently from fatty liver, it makes the situation more complicated, and a detailed evaluation of the patient is required for the outcome. This evaluation should involve, apart from a thorough clinical examination, clinical chemistry and haematological analysis. Although cattle haematology still remains underestimated, in the few last years it has attracted great attention worldwide.

The implication that the concurrence of recumbency and various degree of fatty liver may have on the haematological status of dairy cows is uncertain. The published information on this is lacking.

Considering all the above, the purposes of the present study were: 1. to estimate the haematological values in downer dairy cows with concurrent fatty liver of different degrees, 2. to evaluate the relationship and joint occurrence of the severity of fatty liver and haematology in downer cows, and 3. to compare haematology values in the downer animals with concurrent fatty liver that finally died or were cured.

Materials and methods

Animal selection

Thirty-six Holstein cows (6 of which were first-calf heifers) from 23 dairy farms (fewer than 3 from each farm) in Greece were used. Cows that became recumbent in the first week of lactation were eligible for inclusion in the study and were referred by the local veterinarians who serviced the herds. All cows were sampled at the earliest possible time after recumbency (time between referred recumbency and sampling ranged from 30 min to 6 h) and before any treatments were administered. Data collected when the cows were examined included age, parity, date of calving, recent health and production problems, time (hours) from the onset of recumbency, and body condition score (BCS) (scale of 1 to 5). A thorough physical examination was performed on each cow including rectal temperature, pulse rate, inspection of mucous membranes and examination for mastitis, metritis, and bone fractures. Then blood samples were collected and right after liver biopsy was performed. After sampling, cows without signs of disease other than hypocalcaemia were then treated with 500 mL of 23% calcium borogluconate intravenously, 250 mL of 23% calcium borogluconate subcutaneously, 500 mL of 35% dextrose intravenously, and 150 mL propylene glycol orally.

Samples were collected in a 6-month period. The study protocol was performed in compliance with institutional guidelines, local Ethical Committee approval, and European Union legislation for research on animals. All owners gave informed consent for cattle to be included in the study and undergo the testing procedures.

A total of 74 cows were initially referred to the study. From these, 11 were not sampled because they had already been treated. An additional 12 cows were excluded from the study because they showed evidence of infectious or overt disease (e.g., fever, vaginal discharge, and mastitis). Of the 51 remaining cows, 15 responded to treatment (30 min to 3 h post-treatment) and were also excluded from the study. The remaining 36 cows that were finally included in the study fit the definition of "downer cows". These cows had failed to rise within 6 h after the 1st treatment and were between 10 and 72 h after calving. None of them had history of musculoskeletal injury. The clinical outcome of these 36 downer cows was determined on the 7th day in milk (DIM) and cases were followed up by telephone. Each cow was assigned 1 of 2 clinical outcomes as follows: 1) cured (able to stand and return to good general health by 7 DIM) or 2) died (dead within the first 7 DIM or still recumbent and euthanised by the 7th DIM).

Reference healthy fresh cows

The reference range for haematological variables evaluated in the study was determined using blood and liver tissue samples from 10 healthy Holstein cows within the same 23 farms (no more than 1 per farm) that contributed cows to the study. The selected cows were within the first 7 days in milk, had no history of disease for the current lactation, were clinically healthy at the time of sampling, and had no histologically visible fat in the liver (Grades of Fatty Liver - GFL < 2) as determined later by histopathological evaluation. All of the variables were measured using the same methods used for the diseased cattle of the study. The minimum and maximum values obtained from these 10 cows were used as lower and upper ends of the reference range, respectively.

Sample collection

Blood samples were collected from each cow by jugular vein puncture with an 18-gauge needle in plain vacuum glass tubes and in vacuum glass tubes containing sodium citrate 3.8% as anticoagulant, supplied with the haematological diagnostic kit (IDEXX, Bovine Sample Preparation Kit, QBC® VetAutoread™ Hematology Analyzer, sample preparation procedures for Bovine venous blood. Idexx LaBor.). Whole blood samples were transferred to the laboratory and measured within 6 h of collection.

Liver tissue was obtained by transcutaneous biopsy through the 11th right intercostal space using a liver biopsy needle (Berlin Model, 2.5 mm × 25 cm, Eickemeyer Medizintechnik für Tierärzte, Tuttlingen, Germany). Biopsy specimens (150 to 350 mg of tissue) were divided into 2 parts; one sample of liver tissue was fixed in neutral-buffered 10% formalin for histological examination. The other sample was stored at -20 °C for determination of total lipids (tLPD) and triglycerides (TG).

Haematological analysis

Blood analysis including the determination of haematocrit (HCT), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), white blood count (WBC), granular cell count (GRAN), lymphocytes count (LM), and platelets count (PLT) was performed with the aid of the veterinary haematology analyzer IDEXX QBC®, using the specific procedure for bovine samples.

Liver biochemical analysis

Total lipid concentration in liver tissue was measured using chloroform-methanol-water extraction (11). Liver triglyceride concentration as determined by first saponifying the extracted lipids with 1 mL of 0.5 N potassium hydroxide solution and 1 mL of absolute ethanol for 60 min at 70 °C. The resultant triacylglycerols were measured by the method of Eggstein and Kuhlmann (12). Both liver lipid and triglyceride content were reported as mg/g of wet liver tissue. For triglyceride measurement was used a spectrophotometer (Hitachi U-2000, Hitachi Ltd, Tokyo, Japan).

Histological examination

Biopsy specimens were fixed in neutral-buffered 10% formalin solution, cut to sections 3 to 4 μm in thickness, and stained with H&E. The specimens were examined via light microscopy for lipid content. Liver fat content was classified according to a 6-point scale of severity of fatty infiltration named Grades of Fatty Liver (GFL) (13). The range of GFL scores was from 0 (no fat droplets visible) to 5 (panlobular fatty infiltration). Liver tissue was evaluated in 3 concentric regions in the area from the central vein to the portal triad of the hepatic lobule. In each of those regions, scores were assigned according to the following guidelines: no lesion = 0 points; cloudy swelling = 0.5 points; cloudy swelling with small vacuoles (representing lipids washed out by alcohol during staining) = 1.0 point; many small vacuoles = 2.0 points; medium-sized vacuoles = 3.0 points; large vacuoles = 4.0 points; and appearance of stamp cells (hepatic cells that contain a large volume of lipid to the extent that cell contour is altered and nuclei are displaced) = 5.0 points. The most substantial lesion was used to assign the score for each region of the hepatic lobule.

For example, if in a region large vacuoles (4.0 points) were observed in some cells but small vacuoles and cloudy swelling (1.0 point) were observed in other cells, a score of 4.0 points was assigned. Points were summed and a score for the lobule was obtained. For every specimen, 5 lobules were scored and the mean score was determined. From mean scores, each cow

was classified as having 1 of 6 degrees of fatty liver (GFL 0 to 5) according to the following index: GFL 0 = 0 points; GFL 1 = 0.5 to 1.0 points; GFL 2 = 1.5 to 4.5 points; GFL 3 = 5.0 to 7.0 points; GFL 4 = 7.5 to 9.5 points; and GFL 5 > 9.5 points.

Grouping by severity of fatty liver

Cows were assigned to 1 of 3 groups of fatty liver infiltration based on GFL and TG liver content, as follows: 1) mild fatty liver (cows with GFL = 2 and liver TG < 20 mg/g), 2) moderate fatty liver (cows with GFL = 3 or 4 and liver TG = 20-50 mg/g), and 3) severe fatty liver (cows with GFL = 5 and liver TG > 50 mg/g). No cows were classified as GFL 0 or 1.

Statistical analysis

Analysis was performed using a commercial software program (SPSS, version 16.0, SPSS Inc, Chicago, IL, USA). The Spearman rank bivariate correlation was used to investigate the relationship between variables. Because the assumptions of ANOVA (homogeneity of variances and normality) were not satisfied we compared biochemical and heamatological parameter measurements among the 3 groupings of fatty liver severity using a Kruskal-Wallis non-parametrical test. When significant differences among groups were observed, the Mann-Whitney test was used for pairwise comparisons, in order to identify which group medians were significantly different.

Fisher's exact chi-squared test was used to assess independence between fatty liver group classification and clinical outcome. Finally, a Mann-Whitney test was used to compare medians of biochemical and heamatological parameter measurements in the 2 different clinical outcome groups (cured - died). For all tests, values of $P < 0.05$ were considered significant.

Results

Liver evaluation

Histological evaluation

Liver tissues from all 36 downer cows contained at least some lipids (visible as vacuoles under light microscopy) and were classified as GFL of 2 or higher. Four cows were classified as having mild fatty liver (including 1 first lactation animal), 16 were classified as having moderate fatty liver, and 16 were classified as having severe fatty liver including 5 firstly lactated animals (Table 1).

Liver biochemical analysis

Total lipids and triglyceride concentrations in hepatic tissue increased with increasing GFL. In the severe fatty liver group some very high triglyceride concentrations (up to 135 mg/g) were observed (Table 1). There was no difference in tLPD concentration between the reference cows and mild fatty liver group (Table 1). There were significant differences in liver TG concentration between the reference cows and mild fatty liver group (Table 1). Total lipids and triglyceride content were significantly and highly correlated ($r = 0.635$) (Table 2).

Haematology

HCT median values did not show any significant difference between the reference and the downer cows, irrespective of the fatty liver severity (Table 1). In the same way, Hb was significantly lower only in the severe fatty liver group, while the other downer cows did not show any difference from the reference ones (Table 1). HCT and Hb were highly correlated ($r = 0.897$) (Table 2).

MCHC median values did not show any significant difference between the reference and the downer cows, irrespective of the fatty liver severity, and almost no other significant correlations were recorded (Tables 1 and 2).

White blood cells numbers were significantly higher in moderate and in severe fatty liver downer cows in comparison to reference and mild fatty liver ones (Table 1). The same significant increases were recorded for GRAN numbers in moderate and severe fatty liver cows (Table 1). On the other hand, lymphocytes (LM) did not show any significant difference between any group of downer and reference cows (Table 1). Especially GRAN were significantly correlated with PLT (Table 2).

Platelet values were of wide range in all groups; a significant difference was recorded only among the moderate fatty liver and the reference cows (Table 1).

Clinical outcome

Classification of cows according to the outcome and the severity of fatty liver is shown in Table 3. The time frame for their recovery was between 1 and

Table 1. Results of biochemical and hematological analyses, age, and body condition scores from healthy reference cows and downer cows with mild, moderate, or severe fatty liver.

Parameter	Reference Healthy Fresh Cows < 7 DIM, n = 10		Downer Cows Mild Fatty Liver, n = 4		Downer Cows Moderate Fatty Liver, n = 16		Downer Cows Severe Fatty Liver, n = 16	
	Mean ± SE (range min-max)	Median	**Mean ± SE (range min-max)**	Median	**Mean ± SE (range min-max)**	Median	**Mean ± SE (range min-max)**	Median
HCT (%)	31.27 ± 2.11 (21.0-39.4)	30.40 [a]	29.86 ± 2.70 (24.4-34.8)	30.15 [a]	29.61 ± 1.32 (20.8-37.3)	28.35 [a]	27.18 ± 0.94 (21.0-32.8)	27.20 [a]
Hb (g/dL)	10.30 ± 0.74 (7.0-13.9)	9.90 [a]	10.35 ± 1.13 (7.5-12.3)	10.80 [a]	10.38 ± 0.39 (8.0-13.0)	10.05 [a]	9.19 ± 0.35 (7.2-11.7)	9.05 [b]
MCHC	33.07 ± 0.32 (31.2-35.3)	33.2 [a]	34.18 ± 1.15 (31.7-36.8)	34.1 [a]	34.14 ± 0.31 (31.9-35.9)	34.35 [a]	33.83 ± 0.35 (31.0-35.8)	33.7 [a]
WBC (×10⁹/L)	7.57 ± 0.78 (4.7-11.4)	6.90 [a]	8.76 ± 1.78 (5.0-12.1)	8.30 [a]	9.34 ± 1.32 (2.3-21.5)	9.55 [b]	11.85 ± 1.50 (3.8-25.1)	9.85 [b]
GRAN (×10⁹/L)	3.01 ± 0.76 (0.9-9.2)	2.30 [a]	2.93 ± 1.48 (0.9-7.2)	1.80 [a]	4.81 ± 0.97 (0.3-14.2)	5.20 [b]	6.07 ± 1.21 (0.8-18.2)	5.95 [b]
GRAN (%)	37.91 ± 6.23 (8.0-81.0)	42.0 [a]	32.00 ± 11.43 (8.0-60.0)	30.0 [a]	44.06 ± 6.32 (6.0-77.0)	55.0 [b]	51.00 ± 6.19 (7.0-79.0)	57.50 [b]
LM (×10⁹/L)	4.56 ± 0.66 (2.2-9.9)	4.20 [a]	5.85 ± 1.60 (3.9-10.6)	4.45 [a]	4.82 ± 0.92 (2.0-15.2)	4.35 [a]	5.75 ± 1.19 (2.0-18.0)	3.70 [a]
LM (%)	62.09 ± 6.23 (19.0-92.0)	58.0 [a]	68.00 ± 11.43 (40.0-92.0)	70.0 [a]	55.94 ± 6.32 (23.0-94.0)	45.0 [a]	49.00 ± 6.19 (21.0-93.0)	42.50 [a]
PLT (×10⁹/L)	388.1 ± 28.9 (169-517)	387.0 [a,b]	375.0 ± 71.9 (240-577)	341.5 [a]	330.1 ± 53.2 (86-836)	2 73.0 [a,c]	325.4 ± 44.7 (96-785)	301.5 [a]
tLPD (mg/g)	136.2 ± 12.6 (80-188)	137.5 [a]	127.8 ± 23.3 (92-195)	129.0 [a]	228.6 ± 10.1 (162-330)	224.0 [b]	282.2 ± 14.6 (162-360)	300.0 [c]
TG (mg/g)	10.53 ± 0.61 (7.6-13)	10.8 [a]	15.94 ± 2.18 (9.5-19)	17.2 [b]	36.53 ± 1.77 (24-48)	37.1 [c]	77.74 ± 7.49 (51-135)	62.0 [d]
Age (years)	5.4 ± 0.37 (4-8)	4.5 [a]	4.4 ± 0.47 (2-8)	5 [a]	5.9 ± 0.39 (2.5-10)	6 [b]	6.2 ± 0.39 (3-9)	5.5 [b]
BCS	3.2 ± 0.18 (2.5-4)	3 [a]	2.8 ± 0.13 (2.5-3)	3 [a]	3.1 ± 0.17 (2-5)	3 [a]	2.9 ± 0.19 (2-5)	3 [a]

*Within a row, different superscripts denote significant ($P < 0.05$) differences among groups
Triglyceride (TG) and total lipids (tLPD) values are in wet liver tissue.

6 days after the 1st treatment. Nine of the 36 cows were classified as cured by 7 DIM. Among the cured cows 3 (33.33%) had mild, 4 (44.44%) had moderate, and 2 (22.22%) had severe fatty liver. The remaining 27 of the 36 cows were confirmed dead by 7 DIM. Among these cows 1 (3.7%) had mild, 12 (44.4%) had moderate, and 14 (51.85%) had severe fatty liver (Table 3).

There was a significant association between clinical outcome (cured or died) and severity of fatty liver, as revealed by Fisher's exact X^2 test ($P < 0.05$). Despite that fact, none of the haematological parameters

Table 2. Spearman correlation coefficients (r) for blood and liver variables, and age and body condition scores from downer cows with fatty liver as in Table 1. All correlation presented was significant ($P < 0.05$).

	Hb	MCHC	WBC	GRAN	GRAN%	tLPD
HCT	0.897					
MCHC						
GRAN			0.549			
GRAN%				0.912		
LM			0.713			
LM%				-0.912	-1.0	
PLT				0.483	0.561	
TG	-0.384					0.635

Table 3. Clinical outcome of the downer cows in relationship with their fatty liver severity.

Downer Cows Groups	Clinical Outcome	
	Cured	Died
Mild Fatty Liver	3	1
Moderate Fatty Liver	4	12
Severe Fatty Liver	2	14

showed any significant difference between the cured vs. animals that died.

Discussion

The present study aimed to evaluate the haematology of downer cows with concurrent fatty liver. All the animals included were field cases and the sample collection was done as soon as possible to time of recumbency. Concerning the haematology, analysis was performed with a fairly simple automatic analyser, since it is easy and practical and can be used by private practitioners or small clinics.

HCT, despite the wide variation of values recorded, was within the normal accepted values (14), whereas no significant difference between fatty liver groups and reference cows was noted. Moreover, despite the stressful situation of the animals, haemoconcentration was not evident in any case and no differences existed between the cows that were cured and the ones that died. All the downer cows of the study were at very early lactation (< 3 DIM), when HCT is generally in the lower values. The fact that the downer cows had similar HCT values with the healthy reference cows reveals that the concurrence and severity of fatty liver do not have any significant impact in HCT values.

Similar results with HCT were recorded for Hb. A possible explanation for this may be the fact that sampling was done soon after recumbency, before the fatty liver aggravates the degree of anorexia. Another explanation could be the very early days in lactation of the downer cows. Late pregnancy and onset of lactation is a period when slight anaemia exists (15). In an earlier study of fatty liver without downer cow syndrome, no significant impact of liver triglyceride content on Hb was recorded (10). In reports where Hb appeared to be increased, it was attributed to haemoconcentration (16), a fact that was not recorded here.

The MCHC was unaffected by the concurrency of recumbency and the severity of fatty liver, since no differences between them and the reference cows were recorded. Compared to the normal range (14), all MCHC values of the study for both reference and downer cows were lower. Despite the fact that soon after calving Hb and MCHC are usually decreased, it is accepted that the wide variation of normal ranges and the anisocytosis that usually appear (17) make the diagnostic value of MCHC for cows with fatty liver poor.

The white blood cell count was significantly increased in downer cows with moderate and severe fatty liver in comparison to the reference and mild fatty liver ones. However, even in the moderate and severe fatty liver group both the mean and the median values of WBC were within the normal range (14). In the present study some cases of leukocytosis (25.1×10^9/L) were recorded in the severe fatty liver group, but in other cases of the same group leukopenia (3.5×10^9/L) was also recorded. Despite this wide variation, no difference was recorded between the animals that were cured vs. the ones that died. Due to the strict selection criteria, eligible for inclusion in the study were only those downer cows without any prominent infectious disease, so as to avoid any possible interference of infection in haematology. Earlier observations in fatty liver non-downer cows revealed that WBC did not differ significantly between animals with severe and mild fatty liver (18). In another research, WBC before and after calving was not different between cows with or without fatty liver (19). On the other hand, other studies in fatty liver cows, without being downer reported increased (20) or decreased WBC (3,8). WBC is generally affected even by the stress of sampling (17,21). Since recumbency is a heavier stressor, WBC should be carefully evaluated for diagnosis in downer cows. WBC is not considered of great diagnostic value in the bovine medicine (22), which is in agreement with the results of the present study.

The total GRAN count showed similar pattern changes with WBC, unlike the LM numbers that were not significantly different between the fatty liver groups. Consequently, the WBC increase is attributed to the increase of the GRAN only. There are previous reports about decreased WBC and simultaneous decrease in both GRAN and LM in fatty liver non-downer cows (3,10). It is accepted that fatty liver usually induces low leukocyte counts in the peripheral blood (10,23) and the reduction of total leucocytes is higher with increasing severity of fatty liver (16).

In contrast to WBC, the relative numbers of different white cells and their percentages change are of clinical interest in diagnosing diseases in bovine medicine (24). The normal proportion of GRAN (mainly neutrophils) to LM in adult cattle is 1:2 (22). In our study, it was inversed as in WBC the GRAN were 55% and the LM 45% in the moderate fatty liver group and 57.5%:42.5% in the severe fatty liver group, respectively. These results probably reflect a subclinical acute inflammation, since other overt inflammatory conditions were excluded by clinical examination. The fact that in both moderate and severe fatty liver groups the median GRAN number was within the normal values diminish their diagnostic importance in downer cows with fatty liver.

The PLT count appeared to have a wide variation and did not have any significant diagnostic value for the downer cows of the study. The present PLT values are in accordance with the usual bovine PLT pattern, which is characterised by wide variation in numbers (normal range 210,000-700,000 cells/μL) (14) and lacks significant diagnostic value.

To conclude, the haematology of downer cows with concurrent fatty liver did not offer sound help in the evaluation of the patient. There was not any correlation between WBC, GRAN, or LM with the liver TG content or other liver diagnostic parameter. Furthermore, no significant difference was recorded for any haematological parameter between animals that finally died compared to those cured.

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