AN EVALUATION OF CHANGES OVER TIME IN SERUM CREATINE KINASE ACTIVITY AND C-REACTIVE PROTEIN CONCENTRATION IN DOGS UNDERGOING HEMILAMINECTOMY OR OVARIOHYSTERECTOMY

by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APPs	Acute phase proteins
AUC	Area under the curve
BVSc	Bachelor of Veterinary Science
CK	Creatine kinase
CRP	C-reactive protein
OVAH	Onderstepoort Veterinary Academic Hospital
SD	Standard deviation
WCC	White cell count

SUMMARY

Trauma of diverse origins is a common reason for presentation of pets for treatment. It is often difficult clinically to objectively measure the severity of any trauma to an animal. One approach is to measure the changes in the various serum parameters which are known to alter in response to trauma or inflammation. If the changes over time of relevant and easily measurable parameters can be established under two controlled but different conditions of surgical trauma, it may provide the foundation for evaluating their future use in establishing the severity of trauma in a patient.

A prospective study was performed on animals presented to the Onderstepoort Veterinary Academic Hospital for either thoracolumbar disc disease or for elective ovariohysterectomy. The two surgical procedures chosen for the study involved significant surgical trauma, particularly to muscle, in the case of thoracolumbar decompression and relatively minor surgical trauma in the case of ovariohysterectomy. Serial evaluation of creatine kinase (CK) and C-reactive protein (CRP) were performed both pre- and post-operatively on two sets of patients derived from the two surgical categories. CK is an enzyme found predominantly in skeletal muscle and significantly elevated serum activity is largely associated with muscle damage. CRP is an acute phase protein which shows elevated serum concentration in response to a broad range of inflammatory stimuli.

Analysis of the data showed a very wide range of results at each time point for both CK and CRP. There were no significant differences between the two surgical groups for either analyte preoperatively. Thereafter CK results were markedly and significantly different between the two groups. CRP results were very similar in the two groups with no statistical difference at any time point. The results of this study suggest that the evaluation of CK and CRP at any one time point in a traumatized animal is of limited value. However the evaluation of the trend of these two analytes, even over a relatively short time period, may allow for useful prognostication in clinical cases.

CHAPTER 1

LITERATURE REVIEW

Thoracolumbar disc disease is commonly seen in small animal veterinary practice. Most of the dogs seen are chondrodystrophic breeds, in particular dachshunds.[36] It is a degenerative condition of the intervertebral discs of the thoracolumbar region commonly leading to disc protrusion or extrusion. At the Onderstepoort Veterinary Academic Hospital (OVAH) approximately 80 dogs were diagnosed with this condition in 2005. Some of these animals were seen as first opinion cases at the Outpatients section of the hospital and others were referred to the hospital by private practitioners. At the OVAH, animals suspected of suffering from thoracolumbar disc disease after clinical and neurological examination, undergo radiographic and myelographic examination to confirm the diagnosis. If the diagnosis is confirmed, and if the neurological condition warrants it, the animals are operated on immediately post myelography. Currently the method of choice in the surgery department at the OVAH is decompression of the thoracolumbar disc extrusion by means of a hemilaminectomy or pediculectomy over the affected intervertebral space. Fenestration is not routinely performed. The surgical approach involves extensive elevation and retraction of the epaxial musculature over the affected disc space resulting in surgical trauma primarily to skeletal muscle[30,36]

Ovariohysterectomies are performed routinely at the OVAH. These are healthy pets, generally under a year of age. The standard method of ovariohysterectomy is by means of a midline coeliotomy through the linea alba, with removal of the ovaries and uterus up to the cervix. The linea alba is a midline tendinous aponeurosis of the abdominal muscles. As such there is minimal skeletal muscle damage during the procedure with surgical trauma confined to the genital tract.

Many different parameters have been used as measures of trauma. Studies in humans have employed both CRP and CK in attempts to quantitatively compare the degree of tissue trauma between different surgical techniques.[6,22,23] CK has been used in human studies to evaluate the extent and cause of muscle damage resulting from lumbar back surgery.[24,25,28]

1.1 CREATINE KINASE (CK)

CK is essential as a catalyst in the production of energy in muscle cells. It facilitates the transfer of high energy phosphate bonds from creatine phosphate to adenosine diphosphate to make adenosine triphosphate (ATP) available.[3,8,16] Two subunits are recognised, namely M and B. These subunits can combine into pairs to form three isoenzymes: CK1 or BB, CK2 or MB and CK3 or MM. CK3 (MM) is found mostly in skeletal muscle and also in cardiac muscle. CK2 (MB) is found in cardiac muscle with small amounts in skeletal muscle. CK1(BB) is found in the central nervous system, cerebrospinal fluid and in the smooth muscle of the gastrointestinal and genital tracts.[8] Uterine CK activity may be greater in cows than in dogs and has been used as an indicator of endometritis in the former.[35] The different isoenzymes can be measured individually, but this is seldom done in veterinary medicine as CK is one of the most organ specific serum enzymes in the dog and most serum CK is of skeletal muscle origin.[8,16] In humans, the CK2 (MB) activity in serum has been used as a sensitive measure of the extent of heart muscle damage in myocardial infarction. The dog has been used as an experimental model for this condition.[3]

CK is an extremely sensitive indicator of muscle damage but is not very specific as to cause.[16,38] Serum elevations in dogs are associated with cell membrane leakage and will therefore be seen with any condition associated with muscular inflammation, necrosis or degeneration. Peak serum activity is expected between 3 and 12 hours after muscular insult.[1,5,38] The elevation is roughly proportional to the amount of muscle tissue involved. It is also possible to quantify the mass of muscle damage if the changes in CK values over time after a particular insult are plotted.[1,3,4,38] CK reaches the general circulation by way of the lymphatic system.[1,4] As a result, studies measuring CK under differing conditions of anaesthesia showed no change during anaesthesia (unless an animal's limbs were passively flexed), but only when animals started moving again.[1] CK is such a sensitive indicator of muscle damage that often only very large or persistent elevations are regarded as clinically significant.[8,33] Mild to moderate elevations, up to 23 times baseline values, can be seen with relatively minor insults such as intramuscular injections, electromyographic studies, shivering and convulsions.[3,33,38] Poorly performed venipuncture has been shown to result in

CK levels higher than control samples, probably because of damage to underlying muscle.[8,17] Prolonged recumbency has been found to lead to elevations of up to 12 times normal.[3,16,33] Young animals can have serum CK levels up to five times higher than that of an adult with mature levels reportedly reached at about 7 months of age.[8] There is no measurable difference between males and females.[3,8,16] The serum half-life in dogs is relatively short, about 2 hours. Persistently high serum activity is therefore regarded as indicative of ongoing muscle damage. Conversely, this short half-life can also lead to false negative evaluations, depending on how long after the muscle damage sampling occurs.[2,3,37] Artefactual increases may be seen if haemolysis of a collected blood sample occurs, although there is very little CK in red blood cells.[8] Slight haemolysis in a serum sample does not affect results.[2,16]

A study evaluating the differences in pre and postoperative CK values with laparoscopic and conventional ovariohysterectomy techniques in dogs found CK to be a poor measure of surgical stress.[7] The authors may however have underestimated the influence on serum CK of preoperative intramuscular injections and also drew their conclusions from limited sampling.[1,3,13] Aktas *et al* showed that halothane anaesthesia leads to very high increases in serum CK levels leading the authors to conclude that halothane should not be used for anaesthesia in studies measuring CK activity. This same study showed minimal increases in serum CK when propofol was used for anaesthesia of the same duration as the halothane. The investigators concluded that elevations in serum CK seen after anaesthetic recumbency in their study were due to the affects of halothane and not muscle trauma secondary to pressure.[1] Conversely, another study measuring serum CK activity after 30 minutes of isoflurane anaesthesia, found that the elevation was still within the normal reference range.[38] Moderate exercise, even in unfit dogs, leads to minor and transient CK elevations.[12]

The brain isoenzyme is not found in significant levels in serum even in cases of neurologic disease.[8] In experimental studies performed on dogs, CK1 (BB) isoenzyme was measurable in cerebral venous blood after severe cerebral trauma, but not in peripheral venous blood. The reasons for this were thought to be the shorter half-life of CK1 (BB) and the effectiveness of the blood brain barrier.[29] Elevations in total serum CK seen in neurologic diseases are probably due to fitting, tremors or prolonged recumbency.[3,37] Similarly, an increased CK activity seen in dogs presented

for thoracolumbar disc prolapse is likely to be due to the involuntary recumbency and not due to the disc prolapse itself.[36]

CK is routinely used in cattle together with other serum parameters associated with muscle damage, like serum aspartate aminotranferase (AST) in the diagnosis and serial evaluation of "Downer Cow Syndrome". This condition is associated with muscle damage secondary to recumbency.[31] Similarly in horses, CK is measured for staging and prognostication secondary to muscle damage in Equine Rhabdomyolysis Syndrome.[19]

1.2 C-REACTIVE PROTEIN (CRP)

CRP is a member of a large group of plasma proteins called acute phase proteins (APPs). These proteins are integral to the acute phase response which is the body's rapid initial systemic inflammatory reaction to any non-specific tissue injury.[11,15,32,40] Examples of other acute phase proteins are fibrinogen, serum amyloid A, haptoglobin and ceruloplasmin. Most of the APPs including CRP, are positive, in that they increase in serum concentration in response to inflammation. Others are negative or in other words show a decreased serum concentration in response to inflammation.[11,34] A well known negative APP is the plasma protein albumin.

The synthesis, predominantly in the liver, of positive APPs, is stimulated by various proinflammatory cytokines in response to tissue injury or infection. The main stimulatory cytokines are interleukin-1, interleukin-6 and tumour necrosis factor alpha.[11,18,32,40] The exact functions of the APPs are not completely understood, but it is known that they regulate and coordinate the body's response to tissue injury. The functions of CRP include suppressing microbial growth, the clearance of damaged tissues and regulation of the inflammatory response.[32,34]

CRP was named originally because of its ability to bind the C-polysaccharide of *Pneumocoocus pneumoniae*.[11,34] It is probably the most intensively studied of all the APPs in the dog because the pattern of its' production has been found to be similar to that in humans.[15] Interestingly CRP does not appear to be involved in the acute phase response in cats.[11,32]

By definition the acute phase response is a very rapid response and is often measurable before clinical signs develop. As such the APPs, as a reflection of the acute phase response, are very early and sensitive markers of any tissue injury. The acute phase response is however also a non specific response in that it occurs in response to any condition that leads to tissue injury be that infectious, traumatic or neoplastic.[11,34,40] Major APPs which include CRP, show a rapid and marked serum elevation in the acute phase response and a rapid decline in the absence of further insult.[11,34] Persistently high serum concentrations may indicate ongoing inflammation or infection.[15] Human studies have found CRP to be strongly predictive of cardiovascular

risk both in healthy people and those with a history of coronary disease.[34,39] However studies following trauma showed a poor correlation between serum CRP and severity of injury or prediction of survival.[18] Studies measuring the serum response of CRP in dogs following surgical trauma have shown a maximal response relative to baseline levels within 24hrs post surgery and a gradual decline over several days thereafter. The degree of response was thought to be directly related to the intensity of trauma.[32,40] In a comparison of serum CRP concentration after ovariohysterectomy and orthopaedic surgery, Caspi *et al* found that CRP was higher after 24hrs in the ovariohysterectomy group.[10] In contrast, a later study by Yamamoto *et al* which also compared these two types of surgery, found CRP levels to be higher after orthopaedic surgery.[40] Yamamoto *et al* also found CRP to be a more accurate reflection of the severity and improvement or otherwise of inflammation than the total white blood cell count (WCC). In their study WCC was also elevated postsurgically, but remained elevated at the time of suture removal (8 days post operatively) despite obvious clinical improvement.[40] Conversely a later study by Burton *et al* concluded that CRP held no advantage over WCC as a measure of post surgical inflammation and would be of most use in animals with an abnormal bone marrow response.[9]

No significant age related differences have been found in baseline levels of APPs including CRP, in healthy animals. Very young animals do however appear to show a less marked acute phase response to inflammation. This was demonstrated in a study where serum APP levels were measured in dogs after induced inflammation. Significantly greater concentrations of CRP were found in 3 and 18 month old dogs compared to 1 month old puppies.[20] As with other proteins, CRP is broken down by the liver and thus the presence of liver disease may influence the speed of removal from the bloodstream.[11]

CHAPTER 2

STUDY OBJECTIVES

Clinically it can be challenging for clinicians to quantify the severity of any trauma suffered by a patient. A study was proposed to improve the understanding of the changing serum values over time of CK and CRP in two groups of animals undergoing two differing surgical procedures. The information gained from this study may contribute to the future use of CK and CRP as parameters for the clinical evaluation of trauma patients.

2.1 SPECIFIC OBJECTIVES OF THE STUDY.

- To serially determine serum CK and CRP levels under controlled conditions in two groups of dogs experiencing two different surgical procedures of a very different nature with respect to the level of tissue trauma. The first group of dogs was presented for surgical treatment of thoracolumbar disc prolapse. The second group of dogs was presented for elective ovariohysterectomy.
- To establish expected baseline values and the changes over a set time period of serum CK and CRP in dogs undergoing a procedure involving significant muscle trauma.
- To establish expected baseline parameters and the changes over a set time period of serum CK and CRP in dogs undergoing a procedure involving relatively minor muscle trauma.

2.2 HYPOTHESES

2.2.1 DOGS PRESENTED FOR OVARIOHYSTERECTOMY. (SPAY GROUP)

- CRP and CK levels will both be within the normal reference range on presentation.
- CRP and CK levels will increase markedly after surgery and then decline to normal levels.

2.2.2 DOGS PRESENTED FOR THORACOLUMBAR DISC PROLAPSE. (SPINE GROUP)

2.2.2.1 C-REACTIVE PROTEIN (CRP)

- will be elevated on presentation.
- will show a similar pattern to the spay group postoperatively.

2.2.2.2 CREATINE KINASE (CK)

- will be elevated on presentation.
- will increase to very high levels postoperatively and then decline but will be significantly elevated over the spay group at all time points.

CHAPTER 3

MATERIALS AND METHODS

The Animal Use and Care Committee of the Faculty of Veterinary Science approved the research protocol used in this project. (Protocol V006/06)

3.1 MODEL SYSTEM

This project was a prospective descriptive study on dogs presented to the Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria, South Africa.

3.2 EXPERIMENTAL DESIGN

Forty-three dogs presented at the OVAH between August 2006 and April 2007 were included in this study. Two distinct groups of dogs were identified. Twenty-two dogs were presented for surgical treatment of thoracolumbar disc prolapse. A further twenty-one dogs were presented for elective ovariohysterectomy.
Owner's consent was obtained prior to inclusion of any dog to the study. (Appendix 1)

3.2.1 INCLUSION CRITERIA:

- All dogs were at least 7 months of age.
- No dogs were in season or pregnant.
- No history of any concurrent disease process was identified in the history of the patient.
- No obvious signs of inflammation were detected on clinical evaluation or evaluation of a thin peripheral blood smear.
- No patient had undergone halothane anaesthesia within the previous month.
- No patient had a history of trauma within the previous month except for the occurrence of the disc prolapse in the case of the spine group.
- Dogs selected for the spine group were of either sex, entire or neutered.

3.3 EXPERIMENTAL PROCEDURES

3.3.1 ALL DOGS ADMITTED TO THE STUDY:

- the final year BVSc student to whom the case was assigned performed a standard clinical evaluation. This evaluation included assessment of temperature, pulse and respiration and the drawing of a drop of blood from the pinna for the evaluation of a thin peripheral bloodsmear. (Appendix 2)

- an indwelling 16 gauge over the needle catheter was placed in a jugular vein shortly after anaesthetic induction.

- 2ml of blood was collected from the jugular vein using the indwelling catheter shortly after induction, immediately after completion of the surgical procedure and thereafter at 4, 6, 8, 12, 24 and 48 hours postoperatively.
  No differentiation was made between fasting and non – fasting samples.

- all blood samples collected were placed in a serum tube which was labelled with the date, patient details and time of collection. The samples were sent to the Clinical Pathology laboratory at the OVAH immediately after collection. The samples were centrifuged for 10 minutes at 1520g and the serum harvested. In cases where samples were collected after hours, the samples were centrifuged in the intensive care unit laboratory, the separated serum collected and placed in a labelled serum tube and stored at -20°C. The CK assay was performed on the day of collection or on the first working day thereafter in the case of an after hours sample. The CRP assay was performed as a batch at the end of the trial to avoid inter-assay differences. All CRP samples were stored at -80°C until the assay was performed.

- a final year BVSc student monitored each animal during anaesthesia under the supervision of the anaesthetist on duty.

- postoperatively and for the duration of the study, patients were housed in the intensive care unit of the OVAH. Postoperative care and housing was standardized as far as possible for all animals.

3.3.2 SPAY GROUP

- each dog had an intravenous catheter placed in the cephalic vein by the anaesthetist on duty and premedication was administered intravenously.
- each dog was placed on intravenous fluids (Ringer's Lactate) at 10ml/kg/hr for the duration of the surgical procedure.
- anaesthesia was induced with intravenous thiopentone and an endotracheal tube placed. Anaesthesia was maintained with isoflurane for the duration of the surgical procedure.
- ovariohysterectomy was performed by the same surgeon (the investigator) in all the patients by means of a standard midline coeliotomy. The operative procedure was standardized as far as possible for all patients. Both ovaries and the uterus up to the level of the cervix were removed and ligated with chromic catgut. Clamping of the genital tract was avoided. The linea alba was closed with monofilament absorbable suture material and the skin with nylon.

3.3.3 SPINE GROUP

- a neurological examination was performed and the results noted. (Appendix 2)
- an intravenous cephalic catheter was placed. Any premedication given was given intravenously. The dog was induced for anaesthesia, an endotracheal tube was placed and anaesthesia was subsequently maintained with isoflurane.
- each dog was placed on intravenous fluids (Ringer's Lactate) at 10ml/kg/hr for the duration of the procedure and for as long postoperatively as was deemed necessary by the surgeon.
- lumbar myelography was performed by the radiologist on duty at the diagnostic imaging section of the OVAH. After myelography, the patient was transferred to theatre for standard surgical preparation.
- surgical decompression was performed by the same surgeon (the investigator) in all patients. The operative procedure was standardized as far as possible for

all patients. A dorsolateral approach to the affected intervertebral space was made and the epaxial muscles retracted. A hemilaminectomy was performed over the affected disc space. Bipolar cautery was used if required. Monofilament absorbable suture material was used for closure of the thoracolumbar fascia and monofilament nylon for the skin.

3.4 OBSERVATIONS

The blood samples collected were tested to determine the CRP and CK values at predetermined intervals for both groups of dogs.

The following collection times were observed for all animals:

- Immediately preoperatively
- Immediately postoperatively
- Thereafter at 4, 6, 8, 12, 24 and 48 hours postoperatively.

If a scheduled collection was missed, the animal was still included in the study as it was felt that the individual time points sampled as well as the overall trend would still be valid. An additional two spinal cases and one spay case which had been sampled correctly were incorporated into the study for this reason.

CK activity in serum samples were measured using an automated colorimetric assay validated for use in dogs. The analysis was performed using an automated analyser (Nexct, Alfa Wasserman, The Netherlands) according to the manufacturer's instructions. The assay temperature was 37 $^{\circ}$C and no reducing agent was used. The precision of the assay was 2.35% and the assay was linear up to 1685U/L. Autodilution was performed as required according to the analyser programming with a diluent supplied by the manufacturer.

CRP concentrations in serum samples were measured using an automated human C-Reactive Protein Turbidometric Immunoassay (TIA), (Bayer CRPTIA, Newbury, UK). The assay was previously validated for use in dogs.[26] The analysis was performed using an automated analyser (Nexct, Alfa Wasserman, The Netherlands) according to the manufacturer's instructions.

3.5 DATA CAPTURE AND ANALYSIS

The data generated was initially recorded manually (Appendix 3) and then transferred onto a spreadsheet program (Microsoft Excel, Microsoft Corp. USA) for later analysis.

The data generated were statistically analyzed. Normality was assessed by visual inspection of histograms and using the Shapiro-Wilk test. All data were then log-transformed to achieve near-normality. No identification or removal of outliers was performed. Log-transformed values of CK and CRP for spay and spine groups were plotted over time in order to examine the changes over time in each group. Within each group, geometric mean values at each time point were compared with the pre-operative geometric means using repeated measures analysis of variance and the Bonferroni-Holm multiple comparison test. Comparisons between groups were also done at each time point, adjusting for sex and body mass. Median times to maximum concentration of CK and CRP were compared between groups using the Wilcoxon rank-sum test. Log-transformed areas under the curve were compared between groups using multiple regression, adjusting for sex and body mass. A significance level of $\alpha = 0.05$ was used throughout. All analyses were done using Stata 10.0 (StataCorp, College Station, TX, U.S.A.).

CHAPTER 4.

RESULTS

Twenty- one dogs which underwent ovariohysterectomy (spay group) and twenty two dogs which underwent surgical decompression for thoracolumbar disc extrusion (spine group) were admitted to this study. Samples from a few individual dogs for certain scheduled times were not available. These were either not collected at all due to time pressure or were misplaced after collection. The animals concerned were still included in the study.

Table 4.1 describes the signalment (patient number, mass, sex, age and breed) of each dog included in the study.

Tables 4.2A and 4.2B list the results of CK evaluation on samples collected from both the spay and spine groups.

Tables 4.3A and 4.3B list the results of CRP evaluation on samples collected from both the spay and spine groups.

	Signalment data of dogs in the study			
Patient number	Mass (kg)	Sex	Age (years)	Breed
SPAY GROUP				
2	3.5	F	9 months	Maltese Poodle
4	2.68	F	7 months	Miniature Pinscher
7	5	F	18 months	Dachshund
10	4	F	10 years	Miniature Pinscher
12	5.6	F	3 years	Dachshund
13	6.3	F	9 months	Dachshund
14	6	F	7 months	Dachshund
15	6.4	F	4 years	Terrier Cross
17	4	F	8 months	Pomeranian
18	5.7	F	7 months	Jack Russel Terrier
20	6.3	F	3 years	Jack Russel Terrier
21	8.7	F	18 months	Fox Terrier
22	2.26	F	9 months	Chihuahua Cross
27	5.82	F	11 months	Fox Terrier
33	10.45	F	15 months	Jack Russel Terrier
34	5.4	F	11 months	Dachshund
38	4.66	F	18 months	Jack Russel Terrier
40	3	F	10 months	Dachshund
41	6.3	F	4 years	Dachshund
42	7	F	2 years	Dachshund
43	5.5	F	4 years	Pekingese
SPINE GROUP				
1	6	F	3 years	Dachshund
3	5.8	F	7 years	Dachshund
5	7.6	F	7 years	Dachshund
6	10.8	F	6 years	Dachshund
8	10.4	M	5 years	Dachshund
9	6.14	M	4 years	Dachshund
11	6.5	M	6 years	Dachshund
16	4.38	F	4 years	Pekingese
19	3.72	M	5 years	Dachshund
23	6.46	F	6 years	Dachshund
24	6	F	8 years	Pekingese
25	14.9	F	6 years	Dachshund
26	6.3	F	5 years	Dachshund
28	8.4	F	5 years	Dachshund
29	9.3	M	6 years	Maltese Poodle
30	6.3	F	5 years	Dachshund
31	6.5	F	5 years	Dachshund
32	9.58	M	5 years	Dachshund
35	7.8	F	2 years	Dachshund
36	4	F	6 years	Dachshund
37	5.6	M	12 years	Dachshund
39	7.5	M	3 years	Dachshund

Table 4.1 Signalment data of both groups of dogs involved in the study.
F = female; M = male

	CK results from the spay group (U/L)							
Patient number	Preoperative	Postoperative	4 hours postoperative	6 hours postoperative	8 hours postoperative	12 hours postoperative	24 hours postoperative	48 hours postoperative
2	92	81	321	383	298	NR	138	87
4	2754	1977	3776	3362	3979	337	124	1171
7	221	186	290	321	241	229	191	138
10	121	134	181	254	238	NR	142	NR
12	67	57	129	128	91	86	83	46
13	254	163	482	544	392	263	210	124
14	181	126	182	169	221	148	108	100
15	58	230	103	90	73	87	71	62
17	156	99	772	564	472	277	119	177
18	201	214	291	257	256	153	86	156
20	117	87	71	85	73	78	87	78
21	136	117	92	90	91	86	87	64
22	129	86	190	314	271	220	122	69
27	325	309	603	316	286	170	96	67
33	186	96	142	81	63	117	91	380
34	460	183	459	415	418	310	176	76
38	125	78	257	274	284	254	185	47
40	145	140	539	300	256	217	143	122
41	152	165	682	665	744	571	305	201
42	460	337	363	243	277	252	136	293
43	854	896	1190	1064	795	407	119	101

Table 4.2 A CK results (U/L) for each time point from the spay group. Times for which no results were available are labelled NR.

	CK results from the spine group (U/L)							
Patient number	Preoperative	Postoperative	4 hours postoperative	6 hours postoperative	8 hours postoperative	12 hours postoperative	24 hours postoperative	48 hours postoperative
1	185	415	1191	1317	1472	1467	1581	493
3	77	171	1141	NR	1361	1405	1261	695
5	96	219	615	657	546	747	735	220
6	341	244	1055	1100	1032	1275	1211	1176
8	159	265	985	1192	1225	1670	1946	868
9	122	179	1679	3391	3687	4287	2365	1093
11	185	569	1108	1065	1392	NR	1888	378
16	422	425	1107	1635	2685	4160	755	NR
19	936	805	1544	1685	1853	1374	1151	374
23	304	136	1984	924	2134	2931	3468	1100
24	450	487	1029	2159	3741	2893	4501	2832
25	578	535	3221	3140	4940	4907	3556	1128
26	605	876	2652	NR	4527	5100	2473	NR
28	146	250	1234	1477	1242	1596	2231	753
29	442	730	3200	3702	3768	4567	2539	950
30	804	745	1525	2409	3206	2061	1465	600
31	294	484	940	1029	1300	1243	1211	678
32	180	428	3553	4951	6506	9838	6415	3160
35	153	386	2264	3260	2674	3157	2693	1069
36	329	360	NR	780	NR	NR	3248	828
37	299	263	2664	924	1074	846	918	1267
39	306	342	3710	4698	4201	3010	2109	633

Table 4.2 B CK results (U/L) for each time point from the spine group. Times for which no results were available are labelled NR.

	CRP results from the spay group (mg/L)							
Patient number	Preoperative	Postoperative	4 hours postoperative	6 hours postoperative	8 hours postoperative	12 hours postoperative	24 hours postoperative	48 hours postoperative
2	2.50	2.20	12.80	26.60	49.30	NR	44.60	19.70
4	0.10	3.10	35.50	46.60	19.80	69.90	39.30	66.40
7	3.30	3.00	6.50	22.90	48.20	4.80	6.30	28.90
10	2.20	0.20	16.60	37.00	54.40	NR	87.90	NR
12	6.30	20.40	13.20	23.50	30.80	30.50	23.10	24.60
13	58.70	54.60	62.50	68.50	54.10	52.50	67.00	34.60
14	9.00	8.10	11.50	21.70	32.60	47.30	32.00	16.20
15	6.30	7.70	19.80	31.40	35.70	49.30	62.90	16.30
17	10.10	6.80	23.20	32.60	45.60	68.90	64.70	48.80
18	28.10	28.70	32.50	36.90	46.30	72.40	54.70	41.30
20	10.10	9.70	17.90	29.90	39.00	64.50	61.90	41.10
21	23.60	23.90	25.00	31.00	35.90	51.40	48.10	29.30
22	7.20	11.30	28.60	50.50	54.60	76.40	62.40	33.40
27	17.00	17.70	40.00	34.00	37.20	41.70	29.40	1.40
33	9.00	8.00	9.30	12.50	18.90	21.90	21.40	25.10
34	2.40	1.80	3.80	11.30	22.10	33.30	13.20	23.10
38	2.20	2.10	10.20	21.30	31.10	45.20	22.80	19.30
40	67.00	63.70	80.50	82.70	84.10	88.70	67.10	45.30
41	2.60	2.00	9.80	22.30	37.70	64.30	64.70	38.00
42	5.80	6.40	31.40	29.70	39.00	57.20	45.20	32.40
43	7.70	6.70	22.00	28.90	33.40	46.30	37.50	29.50

Table 4.3A CRP results (mg/L) for each time point from the spay group. Times for which no results were available are labelled NR.

	CRP results from the spine group (mg/L)							
Patient number	Preoperative	Postoperative	4 hours postoperative	6 hours postoperative	8 hours postoperative	12 hours postoperative	24 hours postoperative	48 hours postoperative
1	29.20	28.50	29.80	34.60	41.30	39.90	49.70	47.30
3	10.10	6.40	6.90	NR	21.60	34.10	36.10	30.80
5	5.50	4.50	11.50	16.90	21.00	27.00	38.70	32.40
6	0.00	0.00	13.40	43.20	84.80	53.10	47.70	38.20
8	1.50	2.90	4.20	5.90	7.50	11.80	26.70	19.50
9	59.70	54.20	54.90	58.00	58.20	59.50	48.70	30.00
11	0.00	1.90	4.30	11.40	16.60	NR	30.40	22.90
16	6.10	5.90	5.50	13.80	27.60	24.50	27.50	NR
19	29.50	23.70	31.80	31.80	35.40	44.30	62.50	36.20
23	23.40	17.30	27.90	30.10	64.50	51.10	15.30	42.50
24	15.50	12.50	25.70	35.10	32.10	53.70	107.10	72.90
25	11.50	11.60	9.90	10.20	11.00	13.20	12.20	9.30
26	26.30	30.90	39.80	NR	42.10	53.00	49.90	NR
28	9.00	6.60	9.60	15.20	19.70	21.00	39.50	30.30
29	11.90	13.50	27.50	32.20	42.60	61.20	121.70	162.00
30	34.10	34.50	39.70	45.20	48.00	48.60	54.80	105.30
31	4.80	5.30	16.80	26.70	33.40	48.70	60.50	41.70
32	23.30	22.10	33.50	41.90	53.00	81.60	135.80	101.40
35	3.60	3.40	14.20	47.10	23.20	26.00	37.10	21.40
36	3.00	1.10	NR	18.20	NR	NR	130.00	91.40
37	11.20	13.70	21.30	23.80	30.50	41.70	43.90	29.20
39	25.00	22.30	25.90	48.60	36.70	33.60	32.40	17.20

Table 4.3B CRP results (mg/L) for each time point from the spine group. Times for which no results were available are labelled NR

4.1 ESTABLISHED NORMAL VALUES FOR CREATINE KINASE (CK) AND C-REACTIVE PROTEIN (CRP)

4.1.1 CREATINE KINASE (CK)

As might be expected, different sources show differing normal reference ranges depending on population and measurement techniques used.[2,3,5,16,33,38] The normal CK reference interval of the Clinical Pathology Laboratory at the OVAH (OVAH laboratory) is regarded as 49-146 U/L. The OVAH laboratory range correlates well with a table of reference intervals derived from independent studies and compiled by Aktas *et al*. This table lists a range of normal values for CK of between 4 and 359 U/L with only one report showing an upper normal value of over 200 U/L.[3] Parent describes an upper normal limit of < 460U/L in use in their laboratory.[33]

4.1.2 C-REACTIVE PROTEIN (CRP)

As with CK, different sources show different normal CRP values albeit within a much smaller range. A study by Connor *et al* suggested CRP was undetectable in normal dogs when measured by single radial immunodiffusion assay.[15] Yamamoto *et al* evaluated CRP concentrations in healthy dogs by means of enzyme–linked immunosorbent assay and derived normal CRP values that range between 2.4 and 30.04 mg/L.[40] Caspi *et al* determined a CRP range of between 8 and 67 mg/L in a group of normal dogs although the authors state that they regard ≤5mg/L as normal.[10] A review of acute phase proteins by Ceron *et al* tabulates a selection of normal CRP values derived by other researchers which range from 0.22 to 16.4 mg/L.[11] The normal CRP reference interval of the OVAH laboratory is regarded as <35mg/L and correlates very well with that of the laboratory that validated the automated turbidometric assay for use in dogs.[26]

4.2 RESULTS OF CK AND CRP MEASUREMENTS FOR SPAY AND SPINE GROUPS.

Tables 4.2A, 4.2B, 4.3A and 4.3B list CK and CRP results for both groups of dogs. Time points for which no samples were collected have been labelled NR. The data were found to have a non–parametric distribution and were log-transformed to achieve near-normality and then plotted over time**.**

Figure 4.1 is a graph of the mean log transformed CK values at each time point for both spay and spine groups. The error bars at each time point represent the standard deviation.

Figure 4.2 is a graph of the mean log transformed CRP values at each time point for both spay and spine groups. The error bars at each time point represent the standard deviation.

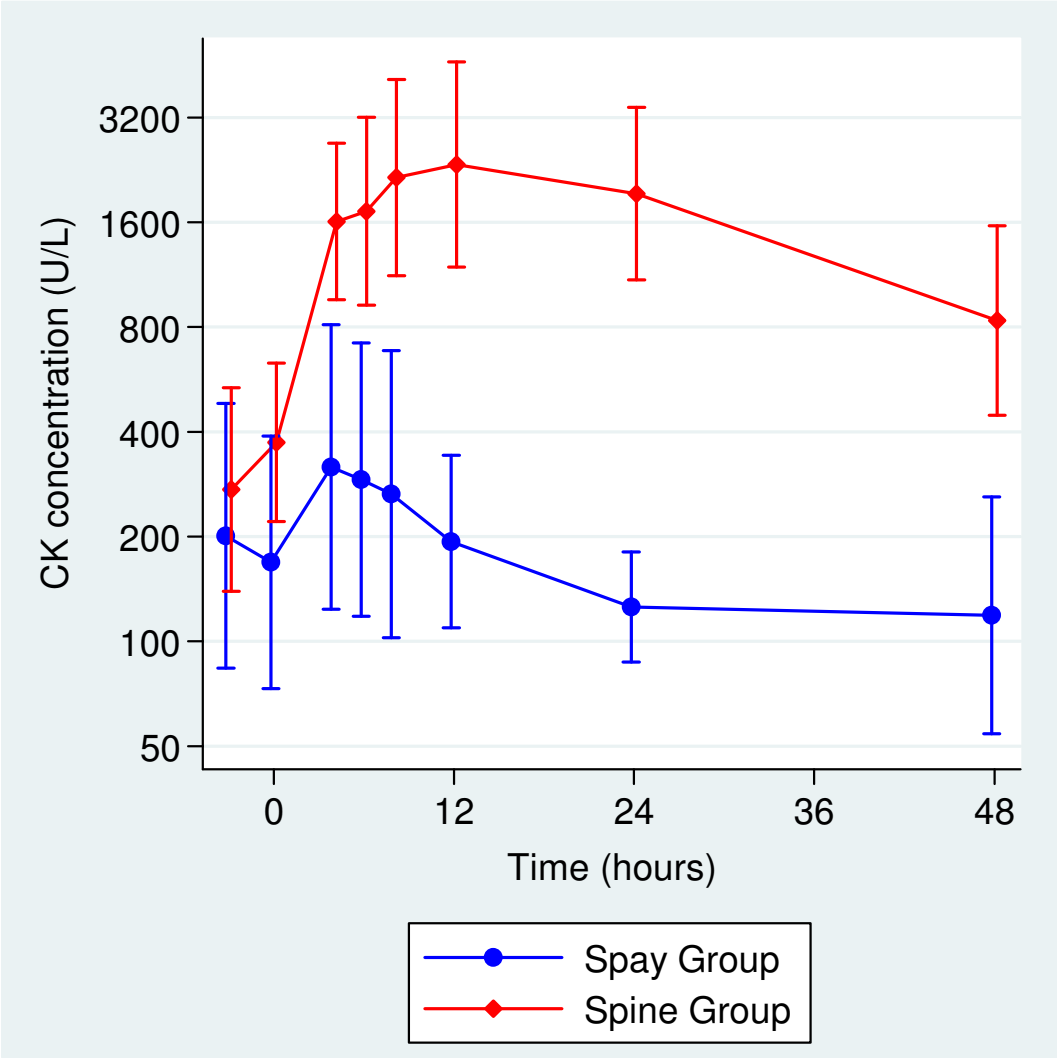

Figure 4.1 Mean log transformed CK values for each time point for both spay and spine groups. The error bars at each time point represent the standard deviation. Time 0 is the immediate postoperative time point. The means were significantly different at all time points except preoperatively.

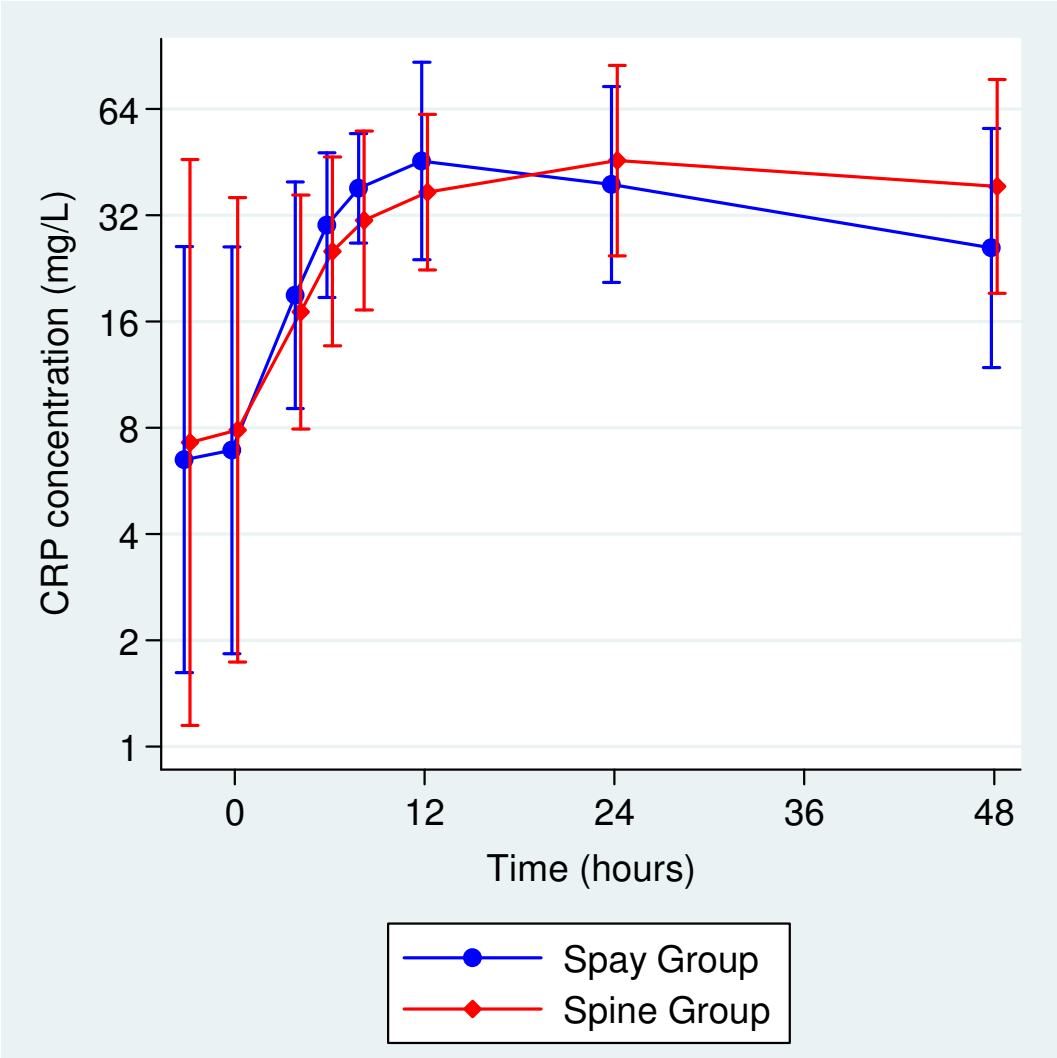

Figure 4.2 Mean log transformed CRP values for each time point for both spay and spine groups. The error bars at each time point represent the standard deviation. Time 0 is the immediate postoperative time point. There was no significant difference between the means at any time point.

4.3 COMPARISON OF BASELINE (PREOPERATIVE) CK AND CRP RESULTS WITH ESTABLISHED NORMAL VALUES.

The preoperative samples taken from each animal in this study provided the baseline results for that animal.

4.3.1 CREATINE KINASE (CK)

These baseline results encompassed a very wide range. The frequency distributions for the two groups are shown as histograms in figure 4.3.

The spay group's lowest result was 58 U/L and the highest result returned was 2754 U/L. Apart from that single extreme value, the next highest result in the spay group was 854 U/L. The median preoperative CK activity for the spay group was 156 with an interquartile range of 125 – 254 U/L. This median is greater than the upper value of the reference interval used by the OVAH laboratory (49-146 U/L).

The spine group's preoperative results ranged between 77 and 936 U/L. Several individuals had values in the high hundreds. The median preoperative CK value for the spine group was 336 U/L with an interquartile range of 159 - 442 U/L. This median was considerably greater than the upper value of the OVAH laboratory reference interval (49-146 U/L).

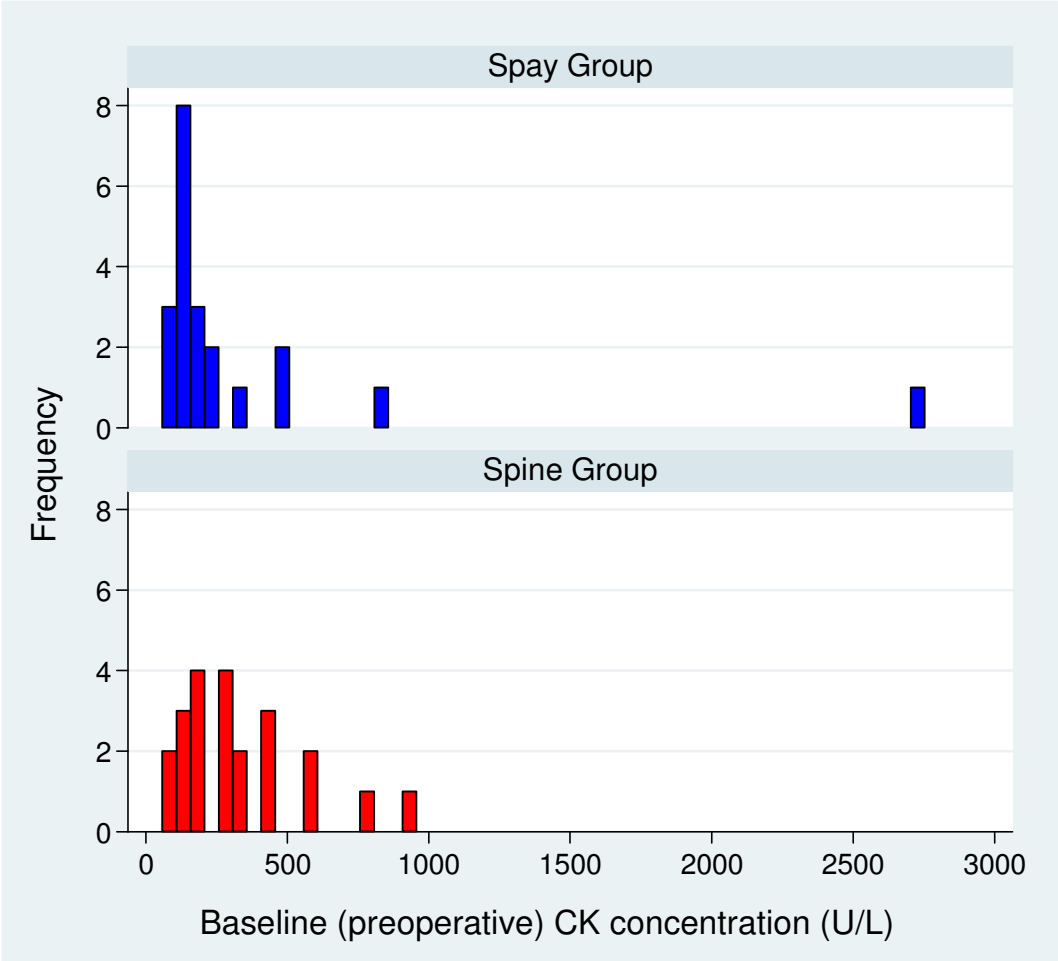

Figure 4.3 CK baseline (preoperative) results for the spay and spine groups. It can be seen that the results for both groups were not normally distributed and encompassed a wide range. The spay group produced the individual with the highest level of all the dogs in the study.

4.3.2 C-REACTIVE PROTEIN (CRP)

The overall range of the baseline results was similar in both groups. Figure 4.4 shows the frequency distributions for the two groups as histograms.

In the spay group, the baseline values for CRP ranged from 0.1 to 67 mg/L. This range is much greater than the normal reference interval of the OVAH laboratory (<35mg/L).

In the spine group, the range of baseline values was between 0.05 and 59.7 mg/L with a median of 11.35 mg/L and an interquartile range of 4.8 – 25 mg/L. Although lower than the spay group, this range was still much greater than the OVAH laboratory reference interval (<35mg/L).

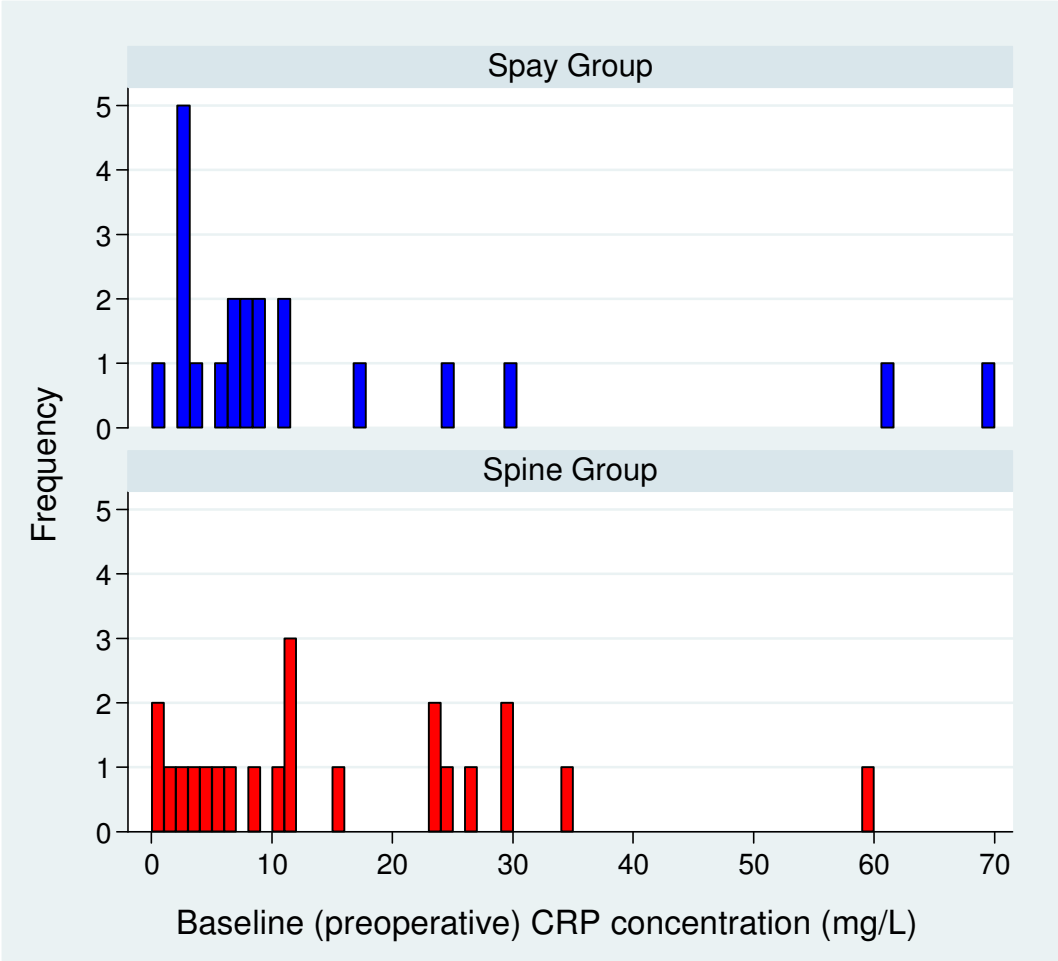

Figure 4.4 CRP baseline (preoperative) results for the spay and spine groups. It can be seen that the results for both groups were not normally distributed and encompassed a wide range. The spay group produced the individual with the highest level of all the dogs in the study.

4.4 COMPARISON OF FINAL (48 HR) RESULTS WITH ESTABLISHED NORMAL VALUES.

4.4.1 CREATINE KINASE (CK)

4.4.1.1 SPAY GROUP

The final (48 hr postoperative) sample for the spay group delivered a range of between 46 and 1171 U/L. The median for this time point was 100.5 U/L with an interquartile range of 68 – 166.5 U/L. The median at this time point thus falls within the reference interval used by the OVAH laboratory (49-146 U/L).[3] The 75th percentile value was slightly higher than the upper value of the OVAH laboratory reference interval.

4.4.1.2 SPINE GROUP

The 48 hr postoperative sample for the spine group delivered a range of between 220 and 3160 U/L. The median was 848 U/L with an interquartile range of 493 to 1176 U/L. This places the median considerably beyond the OVAH reference interval (49-146 U/L). It can however be seen from the graph in fig. 4.1 that there was a marked downward trend for mean CK from the peak at the 12 hours postoperative time point and extending through the 24 and 48 hour time points.

4.4.2 C-REACTIVE PROTEIN (CRP)

4.4.2.1 SPAY GROUP

The final (48 hr postoperative) sample for CRP in the spay group delivered a range of between 1.4 and 66.4 mg/L. This is much greater than the reference interval of the OVAH laboratory (<35mg/L). The median was 29.4 mg/L with an interquartile range of 19.3 – 41.3 mg/L. This places the median within the upper limit of the OVAH laboratory reference interval.

4.4.2.2 SPINE GROUP

The results for the 48 hr postoperative sample in the spine group were broadly similar to that of the spay group with a range of between 9.3 and 162 mg/L, a median of 34.3 mg/L and an interquartile range of 21.4 and 91.4 mg/L. This range is much greater than that of the reference interval of the OVAH laboratory (<35mg/L). The median is just within the upper limit of the OVAH laboratory reference interval.

4.5 EVALUATION OF CK AND CRP RESULTS OVER TIME WITHIN EACH GROUP.

4.5.1 CREATINE KINASE (CK)

Figure 4.1 shows the changes in mean log transformed CK values over the sampling period for the two groups. Repeated measures ANOVA of the geometric means of each time point relative to the pre-operative time point was performed to establish if any significant differences existed. ($\alpha = 0.05$)

4.5.1.1 SPAY GROUP

Fig 4.1 shows a drop in mean CK levels immediately postoperatively although this drop was not statistically significant. The levels then rise and peak at 4 hrs postoperatively. Thereafter there was an even decline to the last measurement at 48 hrs. Only the 4 hr and 48 hr means were found to be significantly different from the preoperative levels; the 4 hr mean being significantly higher and the 48 hr mean being significantly lower. All other time points were not significantly different from the preoperative time point.

4.5.1.2 SPINE GROUP

Fig 4.1 shows an immediate postoperative increase in CK levels and a peak at 12 hrs. Thereafter the levels decline and still suggest a downward trend at 48 hrs. All time point means were found to be significantly elevated from the preoperative level.

4.5.2 C-REACTIVE PROTEIN (CRP)

Figure 4.2 shows the changes in mean CRP values over the sampling period. Repeated measures ANOVA of the geometric means of each time point relative to the pre-operative time point was performed to establish if any significant differences existed.

4.5.2.1 SPAY GROUP AND SPINE GROUP.

CRP results appeared broadly similar in the two groups with an immediate postoperative elevation. However the spay group peaked earlier (12 hrs vs 24 hrs) and at a higher concentration than the spine group. Thereafter the levels in both groups declined and suggested an ongoing downward trend at 48 hrs. All time points, except the immediate postoperative one, were significantly different from the preoperative time point in both spay and spine groups.

4.6 COMPARISON OF CK AND CRP RESULTS OVER TIME BETWEEN THE TWO GROUPS.

Comparisons between the two groups for each time point for both analytes were performed, adjusting for sex and body mass using repeated measures ANOVA and the Bonferroni-Holm multiple comparison test. Median times to maximum concentration of CK and CRP were compared between groups using the Wilcoxon rank-sum test.

4.6.1 CREATINE KINASE (CK)

Figure 4.1 provides a graphic representation of the mean CK values in the two groups at each time point. It is clear that the spine group showed a much greater and more sustained postoperative increase than the spay group. The preoperative time point showed no significant difference between the two groups. All other time points were found to be significantly different. The median time to peak CK levels in the spay group was 4 hours and in the spine group was 12 hours postoperatively. There was a significant difference between the medians of the two groups ($p<0.001$)

4.6.2 C-REACTIVE PROTEIN (CRP)

Figure 4.2 provides a graphic representation of the mean CRP values in the two groups at each time point. An overall broad similarity between the two groups is evident. No significant differences between the two groups were found at any time point. The median time to peak CRP levels in the spay group was at 12 hours postoperatively while the spine group tended to peak later with the median time to peak levels at 24 hours. There was however not a significant difference between the medians of the two groups ($p>0.05$)

4.6.3 Comparison of Areas under the Curve

Log transformed areas under the curve (AUC) were compared between groups using multiple regression, adjusting for sex and mass.

4.6.3.1 Creatine kinase (CK)

The AUC was significantly greater in the spine group compared to the spay group.

4.6.3.2 C-reactive protein (CRP)

There was no significant difference in AUC for CRP between the two groups.

CHAPTER 5

DISCUSSION

The objectives of this study were to establish baseline measurements of two different analytes in two distinct groups of dogs undergoing different surgical procedures, and to measure the changes of these analytes at set time intervals postoperatively. These objectives were achieved satisfactorily. The stated hypotheses were found to be correct with the following two exceptions:

i) CK activity levels on presentation in dogs admitted for ovariohysterectomy were found to be greater than the upper limit of the OVAH reference interval. (see 2.2.1)

ii) CRP concentration was not found to be elevated in the spine group on presentation. (see 2.2.2.1)

5.1 ESTABLISHED NORMAL VALUES FOR CREATINE KINASE (CK) AND C-REACTIVE PROTEIN (CRP)

As might be expected, different research sources present differing normal reference intervals. Reference intervals are also established by different laboratories and may differ according to population, analytical methods and reagents, as well as variable circumstances under which the assays are performed. Although ambient temperature appears to be an important source of variation during CK measurement, Stockham and Scott suggest that with a standard assay temperature, there should be little variation between assay systems.[2,37] The assay method does however appear to be a source of considerable potential variation in CRP evaluation with Connor *et al* even suggesting CRP was undetectable in normal dogs when measured by radial immunodiffusion assay.[10,11,15,40] A comparison of CK ranges show large differences in reported upper normal limits.[3,5,16,33] This is most likely a reflection of the substantial influence preanalytical factors of variance can have on CK estimation.[2] There is less variation in the reported CRP reference intervals.[10,11,40].

5.2 RESULTS OF CK AND CRP MEASUREMENTS FOR SPAY AND SPINE GROUPS.

There was a wide range of results for both CK and CRP at all time points in both groups of dogs in this study. The graphs of the geometric mean values showed an expected basic trend of initial postoperative increase and subsequent decline which enabled useful inter group comparisons. However the wide overall and interquartile ranges obtained suggest that a single evaluation of either analyte at any one time point in a patient would be of limited clinical value unless it was significantly increased.[14]

5.2.1 CREATINE KINASE (CK)

Laboratory reference intervals notwithstanding, some authors feel that increases in serum CK concentration are not clinically relevant unless greatly or persistently elevated. Bender and Parent both suggest that only elevations in the region of 10 000 U/L should be regarded as significant.[8,33] Only a few results in this study were greater than 5000 IU/L and no individual measurement was elevated beyond 10 000 U/L. If one accepts the principle of the above statement, then by implication none of the patients in this study had clinically significant trauma. All of them were of course obviously traumatised, albeit in a controlled manner. DiBartola *et al* had a similar finding in their study of the diagnostic usefulness of CK. Of the dogs in their study that had experienced a traumatic episode (mostly vehicular accidents and dog fights), not one had a CK concentration elevated beyond 8500 U/L with many CK levels below 1000 U/L. The study only included animals with CK levels over 500 U/L and unfortunately did not state at what time after injury these samples were taken.[16] Whatever the exact cut off point of clinical relevance that might be employed, the unavoidable conclusion is that a single time point evaluation of CK in a traumatized patient is of little value.[2,14] In addition the current study suggests that absolute values of CK are probably of less importance than the trend over time.

With specific reference to the use of CK as an indicator of surgical stress, Chanoit and Lefebre queried the validity of the results obtained in a study comparing single pre and postoperative CK samples.[13] The authors of that study concluded that CK was not useful as a measure of surgical stress as the postoperative increases of CK in their

patients differed widely despite them undergoing a fairly uniform procedure (laparoscopic ovariohysterectomy).[7] Chanoit and Lefebre suggested that the aim when utilizing CK as a measure of trauma should not be a comparison between values at certain times. The aim should rather be to obtain a more complete profile of CK activity over time (more measurements) so as to accurately estimate the Area under the Curve (AUC). This calculation has been used to quantify muscle damage in dogs under various circumstances and thus permits accurate comparisons of muscle damage between individuals.[1,5,14,38] This approach may indeed be more accurate and certainly makes a better tool out of CK, however it is still subject to all the preanalytical factors of variance mentioned above and may be difficult to apply in a clinical environment.

5.2.2 C-REACTIVE PROTEIN (CRP)

Single time point values are probably more applicable to CRP than to CK. CRP is recognised as a reliable indicator of inflammation in human medicine and clinically relevant cut off points are utilised routinely in human medicine for various conditions.[39,27,21,27,39] However this has not reached the same level of sophistication in veterinary medicine yet. This is probably largely due to lack of familiarity and availability and at least partly because the measurement of veterinary acute phase proteins has not yet been internationally standardised.[11,34,21,34] CRP assays are also relatively expensive, compared for example to full blood counts, which are routinely used as indicators of inflammation. However the relatively rapid production and clearance of CRP compared to other acute phase proteins should make it a relevant indication of the clinical situation in an animal at any given time or at least provide proof of the presence or otherwise of an inflammatory process.[11,15] A recent study for example, found CRP to be elevated in all cases of spontaneously occurring pancreatitis.[21] However the wide range of baseline results returned in the current study suggest that, for trauma patients at any rate, single measurements, even if elevated, need to be interpreted with caution. The highest baseline CRP result was found in a clinically healthy dog from the spay group. CRP levels may be most applicable in the monitoring of cases where a particular inflammatory condition is known to be present. CRP cut off points can then be used prognostically or the CRP trend can be used to monitor progress and efficacy of treatment.[11,21]

5.3 COMPARISON OF BASELINE (PREOPERATIVE) CK AND CRP RESULTS WITH ESTABLISHED NORMAL VALUES.

Both groups delivered surprisingly wide ranges of baseline values for both CK and CRP.

5.3.1 CREATINE KINASE (CK)

5.3.1.1 SPAY GROUP

We hypothesized that that CK would be within the normal reference range on presentation in the spay group. The high CK median obtained for the spay group was thus surprising. [3,2,3] Endometritis in cows has been shown to lead to increased CK activity. However this study was comprised of a group of clinically normal animals and a median more in line with other normal studies was expected.[35] One can only speculate as to the reason behind the very high levels seen in some of the dogs on presentation. Sex of the animal has been found to have an inconsequential role in CK activity. Individual animals may of course have been exposed to any number of potentially confounding preanalytical events such as exercise, injections or trauma not ascertained by the history. The role of haemolysis is worthy of mention. Haemolysis is a potential consequence of poor blood collection technique. Although erythrocytes do not contain CK, damaged erythrocytes may release haemoglobin, adenine triphosphate and glucose-6-phosphate, all of which can interfere with laboratory assays.[37] Bender states that haemolysis may falsely elevate CK activity in a sample but does not indicate by what margin this might occur.[8] Other authors also caution against haemolysis but add that it will not affect CK values if it is moderate.[3,16] No centrifuged samples were found to be macroscopically hemolysed in this study.

The median value for CK more closely correlates to the studies listed by Aktas *et al* which were performed on groups of younger dogs, mostly less than one year old.[3] In a different study, Aktas *et al* found that dogs between 6 and 12 months of age had a significantly higher mean CK level (73 ± SD 27 U/L) than dogs over one year of age (46 ± SD 22 U/L).[2] On the other hand, Bender reports that mature levels of CK are reached in dogs by the age of seven months.[8] All the dogs in the spay group were over

seven months of age and 50% were less than one year old. They were mostly substantially younger than the spine group.

5.3.1.2 SPINE GROUP

The higher median CK seen in the spine group was more in line with expectations, although statistically there was not a significant difference between preoperative spine and spay group levels. We hypothesized that the spine group would have a raised CK on presentation and this does appear to be so, however whether this increase is directly due to the disc extrusion is debatable. There is some difference of opinion in the literature about what level of the CK1(BB) isoenzyme occurs in the serum of normal dogs. There is however, general agreement that CK1 does not readily breach the blood brain barrier and it is thus unlikely to have done so in sufficient quantities to have influenced the results.[3,8,16,29] There would be no direct muscle trauma to account for any CK elevation and recumbency due to paresis would very likely be the main contributing factor.[16,37]

5.3.2 C-REACTIVE PROTEIN (CRP)

We hypothesized that CRP would be elevated on presentation in the spine group and within the normal reference range in the spay group. Both groups had a wide range on presentation and statistically there was no significant difference between them. Although the CRP median for the spine group was higher than that of the spay group, for both groups it falls well within the normal range of the OVAH laboratory. This makes sense for the spay group where there were no anticipated reasons for inflammation. The array of potential preanalytical factors of variance that loom so large for CK evaluation are far less of an issue for CRP.[11] It is more difficult to explain why the spine group's baseline values were not more elevated. It may be that because the insult experienced by the body after disc extrusion is so localized, the actual trauma is comparatively minor, despite having potentially severe consequences.

5.4 COMPARISON OF FINAL (48 HR) CK AND CRP RESULTS WITH ESTABLISHED NORMAL VALUES.

5.4.1 CREATINE KINASE (CK)

The overall postoperative pattern for both groups unfolded as hypothesized. The maximal elevation of the spay group was perhaps not as high as expected but had declined to near normal activity levels by the 48 hr time point as hypothesized. The clear downward trend at the 48 hr time point in the spine group suggests that preoperative levels would have been attained within the next 12 to 24 hours. It is interesting to speculate whether, if additional time points had been sampled, the spine group would also have shown a tendency for CK to decrease below preoperative levels, as was seen in the spay group. This decrease was found to be statistically significant. In the spay group, the interquartile range at the 48 hour time point (68-166.5 U/L) was closer to the OVAH laboratory reference range (49-146 U/L) than the preoperative interquartile range (125-254 U/L). This perhaps implies that the preoperative CK activity in the spay group was raised by some common preanalytical factor, aside from age as discussed previously. Factors that deserve consideration are unrecorded preoperative trauma, the journey to the OVAH (and associated excitement and physical exercise) and the comprehensive preoperative physical examination (which for example involves a pinprick to the pinna to collect blood for a smear).[3,8,12] Of course the spine group experienced the same influences, which may at least partially account for that group also being elevated preoperatively.

5.4.2 C-REACTIVE PROTEIN (CRP)

CRP levels were still increased over normal at the 48 hour time point in both groups although a similar and gradual decline is suggested by the graph in fig 4.2. This trend would potentially be useful in the monitoring of a trauma case. If baseline CRP concentration was established on presentation, a recovering animal should show a decline in CRP concentration within 48 hours of presentation. A persistently raised or increased CRP concentration would suggest that there is ongoing inflammation and alert the clinician to potential complications such as the presence of infection. In this regard it is worth remembering that both surgical groups in this study were subjected to sterile injuries. Non surgical trauma is much more likely to be associated with infection particularly if the trauma is not acute. As noted previously, infection can lead to significant serum CRP elevations as part of the acute phase response.[11,31,36]

A comparison of fig. 4.2 with the CK graph in fig 4.1 implies a longer half life for CRP than CK. The half life of CK is about 2hrs.[3,2,3,37] The half life of CRP is not specifically mentioned in the literature but is relatively short, amongst the shortest of the acute phase proteins. As discussed previously, it is this rapid synthesis and removal of CRP that make it a potentially useful reflection of an animal's clinical status at the time of sampling.[11,40]

5.5 EVALUATION OF CK AND CRP RESULTS OVER TIME WITHIN EACH GROUP.

5.5.1 CREATINE KINASE (CK)

We hypothesized that CK activity levels would be within the normal reference range in the spay group and elevated in the spine group on presentation and that both groups would show a marked postoperative rise and subsequent decline with the spine group being elevated over the spay group at all time points.

5.5.1.1 SPAY GROUP

Although the CK levels in both the spay and spine groups displayed a postoperative rise to peak and subsequent decline, it is interesting that in the spay group only the 4 hr mean was significantly elevated from the preoperative levels. This peak increase in the spay group was probably less than anticipated as we hypothesized that CK would increase markedly after surgery. This suggests that the degree of muscle trauma in the spay group was not only relatively minor but also not sustained. The reason for this is probably that the surgical approach is made through the linea alba with minimal trauma to striated muscle. In addition the soft tissues and smooth muscle traumatized in the genital tract contain very little CK.[16]

The immediate postoperative drop in mean CK levels, although not statistically significant, is similar to that seen in a study evaluating the influence on serum CK of electromyography under general anaesthetic. In that study, the overall post anaesthetic rise in CK levels was also relatively mild.[38] The influence of anaesthesia on serum CK levels may be worth further evaluation. Aktas *et al* undertook a study comparing muscle damage in dogs anaesthetized with either propofol or halothane in which they found that halothane caused significant muscle damage and that propofol did not.[1] The postoperative CK levels in the latter group of dogs were not however specifically reported.

5.5.1.2 SPINE GROUP

The spine group showed an immediate postoperative rise and was significantly elevated at all time points. We hypothesized that the spine group would demonstrate very high activity postoperatively and certainly the maximum mean was considerably larger than the preoperative mean. The maximum CK activity levels were nonetheless perhaps lower than might have been anticipated when one considers the nature of the surgical procedure. The greatest CK value in the spine group was below 10000 U/L and only four results at any of the time points were over 5000 U/L, with three of those from one dog. This should be considered against reports that a single intramuscular injection can result in CK elevations of greater than 23 times normal values and that in racing sled dogs, for instance, CK activity levels of less than 5000U/L have been regarded as not significant.[3,5,14] These events are of course not directly comparable, but the apparent discrepancies in CK values again call into question the usefulness of isolated CK measurements. It may also be that the surgical approach for a hemilaminectomy is not as traumatic, to muscle at any rate, as one might suppose. Certainly the objective for the surgeon should be to elevate rather than to incise the epaxial musculature whilst making the surgical approach so as to minimize trauma. It is again necessary to bear in mind that using peak serum CK activity levels only, to assess the degree of muscle damage, is not particularly accurate. Quantification of muscle damage is more accurately achieved by determining the area under the CK concentration versus time curve.[2,12]

5.5.2 C-REACTIVE PROTEIN (CRP)

The marked similarities in the results of the two groups are discussed in 4.6. It was hypothesized that the CRP concentration would be within the normal reference range in the spay group and elevated in the spine group on presentation but that both groups would show marked elevation postoperatively with a decline to normal concentrations. The results support the hypothesis, although as discussed in 5.3, it is perhaps surprising that the preoperative CRP results in the spine group were not more elevated relative to the spay group. In a study on a group of dogs undergoing ovariohysterectomy, Burton *et al* found CRP to be significantly elevated above preoperative levels only by 12 hours postoperatively. This contrasts to the current study where a significant difference was found already by 4 hours postoperatively. This difference in the two studies may possibly be accounted for by differences in assay technique or the low numbers in the study by Burton et al where only 6 dogs were included. Both studies however, show a similar peak in CRP levels at about 24 hours postoperatively.[9]

5.6 COMPARISON OF CK AND CRP RESULTS OVER TIME BETWEEN THE TWO GROUPS.

The graphs in figures 4.1 and 4.2 show the striking differences in mean CK results and equally striking similarity in mean CRP results between the two groups.

5.6.1 CREATINE KINASE (CK)

The CK activity in the spay group peaked significantly earlier than in the spine group albeit at a much lower level. The peak was also the only time point significantly increased from the baseline in the spay group. Serum CK is largely of skeletal muscle origin and the CK results directly reflect the difference in degree of muscle trauma between the two surgical procedures. CK activity levels peaked at a higher level and at a later time point in the spine group suggesting that not only was a greater amount of CK released, but that the release was more sustained. Bearing in mind the short half life of CK of about 2 hours, this suggests that there was ongoing CK release, well beyond the surgical insult, with the peak reached sometime between 12 and 24 hours. More frequent sampling would have pinpointed the peak time more accurately. Estimation of the AUC again supports the conclusion that significantly greater skeletal muscle damage occurred in the spine group compared to the spay group.

6.2 C-REACTIVE PROTEIN (CRP)

The similarity of the CRP results between the two groups, as reflected by the graphs and the calculation of the AUC, was perhaps a little surprising. In their review of acute phase proteins, Ceron *et al* state that postoperative CRP increases are approximately proportional to the intensity of surgical trauma.[11] Macroscopically it seems that the surgical procedure for the spinal decompression is much more traumatic than an ovariohysterectomy and that this should be reflected by higher CRP concentrations. CRP values were found to have a much greater increase in orthopaedic surgery compared to ovariohysterectomy in one study. The authors concluded that the rise in CRP was indeed proportional to the intensity of surgical trauma. The strength of their conclusions may have been compromised by the small number of animals in the two

groups (3 and 4 dogs respectively).[40] Another larger study found the opposite, with the median CRP concentration 24 hrs postoperatively being higher in dogs undergoing ovariohysterectomy compared to dogs undergoing orthopaedic procedures.[10] These results are more in line with this current study. The solution to this apparent contradiction may lie in human studies, where no correlation has been found between serum CRP concentration and severity of injury or survival.[18] The conclusion seems to be that CRP is much more of a general rather than a specific measure of inflammation and that this inflammation is not necessarily related to macroscopic appearance. In other words CRP is a sensitive indicator of trauma but cannot be used as a reliable quantitative measure of trauma.

The peak in CRP concentration was also at a point between 12 and 24 hours for both groups as it was for CK in the spine group. This time period is thus likely to be the most worthwhile and informative interval in which to monitor CK and CRP levels in traumatized animals. As discussed previously, it would be preferable to have baseline levels against which to compare the trend, as isolated evaluations of both CK and CRP may be of limited value.

CHAPTER 6

CONCLUSION

The ability to quantitatively measure the degree of trauma in an injured animal would be useful therapeutically and prognostically in the clinical situation. CRP and CK have both been used previously as measures of trauma and were thus evaluated and compared in this study. A wide range of results was obtained for each time point for both CRP and CK, although as groups they followed a predictable pattern over time after trauma. The results of this study suggest that measuring the serum levels of CK and CRP in a traumatized dog at a single time point, for instance on presentation, is of limited value. Establishing the maximal CK and/or CRP levels is similarly of little clinical use. The information gained does not necessarily accurately reflect the degree or severity of trauma suffered by the animal, particularly in the case of CRP. The time interval 12 to 24 hours post trauma is potentially the most informative, especially if a baseline value is available for comparison. However the trend over time, even a relatively short time period of 48 hours, may deliver the most meaningful information.

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APPENDICES

APPENDIX 1

TRAUMA STUDY CONSENT FORM

Dear Client
The Onderstepoort Veterinary Academic Hospital is currently running a study on the effects of surgery in dogs. We hope to gain additional insights into certain blood parameters affected by surgery to enable us to treat future patients more effectively. We would be very grateful if you would allow us to include your pet in this study. The only additional procedures that will be performed will be daily collections of a small amount of blood.
We ask that dogs admitted for sterilisation (spay) stay an additional night in hospital. There will be no additional costs for yourself.

Pet's name: Breed: Age: (Please place sticker)

I hereby give consent to the inclusion of my pet to the study as described above.

Client signature _____

Client name: _____

Client no: _____

Telephone: _____

Date: _____

Thank you for allowing us to include your pet in this study, your participation is greatly appreciated.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

APPENDIX 2

SURGICAL TRAUMA STUDY NB NO INTRAMUSCULAR INJECTIONS PLEASE!

Owner

Patient

(Please place sticker)

Basic Clinical Examination
(Spays and Spines)

Condition_____

Temp_____Pulse_____Respiration_____

Bloodsmear _____

_____

_____

Any inflammatory conditions? (eg pyoderma/ otitis ext)

_____

_____

_____

Comments _____

_____

Date of admission _____

Reason for admission: **Spay / spine** (Circle)

NB: Any history of trauma, injections , surgery within the last month? **Yes / No** (Circle)

If yes' describe briefly_____

_____

Spinal Neurological Examination

Date symptoms first seen. _____

Any prior treatment given? _____

_____

Ambulatory? _____

Hindquarter paresis/ paralysis? Left_____Right_____

Proprioception: Left_____Right_____

Superficial pain: Left_____Right_____

Deep pain: Left_____Right_____

Hyperpathic zone (where?) _____

Panniculus reflex localises to: _____

Patellar Reflex: Left_____Right_____

Any other comments. _____

_____

APPENDIX 3

SURGICAL TRAUMA STUDY

Patient Sampling Record

Owner Patient (Please place sticker)

Reason for admission: Spay / spine (Circle)

Date of surgery_____

Sample	Time due	Date due	Tick	Comments
Preop (immediately after induction)				
Postop (immediately postop)				
4hrs postop				
6hrs				
8hrs				
12hrs				
24hrs				
48hrs				