COMPARISON OF INTERNAL AND EXTERNAL BILIARY DRAINAGE PROCEDURES IN PREPARATION FOR SURGERY.

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By

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Statement of Originality

The work on which this thesis is based is my own independent work except where acknowledged

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Abbreviations

99mTc	Technetium-99m
ADH	Antidiuretic Hormone
ALP	Alkaline Phosphatase
ANP	Atrial Natriuretic Peptide
APACHE	Acute Physiology and Chronic Health Evaluation
AST	Aspartate Aminotransferase
BDL	Bile Duct Ligation
BMI	Body Mass Index
cAMP	adenosine 3',5'-cyclic monophosphate
CD	Cluster of Differentiation
cGMP	guanosine cyclic 3',5'- monophosphate
CRP	C Reactive Protein
DTPA	Diethylene Triamine Pentaacetic Acid
ERCP	Endoscopic Retrograde Cholangiopancreatography
GGT	Gamma-glutamyltransferase
H+	Hydrogen ion
Hb	Haemoglobin
HLA-DR	Human Leukocyte Antigen DR-1
IgA	Immunoglobulin A
IL-1	Interleukin 1
IL-10	Interleukin 10
IL-6	Interleukin 6
iNOS	Inducible Nitric Oxide Synthase
kDa	kilodaltons
mRNA	messenger RiboNucleic Acid
MVR	Major Visceral Resection
Na+	Sodium ion
PGF1	Platelet Derived Growth Factor 1
PTC	Percutaneous Transhepatic Cholangiography
RNA	RiboNucleic Acid
TNF α	Tumour Necrosis Factor Alpha
TNM	Tumour Node Metastasis
TPN	Total Parenteral Nutrition

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1.1: Clinical Problem

The presence of obstructive jaundice in the surgical patient is associated with increased post operative morbidity and mortality, irrespective of the type of procedure being performed [1, 2]. Obstructive jaundice produces a range of pathophysiological changes including renal impairment [3] increased rates of gut bacterial translocation and endotoxaemia [4] and impairment of immunological function [5, 6].

Hypoalbuminaemia in acutely ill patients is associated with poor outcomes in terms of increased rates of morbidity and mortality, as well as prolonged stays in intensive care units and in hospital [7]. The development of hypoalbuminaemia in obstructive jaundice is also well described [3]. It is often impossible however, to increase the serum albumin concentrations by enteral or parenteral administration in the setting of obstructive jaundice [8] although albumin levels have been shown to be restored after biliary drainage in both animal [9] and human studies [10].

Preoperative biliary drainage can be used as a means of reversing the pathophysiological disturbances seen in jaundiced patients, although the efficacy remains a contentious issue.

Preoperative internal biliary drainage has been found to cause bacterial contamination of bile which is associated in some clinical studies with increased postoperative complications [11, 12] and death [11] while others studies have shown no detrimental effects of preoperative internal biliary drainage [13]. The benefits of internal

and external drainage in some studies has been offset by complications and morbidity caused by the drainage procedure itself such as infection and haemorrhage [14-16]. Other authors have found that drainage procedures do not increase either the risk of morbidity or mortality however, nor do they convey benefit either, thereby creating an argument against preoperative drainage where feasible [17]. Conversely studies quoting a minimal or zero complication rate following external drainage have shown a subsequent reduced mortality following definitive surgical management [18].

One published meta-analysis by Sewnath *et al* examined preoperative biliary drainage (both internal and external) versus no biliary drainage and found no difference in the overall postoperative death rate between patients who had drainage procedures and those who had surgery without drainage. This study did not look for superiority of one drainage type over the other however, and the conclusions amalgamate all types of biliary drainage procedures together. The overall postoperative complication rate in the level one studies examined was significantly lower in the drainage group (29.9 vs 41.9%), but balanced against significant complication rates due to the biliary drainage procedures itself (5.1% mortality and 27.4% procedure related complication rate) reduced the overall benefit [19].

Currently the indications for pre-operative biliary drainage, if any, remain poorly defined. No clear evidence has emerged whether preoperative biliary drainage (either external or internal) is superior to no drainage. The presence of hypoalbuminaemia in conjunction with obstructive jaundice and the potential to restore albumin levels with drainage may be an argument for preoperative biliary drainage.

1.2: Trends in malignant biliary obstruction

Obstructive jaundice may result from both benign and malignant processes. The most common cause of benign biliary obstruction are common bile duct stones which can usually be managed either surgically or with a combination of endoscopic (ERCP - Endoscopic Retrograde Cholangiopancreatography) and surgical approaches.

Malignant biliary obstruction is most often caused by adenocarcinoma of the pancreas, followed by cholangiocarcinoma and carcinoma of the ampulla of Vater (Dept of Health Series DH2 mortality data 1982-2003). Pancreatic cancer is the tenth most common cancer in males and eleventh in females but accounts for the 7th most common cause of cancer deaths in men and the 5th most common in females (Figure 1.21). In 1997 the incidence was just below 10 per 100,000 in males and 7 per 100,000 in females. The main risk factor in the development is smoking with a two to six fold increase in risk compared to non smokers [20]. Other factors such as hereditary pancreatitis and diabetes have also been implicated [21]. Similar incidences are seen in developing countries such as Japan and the USA while less developed countries such as Singapore and India the incidence of pancreatic cancer is lower [22].

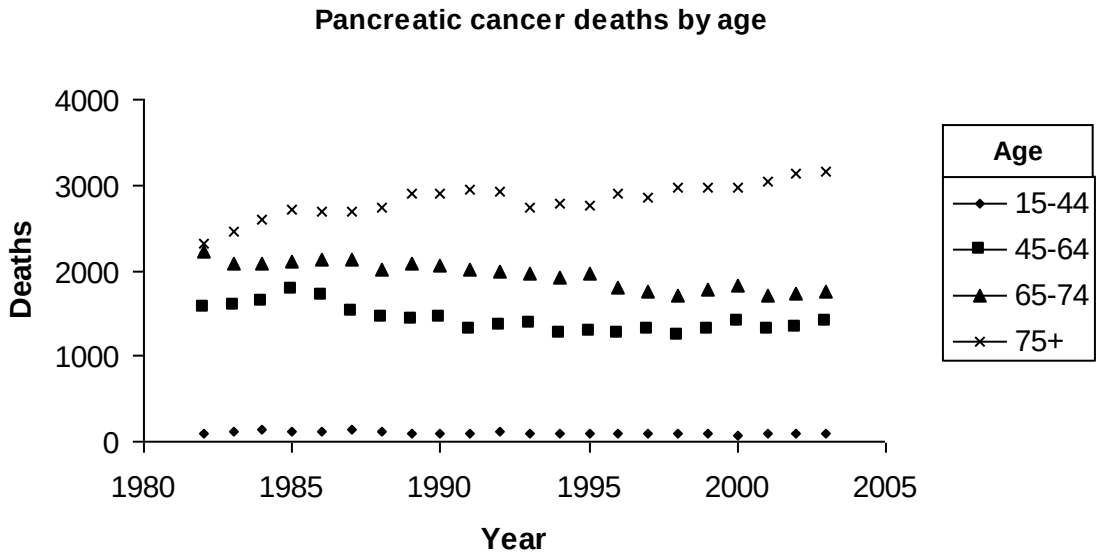


Figure 1.21: UK Death rates due to pancreatic cancer 1982-2003. Extracted from Series DH2. Mortality by cause. London, The Stationary Office 1982-2003.

The incidence of pancreatic cancer in men has fallen since to 1980's and in women the incidence rose in the 1970's and 1980's before starting to fall in the 1990's. The male: female age standard ratio is 1.4:1 in 1997 as compared to a ratio of 1.7:1 in 1971 [22]. Over 90% of cases are diagnosed in the over 55 age group and 40% reported as arising in the head of pancreas, 3% body and 2% tail of pancreas. The remainder are unclassified in the Office of National Statistics database [22]. Histological diagnosis is recorded as two thirds being non specific epithelial pancreatic neoplasms, 20% as pancreatic adenocarcinomas and 2% mucinous neoplasms [22].

Trends in males have shown no increase in total number of deaths over 20 years (Figure 1.22) although there has been a reduction since the start of the First World War. In females the total number of deaths in the over 75 group is increasing (Figure 1.23) but

this may be a reflection of the increased number of elderly females in the population, rather than any change in the underlying death rate.

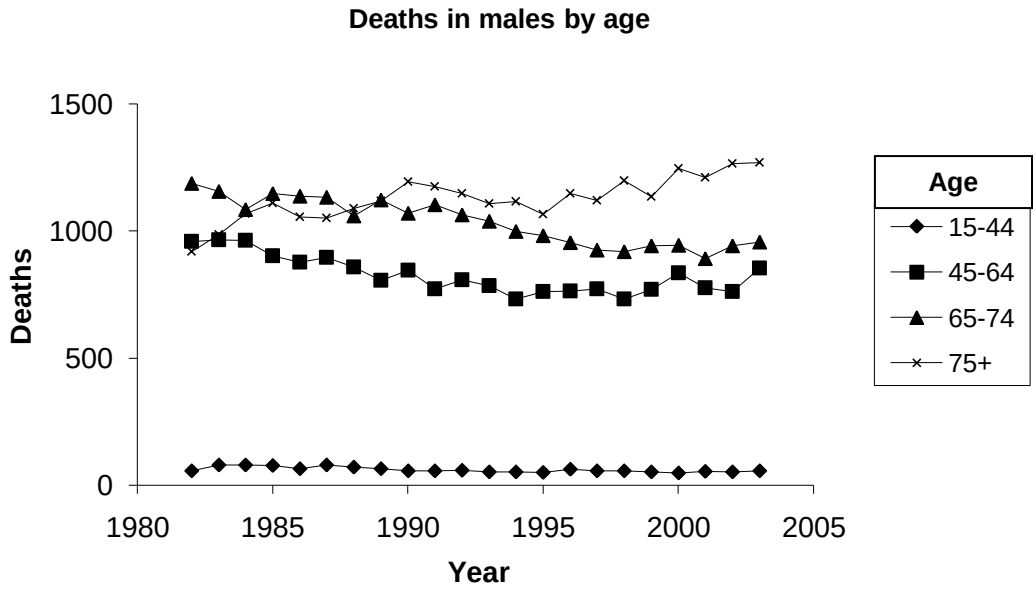


Figure 1.22: UK Death rates due to pancreatic cancer in males 1982-2003. Extracted from Series DH2. Mortality by cause. London, The Stationary Office 1982-2003.

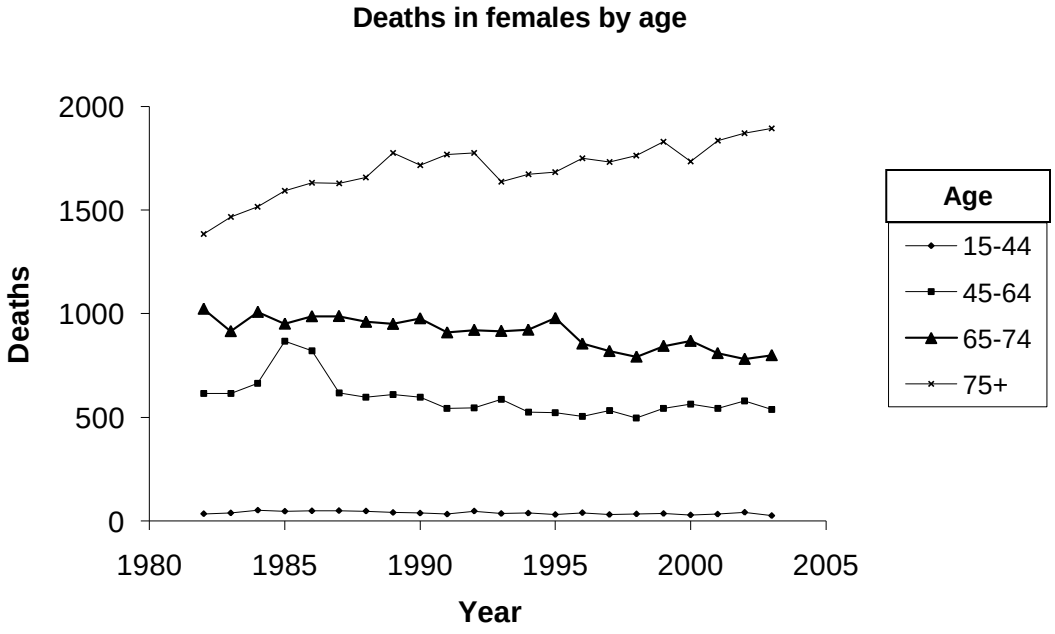


Figure 1.23: UK Death rates in females due to pancreatic cancer 1982-2003. Extracted from Series DH2. Mortality by cause. London, The Stationary Office 1982-2003.

In 2001 the Department of national statistics switched from the ICD-9 to ICD-10 classification in line with the World Health Organisation guidelines. This new classification system increased the overall deaths from neoplasm by 3.3% when compared to ICD-9. However a bridging study published by the Office of National Statistics did not show any effect on deaths from pancreatic cancer following this change in classification [23].

Survival rates for pancreatic cancer are particularly poor, with average one year survival being 13% and 5 year survival ranging from 0.4 [24] to 5.2% [25]. This is a reflection of the fact that the disease is often advanced by the time of diagnosis with only approximately 10% of patients presenting with operable disease [25]. Long term survival after pancreaticoduodenectomy for adenocarcinoma of the pancreas ranges from 7% up to 23% with the higher figure seen in patients with clear resection margins, negative nodes, no perineural invasion and tumours less than 2cm [26]. Postoperative survival is increased with adjuvant chemotherapy [27] but no survival benefit is seen with the addition of chemoradiotherapy [27, 28].

1.3: Albumin

Serum albumin is the most abundant protein in the body. Its name is derived from the Latin *albus* meaning white [29]. It serves a large number of processes including maintenance of oncotic pressure, acid/base buffer, antioxidant functions and the transport and binding of a number of substances such as drugs, hormones, vitamins, minerals and metals in addition to bilirubin [30]. Albumin specific receptors on the surface of hepatocytes mediate the uptake of many of these substances [31].

1.3.i: Biosynthesis and structure

The gene for human albumin lies on the long arm of chromosome 4, near the centromere at position q11-22 [32]. The gene for the rat albumin family lies on chromosome 14 [33]. Albumins are found throughout the body and 60% of total body albumin is found in the extravascular space [34].

Albumin is produced in the liver as a single protein chain made up of 585 amino acids with a molecular weight of 65kDa [35]. It is a heart shaped molecule that has three alpha-helical domains (I-III) which are structurally similar to each other and subdivided further into subdomains A and B [34]. Albumin is not stored in hepatocytes but passes rapidly into secretory channels where it becomes folded into a unique series of nine double loops held together by 17 disulphide bonds [36].

Following transcription, albumin mRNA leaves the nucleus and is translated by cytoplasmic ribosomes leading to the formation of the albumin precursor preproalbumin.

(See Figure 1.31). Preproalbumin is characterised by a 24 amino acid residue extension at the amino terminus of which the first 18 act as a signal for endoplasmic reticulum insertion, after which this extension is cleaved to form proalbumin within the endoplasmic reticulum [29]. The proalbumin within the endoplasmic reticulum is transported to the Golgi apparatus for further processing and secretion. Proalbumin is characterized by a hexapeptide extension on the amino terminus [29] which is cleaved within the Golgi apparatus to form albumin, and this cleavage process is required for the secretory process to continue [37]. Albumin is then secreted via the Golgi apparatus to the cell surface, facilitated by the cytoplasmic microtubules [38]. It is estimated that albumin may emerge from the hepatocyte approximately 20 minutes after its synthesis [39]. The highly folded structure of albumin, along with its hydrophilic regions results in the ability to bind and transport the many substances discussed above [39].

Rat albumin differs from human and bovine albumin in that it has a sequence of amino acids within its structure that may allow it to be glycosylated [37]. However between bovine, human and rat albumins 61% of the amino acid sequence is conserved [36].

1.3.ii: Factors affecting albumin synthesis and hypoalbuminaemia

The adult human liver produces about 15g (200mg/kg body weight) of albumin per day and the rate of secretion matches production as hepatocytes do not store albumin (i.e. constitutive secretion). Serum albumin levels are not controlled by one factor but rather by a combination of synthesis and secretion, distribution and breakdown. The half life of albumin is approximately 19 [40] to 21 [41] days in humans whereas rat albumin

half life is much shorter, being between 22 hours [42] and 48 hours [43]. Once within the intravascular space approximately 5% of the intravascular albumin escapes into the interstitial space per hour, and is ultimately returned via lymphatics [44].

Regulation of production under normal physiological conditions may be via the effect of colloidal oncotic pressure on the hepatocyte [39] as albumin regulates plasma oncotic pressure. This may be mediated via changes in hepatic interstitial volume as albumin production increases as hepatic interstitial volume decreases [45] and may be mediated by down regulation of albumin translation at the molecular level although the precise mechanism by which this occurs is unknown [46].

Steroids [47] and thyroid hormones [48] have an effect on albumin production. Thyroid hormones stimulate mRNA production and insulin stimulate albumin mRNA production and increases the rate of albumin secretion in rats [49]. Alcohol and its metabolites have a directly toxic effect on cytoplasmic microtubules and thus reduced albumin secretion [50].

As with all proteins, the synthesis of albumin requires a supply of amino acids, and albumin synthesis is particularly sensitive to dietary restriction. Protein restriction in rats reduces in vivo production of albumin by 70% [51] although in situations of total starvation the fall in albumin production is not so pronounced [52] as a result of catabolism of the body's own tissue protein stores.

Malnutrition and development of hypoalbuminaemia is well established [53]. In models, malnutrition results in loss of cellular RNA, disruption in membrane bound ribosomes and decreased albumin synthesis [53-55]. Albumin synthesis is reduced by up to a third after a fast as short as 24 to 48 hours and the ability to synthesise albumin by hepatocytes is restored within 30 minutes after eating [54].

Hypoalbuminaemia is also seen in situations resulting in systemic upset, such as inflammation, infection, trauma and the post operative setting [56, 57]**.** The mechanisms by which this effect is mediated is complex but includes increased losses due to increased vascular permeability [58] but also an increase in hepatic production of other stress related proteins such as complement, fibrinogen, haptoglobins and antitrypsin [39]. Albumin synthesis is suppressed at a transcriptional level by IL-6, IL-1 and TNF-α [59, 60], production of which are increased in times of stress such as in the perioperative period. Postoperatively patients with high IL-6 levels have lower serum levels of total protein and albumin, higher age and more frequently positive blood cultures compared with those with lower IL-6 levels [61]. Following surgery for rectal cancer, albumin synthesis and total body protein turnover are increased and do not return to baseline levels until 4 months postoperatively [62].

Hypoalbuminaemia is also well described in patients with prolonged jaundice [3] although little is known about the precise mechanism for this.

In burns, hypoalbuminaemia is further exacerbated as skin contains a significant proportion of extravascular albumin which is lost and lymphatic channels are disrupted,

thereby reducing albumin recirculation via lymphatics through the thoracic duct [63]. Burns and surgical trauma further compound hypoalbuminaemia due to the significant increase in metabolic rate and therefore increased protein turnover [64].

Less is known about albumin breakdown than its production and secretion. Albumin breakdown and loss occurs in several areas, including the kidney, gut, muscle and skin but also a proportion is directly broken down by vascular endothelial cells [44].

1.3.iii: Albumin binding to bilirubin

Most bilirubin travels within the vascular system tightly bound to albumin with a small proportion remaining as free pigment with plasma [65]. Albumin in general, binds with the highest affinity to negatively charged hydrophobic molecules [36].

There are two types of bilirubin binding to albumin. The first consists of a bilirubin anion – albumin compound whereby two bilirubin dianions bind to one binding site on albumin. This process is rapid, occurs with a high affinity, is pH independent and reaches an equilibrium within plasma. Smaller amounts of bilirubin may bind to one or two secondary sites [65].

The second form (which is less common in vivo) occurs with co-crystallization of albumin and bilirubin acids at low pH (below 7.4). Large complexes containing several albumin molecules and hundreds of bilirubin molecules form although this process is considerably slower and occurs over hours or days [65].

The principle binding sites for ligand binding are thought to be on subunits IIA and IIIA within hydrophobic cavities [36] in the region of amino acids 124-297 [66] with a lysine residue being suggested as a central amino acid involved with this binding [67]. However more recent work by Petersen *et al* suggests that the binding site may be more dynamic and have a flexible high affinity binding site [68]. It has been postulated that bilirubin-albumin complexes can have an antioxidant effect and protects the constitutive amino acids of bilirubin from photo-oxidative damage [69], and it has also been shown in vitro to protect albumin bound fatty acids from peroxyl radical-mediated oxidation [70].

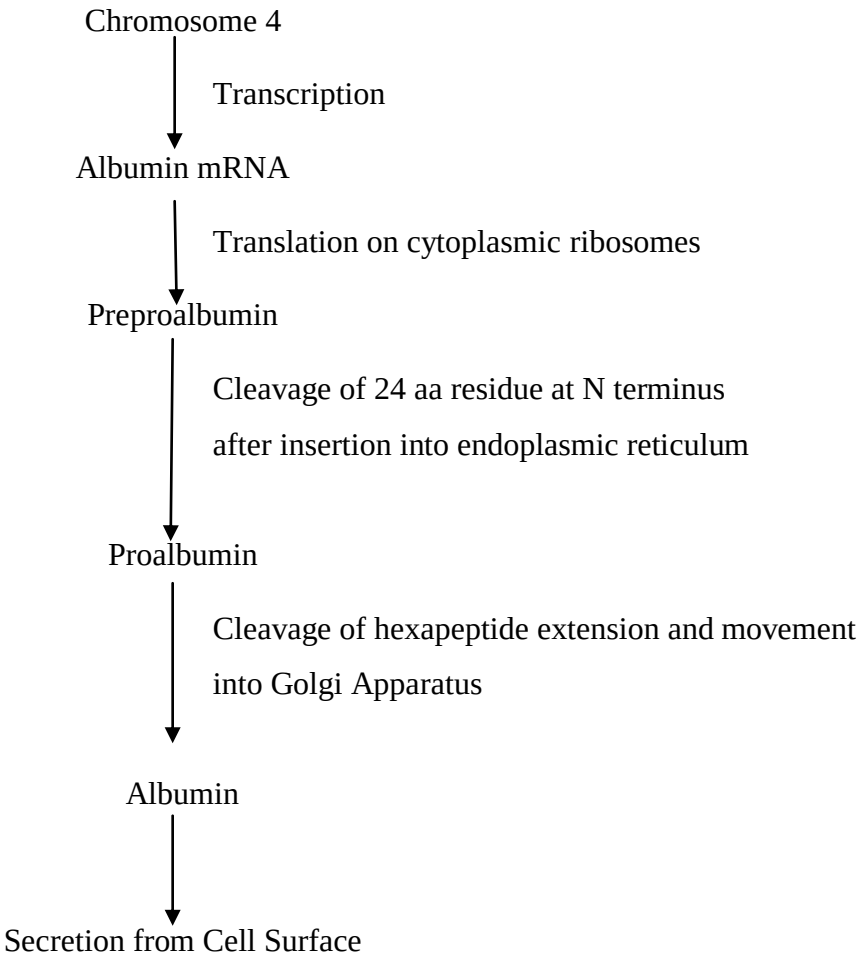


Figure 1.31: Flow diagram for the formation of albumin

1.4: Complications of obstructive jaundice

In this section the physiological effects of obstructive jaundice on infection, the development of renal failure, metabolism and the gastrointestinal tract are examined.

1.4.i: Obstructive jaundice and infection

Obstructive jaundice impairs the function of multiple components of the immune system. The development of sepsis in those with obstructive jaundice is well documented and is associated with bacterial translocation and endotoxaemia [4]. Post operative septic morbidity and mortality is also increased in the setting of obstructive jaundice [71].

Under normal conditions bile is sterile [72], however biliary tract obstruction and stasis predisposes to infection. Prevention of colonization from either the duodenum or portal system is achieved through several mechanisms including the sphincter of Oddi, hepatocyte tight junctions, mechanical clearance through bile flow, bile acids, secretion of immunoglobulin and Kupffer cell function [73]. Routes of biliary infection include ascending spread though the biliary tree, and lymphatic [74] or haematogenous transmission [75].

1.4.i.a: Bacterial translocation, clinical infections and bacterobilia

Greig *et al* observed that jaundiced patients undergoing surgery for the relief of benign and malignant biliary obstructions had a 3.1% wound infection rate and episodes of septicaemia occurred in 3.8% of patients [76], although a correlation

between sepsis and infected bile was not demonstrated. In a comparative study between 373 jaundiced and 760 non jaundiced patients undergoing open procedures, Armstrong *et al* found that wound dehiscence was significantly more common in those with jaundice and this correlated to the presence of a post operative wound infection or intra abdominal sepsis [77].

Shigeta *et al* found that 24% of patients with obstructive jaundice undergoing hepatectomy had post operative bacteraemia, as compared with only 4% of non-jaundiced patients. The presence of bacteraemia increased the risk of multiorgan failure 5 to 10 fold postoperatively [71]. A combination of diabetes and obstructive jaundice may predispose to an especially high risk of septic complications following surgery [78]. Organisms commonly cultured include *Staphylococci, Enterococci, Klebsiella pneumoniae, Enterobacter* [71], *pseudomonas aeruginosa* and *proteus vulgaris* [79]

Studies comparing infection rates between benign and malignant obstructions have demonstrated infected bile in 71.6% of patients with benign disease and 31.3% of those with malignancy [76]. This proportion was also demonstrated by Armstrong *et al*, who found in a series of patients with obstructive jaundice, 60% with benign disease had positive biliary cultures but only 30% with malignant disease had infected cultures [80]. The presence of infected bile in benign disease correlated with age and the presence of biliary stones, which is perhaps not surprising as gallstones act as a nidus for bacterial colonization. Neve *et al* demonstrated similar rates of infected bile in malignant obstruction [79]. Post operative mortality across both groups was 14.2%, but sepsis was the cause of death in 2 patients (1.6%). Non-lethal

infections occurred in 13%, comprising wound infections, septicaemia, cholangitis and sub-phrenic abscesses [80]. All patients with septic complications had infected bile with identical organisms cultured from their point of focal infection.

In a group of patients undergoing elective cholecystectomy for chronic cholecystitis, evidence of bacterial translocation, as defined by positive peritoneal swabs, mesenteric lymph nodes, portal venous blood, liver tissue or bile cultures was only 3.5% as compared to a figure of 24% in those with obstructive jaundice undergoing surgery [4]. Intestinal expression of CD44, which is important for cell-cell and cell-matrix binding, is initially increased in rat models but becomes significantly decreased after 2 weeks of bile duct obstruction [81].

Sakrak *et al* found positive mesenteric lymph node cultures in 63.2% of patients with gall stone biliary obstruction compared to 14.3% of those with symptomatic cholelithiasis without obstruction [82].

Tight junctions permeability between hepatocytes is controlled in part by the hydrostatic pressure in the biliary tree [83]. Electron microscopy of human livers with extrahepatic cholestasis demonstrates alterations in the canaliculi and tight junctions [84]. The theory that bacterobilia is related to biliary tract pressure is supported by Lygidakis *et al* who examined the relationship between intrabiliary pressure and sepsis in human studies. The presence of proximal obstruction was associated with a higher intrabiliary pressure than in those with more distal obstruction. In turn, those patients with higher intrabiliary pressures had higher rates

of bacteraemia [85]. This observation has also been borne out by experimental animal models [86].

1.4.i.b: Cell-mediated immunity

T-cells are involved in the elimination of bacteria through the formation of cytotoxic T-cells, secretion of cytokines and activation of macrophages and natural killer cells (NK cells). The presence of obstructive jaundice has been shown to impair T cell-mediated immunity in rat models [87-91]. Human studies have shown both a decrease in T cell function [92] but also decreased number of T lymphocytes in mesenteric lymph nodes [93] . Internal drainage may be superior to external drainage in terms of recovery of T-cell function due to the presence of bile in the intestinal tract although in this rat study return of immunocompetence took up to 4 weeks following biliary drainage [89].

Depressed T cell function has been demonstrated by the prolonged survival of allogenic skin grafts placed in bile duct ligated rats. When jaundice is resolved by internal drainage survival of the skin grafts is reduced as T-cell mediated graft rejection occurs [94]. Direct causative factors for T-cell dysfunction remain unclear but factors such as hyperbilirubinaemia [95], bile acids [96], endotoxin [97] and abnormal lipoproteins [98] have all been proposed.

1.4.i.c: Humoral immunity

B cell function has been found to be unchanged in obstructive jaundice [99]. Increased concentrations of secretory IgA and circulating IgA have been demonstrated in both animal and human models of obstructive jaundice [100, 101].

1.4.i.d: Neutrophil function

Many of the functions of neutrophils are affected in the setting of obstructive jaundice. The recruitment of neutrophils to areas of inflammation has been shown to be impaired in cholestatic rats [102]. Addition of serum from bile duct ligated rats to neutrophils has been shown to impair bacterial killing [5]. Migration of neutrophils from the plasma into the interstitium first requires adhesion to vascular endothelium, and this process is also defective in the setting of cholestasis [103]. Expression of neutrophil cell surface adhesion receptors L-selectin, CD11a, CD11b, CD11c, and CD15 are reduced in obstructive jaundice and show an impaired response following a bacterial challenge [104].

Neutrophils, under certain conditions, can cause local tissue damage following activation, due to release of enzymes and reactive oxygen metabolites in situations such as sepsis [105] and trauma [106]. Jiang *et al* examined the neutrophil respiratory burst in jaundiced and non jaundiced patients and showed a larger oxidative response in obstructive jaundice [107]. The normal process of priming the neutrophils oxidative response (i.e. a state where neutrophils are activated in order to able to act upon a secondary stimulus) by TNF-alpha, IL-1, IL-6 and IL-8 is reduced in jaundiced patients. In this study the greatest increase in the respiratory burst was seen in patients with the worst outcome, suggesting this may be a factor in peri-

operative complications [107]. This increase in superoxide production is believed to correlate directly with liver damage following bile duct ligation [108]. However, conflicting results are documented with some studies demonstrating decreased production of superoxide in rat models of obstructive jaundice [5].

Neutrophil chemotaxis [109] and phagocytosis have been shown to increase in rats with obstructive jaundice, an effect seen within 12 hours of bile duct ligation in rats [108]. Conversely Roughneen *et al* found that there was no difference in chemotaxis in response to C5a between bile duct ligated, sham operated and control rats, although phagocytic ability was significantly depressed and superoxide production was increased in the jaundiced group [110].

1.4.i.e: Reticuloendothelial system and Kupffer cells

Hepatic Kupffer cells constitute the largest pool of macrophages in the body [100]. The reticuloendothelial system has an important role in the clearance of circulating bacteria, both in the systemic and portal circulations. Macrophages have roles not only in phagocytosis and antigen presentation, but also in production of cytokines, prostaglandins and free radicals [111].

Kupffer cell function is important in postoperative immune response [112] and up to half of patients with obstructive jaundice have evidence of decreased Kupffer cell activity preoperatively [113]. Despite reduced Kupffer cell phagocytic function in obstructive jaundice, the overall number of Kupffer cells increases [114, 115] although these cells may not be functional [116]. Pain *et al* suggest that this reduced

Kupffer cell function results in depression of the normal clearance pathways of endotoxaemia [117].

Following laparotomy, bile duct ligated rats have been shown to have significantly more organisms in lungs, liver, and kidneys further supporting a theory of decreased bacterial killing. In addition, not only are increased amounts of bacteria seen within tissue, but this study also demonstrated that these bacteria remained viable [118]. After a bacterial challenge in rodents, bacteria were seen by electron microscopy within Kupffer cell phagocytic vesicles in both sham and jaundiced animals [119] although only the bile duct ligated rats had persistent bacteraemia.

Depression of Kupffer cell function may be responsible for the overspill of endotoxin into the systemic circulation from the portal circulation [120]. Elevated levels of bile salts may have an effect on Kupffer cell membranes, pinocytosis and phagocytosis [113] and morphology [121, 122]. The production of nitric oxide in response of endotoxin is significantly increased in jaundiced rats and may play a role in hepatic and renal dysfunction [123].

Megison *et al* demonstrated that in bile duct ligated rats hepatic sequestration of radiolabelled *E. coli* is reduced, with a concomitant increase of pulmonary bacterial sequestration when compared to controls. They postulate that the increased bacterial load presented to alveolar macrophages may be overwhelming and contribute to increased infective rates [87]. Katz *et al* not only also demonstrated that a similar compensatory increase in pulmonary phagocytosis occurs due to the decrease in Kupffer cell function, but that bacterial survival is prolonged in the lung

as compared to the liver [94]. A similar decrease in hepatic trapping of organisms within the liver and a concomitant increase in pulmonary organisms has also been demonstrated in the rat model using candida albicans [124].

In rabbits with obstructive jaundice, impairment of reticuloendothelial phagocytic index is seen and remains low for up to 6 months after reversal of jaundice by cholecystojejunostomy [90]. However Megison *et al* found that in rat models phagocytic activity returned to normal only 7 days after internal biliary drainage [87]. This demonstrates the problems of both interspecies variation and differences seen in using different methods to assess phagocytic function.

A summary of the cellular responses seen in obstructive jaundice are shown below in Figure 1.4.

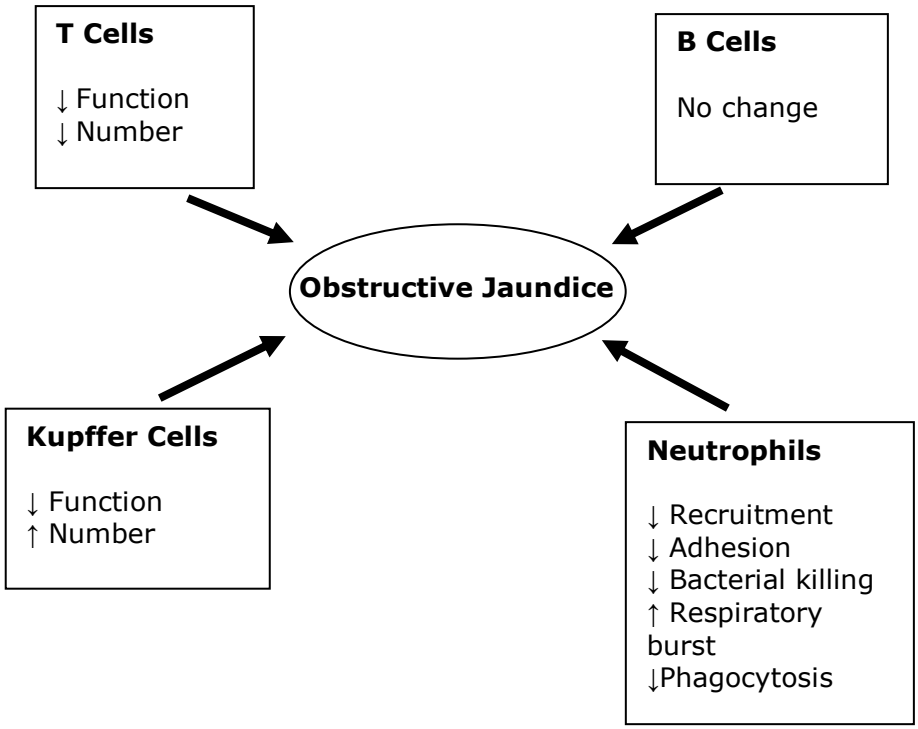


Figure 1.4: Summary of immune cellular changes in response to obstructive jaundice.

1.4.ii: Renal failure and obstructive jaundice

The development of renal impairment and renal failure in the settings of obstructive jaundice and hepatic failure (hepatorenal syndrome) is well described [125, 126], but as yet remains incompletely understood. Walters and Parham stated in 1922 that "Operations on patients with obstructive jaundice offer three avenues of danger, aside from so-called accidents of surgery: haemorrhage, uraemia and hepatic insufficiency" [127].

Following any surgical stress there are well-defined pathophysiological changes that come into effect such as the release of antidiuretic hormone, aldosterone, glucocorticoid and catecholamines. In the kidney these responses are directed toward the retention of fluid in the immediate post operative period, mediated by a number of factors but especially antidiuretic hormone (ADH). Post operative renal failure is a risk factor following any major operation and particularly high risk groups include those undergoing cardiac, vascular and major abdominal surgery and those with pre-operative renal insufficiency [128]. The presence of obstructive jaundice alone is a risk factor for the development of renal failure but this group is at especially high risk following a surgical intervention. The presence of a raised concentration of serum fibrinogen/fibrin degradation products, infection, hypoalbuminaemia and a low glomerular filtration rate has been associated with a poor post operative outcome [129].

As ever, wide variations in the definitions of both obstructive jaundice and renal failure hamper accurate evaluation of the incidence of renal failure in association with obstructive jaundice. In several published series, the incidence of

post operative renal failure in patients with obstructive jaundice ranges from 3%-18% [130-134]. The mortality reported from post operative acute renal failure also varies widely from 0% in some studies [130] to 100% in others [80, 133]. Mairiang *et al* [3] examined a cohort of patients with cholangiocarcinoma and found that 64 of 130 patients who developed postoperative renal failure had evidence of renal dysfunction preoperatively and that the development of renal failure was associated with severe jaundice, gram-negative infections, hypotension, hypoproteinemia, hyponatraemia, and hypokalaemia.

The presence of obstructive jaundice sets in motion a wide range of responses with the end result of renal hypoperfusion and subsequent dysfunction and these are discussed further below.

1.4.ii.a: Changes in renal physiology and fluid balance

Obstructive jaundice has been shown to result in a number of changes in renal physiology including decreased creatinine clearance, increased reabsorption of sodium and water in the proximal tubule, decreased urinary sodium excretion, increased renal prostaglandin production [135] and decreased glomerular filtration rate [129, 136] and renal blood flow. These changes in creatinine clearance and sodium excretion seen after bile duct ligation were not prevented by renal denervation [137]. However some authors have found no reduction in GFR in bile duct ligated rats, with a reduction in tubular sodium reabsorption [138].

Histopathological changes in the kidney are non-specific in the setting of obstructive jaundice and range from minimal histological change to acute tubular

necrosis, with peritubular and glomerular fibrin deposition [139]. Bile pigment may be seen accumulated in the cortex, and in particular the proximal tubules [140]. Changes in the proximal convoluted tubule seen on electron microscopy in bile duct ligated rats include increased pinocytotic organelles in the subapical cytoplasm of the proximal convoluted tubule, possibly due to an increase in bile acid transport [141]. Changes in the basement membrane with swelling of endothelial cells are also seen.

A small study of six patients with obstructive jaundice undergoing hepatobiliary surgery, dynamic 99mTc-DTPA renal scintigraphy done before and eight weeks after surgery, showed decreased glomerular filtration preoperatively in the jaundiced patients compared to the same number of control patients. Glomerular filtration then increased postoperatively in jaundiced patients, but decreased in control patients. Renal blood flow was reduced postoperatively in control patients, but not in jaundiced patients [142].

Alterations in the hormonal mechanisms of water and sodium regulation (high ANP, aldosterone and renin) is seen and are accompanied by a marked depletion of extracellular volume [143]. However, in the early stages of obstructive jaundice these changes in sodium handling appear to be independent of the renin-angiotensin-aldosterone system [135].

Long term common bile duct ligation in dogs is associated with enhanced sodium reabsorption both in the proximal and diluting segments of the nephron, defects in urinary concentration, diminished sodium content in the renal papilla, and impaired excretion of a water load. This suggests that decreased distal delivery of

sodium may underlie the abnormality in the concentrating mechanism and in the inability to normally excrete a water load. In addition, reduced antidiuretic activity may contribute to impaired water diuresis [144]. In this study the dogs were also shown to have hypotonic renal medullae, which may be contributed to by decreased sodium supply to the ascending loop of Henle, a decrease in absorptive capacity or washout of medullary solutes [144].

The percentage of blood flow to the cortex in obstructive jaundice is reduced, while the total medullary blood flow increases, which may be a factor in the washout of medullary solutes and impaired concentrating ability as described above [145]. This alteration in blood flow may be due to increased sensitivity to catecholamines in addition to enhanced alpha-adrenoceptor activity.

Proximal tubular dysfunction may be reversibly affected in obstructive jaundice at a functional level, manifested by the development of uricosuria, glycosuria, phosphaturia and increased excretion of alpha (1) microglobulin [146]. These tubular functional changes are mirrored by morphological changes in proximal renal tubules [147]. Water and electrolyte processing in the kidney is affected by sulphated bile salts via inhibition of the Na+ -H+ antiport in the brush border of proximal tubular cells [148].

Rodrigo *et al* [138] demonstrated that tubular effects in the rat model are more pronounced at day 7 compared to day 14, following bile duct ligation, suggesting the development of compensatory pathways after prolonged periods of jaundice. Indeed after 21 days of obstructive jaundice, tubular function was not

significantly different when compared to sham operated animals. These changes included a near doubling of urinary sodium and chloride excretion with a concomitant decrease in proximal and distal tubular reabsorption of sodium of about 50 and 40%, respectively.

Tajiri *et al* demonstrated that kidneys in obstructive jaundice are more susceptible to ischaemia-reperfusion injury [149] demonstrated by elevated creatinine and urea following an ischaemic insult after 5 days of bile duct ligation in the rat. While the method of producing ischaemia in this experiment i.e. clamping the renal vessels, is not commonly encountered in clinical practice, systemic hypotension and renal hypoperfusion is seen in the jaundiced patient and such hypoperfusion may be critical in ischaemically-sensitized kidneys.

In summary the renal physiological changes seen in obstructive jaundice are due to a combination of alterations in glomerular filtration and creatinine clearance, reduction in renal blood flow, tubular disturbances and ischaemia-reperfusion injury.

1.4.ii.b: Endotoxaemia and bacterial translocation

The presence of endotoxaemia in association with obstructive jaundice is thought to be a major contribution to the development of renal failure [150, 151] and post operative mortality [152]. Endotoxins are the lipopolysaccharide portions of the outer membrane of cell walls of gram negative bacteria. Their action is most likely to be mediated through the activation of the endothelial macrophage system and activation of cytokine cascades and the subsequent inflammatory response [153] rather than a directly toxic effect of endotoxin. They can also cause activation of

platelets, leucocytes, complement cascades and initiate intravascular coagulation [154].

Causes of endotoxaemia

Endotoxaemia has been demonstrated in both animal and human models of obstructive jaundice [155]. There are many factors which culminate in the development of endotoxaemia such as absence of bile and bile salts [156] leading to bacterial overgrowth and translocation [157], increases in intestinal permeability [158] and changes in Kupffer cell function leading to endotoxin entering the circulation.

Bile has been shown to inhibit the growth of gram negative bacteria and therefore a deficiency in the gut lumen may allow for relative bacterial overgrowth. Caecal levels of gram negative bacteria in bile duct ligated mice have been found by some to be increased a hundredfold in those with endotoxaemia compared to controls [157]. On the other hand alterations in caecal flora have not been demonstrated by other authors in rats [159] or in the small bowel in humans [160]. Loss of secretory IgA contained within bile may also play a role in prevention of bacterial translocation within the small bowel [161].

Administration of bile salts preoperatively has been shown to prevent both endotoxemia and reduction in renal function in humans [162] although the administration of the anti-endotoxin polymyxin B does not prevent endotoxaemia or postoperative complications [152]. Cholestyramine has been shown to reduce intestinal endotoxin levels [163] and its use in obstructive jaundice helps maintain

blood pressure, improves splanchnic blood flow, and prevents the fall in renal blood flow seen in bile duct ligated rats [164]. This protective effect has also been demonstrated by the enteral administration of bile to jaundiced patients [165].

The intestine, particularly the large intestine, is the prime source of such endotoxin via bacterial translocation [150], whereby enteric bacteria (or their breakdown products) traverse the mucosal barrier. The obstructed biliary tree may also be an important source of bacterial translocation and endotoxaemia, particularly following biliary intervention [166]. Positive bacterial cultures are seen in both bile and mesenteric lymph nodes in obstructive jaundice as compared to controls [82].

Levels of portal endotoxaemia are also increased in non-jaundiced patients undergoing surgery, but levels are increased to a greater degree when obstructive jaundice is present [167]. This may indicate either that in the setting of jaundice the levels of endotoxaemia developed are higher, or that the ability of innate systems to clear them is reduced. While haematogenous spread of endotoxin is well described one theory suggests that spread via the lymphatics is important in the development of systemic endotoxaemia [168].

Increased oxidative stress has also been demonstrated in intestinal epithelium, which may increase epithelial injury and endotoxin translocation [169]. Further increase in mucosal permeability is also seen after surgical intervention as compared to jaundiced patients treated by endoscopic drainage [170]. Exposure of the abdominal cavity to air, as occurs at laparotomy, promotes lipopolysaccharide

translocation, an effect that is not seen in laparoscopy where carbon dioxide is used for insufflation [171].

Effects of endotoxin on renal function

Although the presence of endotoxinaemia in obstructive jaundice is well documented, how does this relate to an increased risk of renal failure in the postoperative patient?

The presence of obstructive jaundice has been shown to sensitize rats to the effects of endotoxin [172]. Endotoxin increases renal vascular resistance due to intravascular coagulation and fibrin deposition [172] leading to acute tubular necrosis

One theory by Parks *et al* is of a ‘two-hit’ phenomenon, where the initial insult of obstructive jaundice primes the immune system so that any subsequent surgery causes an influx of endotoxin that causes a pronounced inflammatory response [170]. Similarly neutrophils from obstructive jaundiced patients demonstrate an increased oxidative response, which may be due to "pre-priming" in vivo by cytokines, such as IL-6, IL-8, or TNF alpha. Reduced clearance of endotoxin and enhanced TNF-alpha production may play a role in the development of renal failure [173]. This exaggeration of the oxidative response in circulating neutrophils may contribute to the peri-operative complications of patients with obstructive jaundice [107, 174].

1.4.ii.c: Free oxygen radicals

Recent studies suggest that the free oxygen radicals produced in obstructive jaundice may play a significant role in the development of acute renal failure [175], which may be more pronounced after a period of ischaemia reperfusion [176]. Studies on rats demonstrate that oxygen radical production by polymorphonuclear leukocytes is increased in obstructive jaundice as compared to controls [177].

1.4.ii.d: Nitric oxide

In vitro studies of blood vessels from bile duct ligated dogs, show that the contractile response to noradrenaline and serotonin were blunted when compared with controls, an effect that disappeared after removal of the endothelium. Endothelin derived relaxation in response to acetylcholine in vessels already contracted by phenylephrine was increased as compared to controls. This suggests that obstructive jaundice induces a decrease in vascular contractile responses and an increased nitric oxide relaxation response that may be caused by an increase in production of nitric oxide [178].

The number of cells expressing the inducible form of nitric oxide synthase (iNOS) in both hepatic and renal tissue is increased in rat models of obstructive jaundice [179]. This effect may be mediated by the endotoxaemia seen in obstructive jaundice [180]. Administration of lipopolysaccharide to non-jaundiced rats has been shown to increase iNOS staining in tissue macrophages in heart, lung, liver, and kidney. It is also increased in Kupffer cells and hepatocytes, biliary epithelium, mesangial cells, airway epithelium, and nerves supplying mesenteric blood vessels but not detected in vasculature [180]. This nitric peroxide thus produced can react

with oxygen free radicals, resulting in the formation of the peroxynitrite anion ($ONOO^-$), which causes lipid peroxidation and cellular damage [181] Impairment of mitochondrial respiration is mediated by a lipopolysaccharide induced increase in nitric oxide [182].

In vitro studies of canine vascular smooth muscle after bile duct ligation demonstrated enhanced production and/or release of nitric oxide, mainly of endothelial origin, and is associated with reduced vascular responses to contractile agents such as noradrenaline, acetylcholine and 5-hydroxytryptamine [183]. This effect may be mediated through impairment of guanosine cyclic 3',5'-monophosphate (cGMP) mediated vasodilation [140]. The production of such nitric oxide may also play a role in hypotension associated with obstructive jaundice.

1.4.ii.e: Endothelin and thromboxane

Endothelin is a potent renal vasoconstrictor and modulator of the tubular action of vasopressin and has been implicated in the development of acute renal failure. Bile duct ligation has been shown to increase plasma endothelin concentration and decrease glomerular filtration [184, 185] in animals and humans [186]. Renal vascular and inner medullary collecting-duct endothelin synthesis is also increased, which may reduce distal tubular water absorption in jaundiced rats. [184]. These authors have also demonstrated increased urinary excretion of thromboxane and glomerular thromboxane B2 synthesis, that may further affect renal function [187].

Administration of endotoxin to jaundiced rats is associated with significant elevations of plasma thromboxane B2 and early inhibition of plasma PGF1 alpha generation. Deposition of fibrin occurred in jaundiced kidneys following endotoxin and could be prevented using indomethacin, dazoxiben and prostacylin. This suggests that endotoxin-induced thromboxane B2 production can cause renal fibrin deposition in obstructive jaundice, which maybe a contributing factor in the development of renal failure [139]. Elevation of thromboxane in jaundiced rats has also been shown to reduce hepatic blood flow [188].

1.4.ii.f: Prostaglandins

Bile duct ligation in the rat doubles urinary excretion of prostaglandin E2, 6-keto-prostaglandin F1 and thromboxane B2. This renal response to increase prostaglandin production may be in response to counterbalance vasoconstricting substances [137]. This increase in urinary prostaglandin excretion is not affected by renal denervation [137].

In human studies of obstructive jaundice, levels of prostaglandin E2 and angiotensin II are significantly higher than normal controls. In patients whose creatinine clearance falls to normal after biliary drainage, prostaglandin and angiotensin II levels also fall. However in those whose creatinine clearance actually deteriorates following biliary drainage the levels of prostaglandin E2 and angiotensin II raise further, suggesting a link with the renin-angiotensin system [189]. However O'Neill *et al* have not been able to confirm the link between prostaglandins and the renin-angiotensin system, but were able to conclude that elevated prostaglandin levels modulate renal sodium excretion [135]. However the effect of prostaglandins

and the renin-angiotensin system on renal function in jaundice still remains unclear [135]. Decreased renal vascular responsiveness to noradrenaline seen in obstructive jaundice may be mediated by prostaglandins [190].

Production of renal prostaglandin may also help to counter the effects of vasoconstrictor substances and increased urinary prostaglandin excretion has been demonstrated in both animal models [137, 191] and humans [189] with obstructive jaundice.

1.4.ii.g: Elevated bile acids

Bilirubin [192] and bile salts [193] have been directly associated with renal failure in obstructive jaundice [148]. Levels of serum bile acids rise to a peak of twenty times normal value at 72 hours following bile duct ligation in rats, with a concomitant increase in the rate of urinary bile acid excretion [147].

Bile acids can alter the renal handling of electrolytes and water by blocking the Na+-H+ antiport in the tubule. Bile acids cause oxidative damage to tubular cell membranes by stimulating the generation of oxygen free radicals from mitochondria, as well as promoting their release from neutrophils and macrophages. Oxidative stress can promote the formation of a variety of vasoactive mediators which can each affect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient, and thus reduce the glomerular filtration rate. Collectively, these factors contribute to the onset of renal failure in patients with biliary obstruction [194]. Sulphated bile acids may cause direct

inhibition of the human renal Na+-H+ antiporter, which may be also contribute to post operative pH abnormalities and renal failure [148].

1.4.ii.h: Cardiac responses

Cardiac endocrine activity is increased in experimental obstructive jaundice in rabbits, although total cardiac output is reduced [136]. Plasma levels and the number of cardiac cells staining for ANP are increased in bile duct ligated rabbits and this may be involved in the pathogenesis of the renal and water and sodium metabolic disturbances [195].

Jaundiced patients show a reduced cardiac contractile response to inotropes. This refractory response of the myocardium to beta-1 stimulation may contribute to the susceptibility of jaundiced patients to postoperative shock and acute renal failure and is demonstrated in both human [196] and animal models [197].

Summary

The development of renal failure in obstructive jaundice results from a complex interplay between primary renal tubular and physiological dysfunction associated with the effects of endotoxaemia, oxidative damage and the action of vasoactive substances. The effects of surgical stress may exacerbate all of the above factors.

1.4.iii: Obstructive jaundice and metabolic effects

1.4.iii.a: Carbohydrates

Obstructive jaundice causes changes in the metabolism of carbohydrates both in human and animal models of bile duct ligation [198]. Hyperglycaemia may be a factor in determining survival following surgery for malignant biliary obstruction [199].

Reduction in both hepatic glycogen stores and skeletal muscle glycogen stores are seen [200]. There are conflicting studies as to the cause for this, with some authors finding an increase in glycogen phosphorylase activity [201] but others a reduction [200]. Glycogen synthase activity has also been found to be reduced, contributing to the decrease in hepatic glycogen content [200].

Reduced plasma levels of insulin are seen in bile duct ligated dogs [202] with a decrease in the insulin response to an oral glucose tolerance test also seen [203]. The reduction in serum insulin levels is due to a reduction in pancreatic production [202] and is accompanied by a decrease in the ability of insulin and glucagon to bind to hepatic cell membranes [204].

Plasma glucagon levels are increased in obstructive jaundice but glucagon-induced cAMP synthesis in the liver is reduced, and as cAMP is the second messenger responsible for hepatocyte proliferation, bile acid uptake and gluconeogenesis [205], this has important consequences for hepatic function and glucose control.

1.4.iii.b: Proteins

The liver is responsible for the production of a wide range and number of proteins. Protein metabolism and physiology in obstructive jaundice is affected by the underlying disease process causing the obstruction. In malignant conditions the development of an acute phase reaction causes changes in protein metabolism (not necessarily related directly to jaundice), such as the loss of muscle mass seen in tumour cachexia [206]. In biliary sepsis, and obstructive jaundice the change from anabolic synthesis to acute phase protein synthesis may be driven by endotoxin mediated production of tumour necrosis factor, interleukin 6 and nitric oxide [207]. Well established alterations in clotting profiles in obstructive jaundice are due to the reduced production of vitamin K dependent clotting factors [208].

Changes in amino acid levels are seen in both acute and chronic liver diseases [208]. The changes in obstructive jaundice are not clear, and minor but non specific elevations of methionine, phenylalanine, aspartic acid and glutamine have been reported [209]. Others have found elevations in levels of alanine and proline [210], while some authors have suggested that increased levels of ornithine and the simultaneous decrease of arginine suggests an inhibition of the urea cycle [211].

The production of collagen is also reduced in obstructive jaundice which may play a role in poor wound healing [212].

1.4.iii.c: Lipids

Absorption of fats in obstructive jaundice is reduced as expected due to the absence of bile in the intestine [213] while hyperlipidaemia is also commonly seen

[214]. Changes in lipid profiles include elevated serum cholesterol, lecithin, triglycerides and the appearance of lipoprotein X [208], while some have found triglyceride levels to be static but with reductions in levels of unsaturated fatty acids [213]. Other changes include large elevations of low density lipoproteins and marked reduction in high density lipoproteins [215]. The noted elevations in serum cholesterol have been thought to be due to an increased rate of hepatic synthesis [216] or reflux of biliary phospholipids from the biliary tree [215].

The clinical significance of these changes is unclear. Developments of cutaneous xanthomas are well documented in obstructive jaundice [208] but alterations in lipoproteins may also have a systemic effect on the permeability of cell membranes [217].

1.4.iv: Gastrointestinal effects of obstructive jaundice

The presence of obstructive jaundice affects the entire gastrointestinal tract. Mechanical and structural differences are seen throughout the intestine which affect both physiological functions and subsequent systemic manifestations, in particular the role of the intestine and endotoxaemia in obstructive jaundice.

1.4.iv.a: Stomach

Obstructive jaundice is associated with an increase in gastrointestinal bleeding both before and after a surgical procedure, [218-220]. The most common site of bleeding is the stomach, and the most common cause is the development of gastric erosions [220].

In jaundiced rats, a decrease in the basal output and volume of acid secreted is seen in addition to increased mucosal permeability. Normal flow of bile into the duodenum is thought to be required for correct functioning of gastric mucosal protective mechanisms [221]. While rats show decrease in gastric acid production, dogs however have been found to have increased gastric acid production in response to obstructive jaundice [222].

Increased levels of serum bile acids may have a directly toxic effect on gastric mucosa, with reductions in both gastric acid output and mucus secretion seen in rodents following intravenous infusion of bile acids [223]. This study also showed that following application of a stressor, the infusion of bile acids caused the development of gastric

ulceration earlier than in control animals [223], suggesting that operative stress may further increase the risk of ulceration.

One theory concerning the development of gastric ulceration is that a reduction of gastric blood flow occurs in obstructive jaundice, resulting in hypoperfusion of the mucosa. Prostaglandin E2 has a mucosal protective role and levels have been found to be reduced within gastric mucosa in obstructive jaundice, but returning to normal once biliary obstruction is removed [224]. Gastric mucosal noradrenaline is also reduced in jaundiced rats and after a physiological stress, mucosal blood flow falls, an effect that can be reduced by administration of noradrenaline [225]. This reduction in blood flow may be vagally mediated [226].

Systemic administration of endotoxin to rats causes increased mucosal permeability, resulting in lesions within the stomach and intestine and correlating with a disruption in mucosal architecture [227]. Conversely, other studies have shown an increase in the gastric mucosal resistance to injury following endotoxin administration, an effect which may be mediated via nitric oxide mechanisms rather than changes in perfusion [228].

Compared to the volume of published work examining gastric mucosal blood flow and gastric erosions in obstructive jaundice, there are few studies published on the effects of obstructive jaundice and gastric motility and emptying. Jaundiced neonates have delayed gastric emptying which resolves after photodynamic therapy [229]. Food intake in rats is initially reduced after bile duct ligation but this returns to baseline after a

few days and may be related to reduced responsiveness to centrally active substances (such as neuropeptide Y, which is important in central control of food intake) rather than reductions in gastric motility [230].

1.4.iv.b: Small intestine

Slow small bowel transit is seen in obstructive jaundice and may be regulated through increased activity of the endogenous opioid system [231]. Endotoxin may also have an effect on small intestinal hypomotility in jaundiced rats [232]. Rodent studies have shown prolongation of the migratory motor complex in the duodenum of rats which have been jaundiced for up to 3 days, which correlated with bacterial overgrowth and endotoxaemia [233].

There is conflicting evidence regarding the occurrence of bacterial overgrowth in the small bowel in obstructive jaundice. Some authors have found significantly raised levels of small bowel endogenous bacteria in bile duct ligated rats [234], whereas others have found no difference in intestinal flora found in either jejunum or ileum between bile duct ligated and sham operated rats [159]. Loss of intestinal IgA, which is secreted in bile, may aid increased bacterial adherence [235] although rat models may not be representative as over 90% of IgA is secreted through the bile [236].

Histological changes in the small bowel include loss of muscosal thickness and subepithelial oedema [157, 237]. Mucosal damage is caused by loss of villous height

[238], atrophy and in some cases complete mucosal loss with exposure of connective tissue and lymphatics to intestinal contents [169, 239].

In cases where intestinal mucosa appears normal on light microscopy, electron microscopy can demonstrate shortening of the villi, with damage to the mitochondria [240]. Parks *et al* [238] also showed formation of lateral spaces between enterocytes and disruption of desmosomes. Duodenal biopsies in jaundiced patients have shown atrophy of the mucosa without disruption of the epithelium but high levels of oxidative stress [241]. Increased rates of enterocyte apoptosis have also been demonstrated within intestinal crypts [242].

Increased intestinal permeability in the presence of obstructive jaundice has been demonstrated using the lactulose:mannitol ratio [158, 243]. Intestinal tight junctions are integral to the maintenance of mucosal integrity and increased mucosal permeability may be modulated through alterations in tight junction regulatory peptides [241]. Cytokines produced as a consequence of endotoxaemia, may have a directly toxic effect on the structure and function of intestinal tight junctions [244] and subsequent endotoxaemia. Occludin is an integral component of tight junctions and its expression on the intestinal epithelium is lost in obstructive jaundice and may be prevented by administration of neurotensin and bombesin, which are gut regulatory peptides [169], which have also been shown to reduce systemic and portal endotoxaemia. Integrins are involved in mucosal repair by facilitating cell-matrix interactions and enterocyte migration. Platelet activating factor is also reported to cause mucosal injury, levels of which are increased in the liver in obstructive jaundice in rats [245].

1.4.iv.c: Large intestine

The large intestine is also implicated in the development of bacterial translocation and endotoxaemia. Again conflicting results are present with some authors finding over a 100 fold increase in gram negative bacteria within the caecum [157] but with others finding similar overall numbers but with a broader spectrum of gram negative bacteria [159].

Endotoxin related shock has been shown to cause mucosal injury in the colon after 12 hours, with increased levels of intraluminal lactate that may play a role in producing epithelial injury [246]**.** However again other authors have not found mucosal changes in the large bowel either on light microscopy or electron microscopy [238] which may be related to the fact that mucosal lesions are very focal in nature and therefore may be subject to sampling error.

1.5: Obstructive jaundice and operative mortality

The increased risk of postoperative mortality in patients with obstructive jaundice has prompted attempts to find a system of stratifying the risk in such patients.

Pitiakoudis *et al* identified eight factors associated with increased postoperative mortality in 215 patients undergoing surgery for both benign and malignant biliary obstruction between 1998 and 2003. The factors identified are shown in table 1.5. This study had an overall postoperative mortality rate of 21.9% but did not specify the causes of death. They did however state that the presence of only one or two of the factors above was associated with a 100% postoperative survival but the presence of seven or more was related to a 100% postoperative mortality [247].

Pitt *et al* published a study of 155 patients, establishing risk factors in biliary surgery between 1976 and 1979. They identified eight variables out of 15 studied which were associated with increased risk of postoperative death. These were, the presence of malignant obstruction, age greater than 60 years, albumin less than 3g/100 ml, hematocrit less than 30 %, white blood cell count greater than 10,000/mm^3, total bilirubin greater than 10 mg/dL, alkaline phosphatase greater than 100 IU, and creatinine greater than 1.3 mg/dL. The presence of seven or more of these factors was associated with a 100% postoperative mortality, and five or more with increased major postoperative complications including death. The conclusion to this study was that in the presence of malignant biliary obstruction, and in the

presence of five or more risk factors, preoperative biliary decompression should be considered [134].

Risk Factor
Age > 70 years
Malignant disease
WCC >15000/μl,
Temperature > 38.5 °C
Haematocrit ≤30%,
Creatinine > 1.3 mg/dl
Albumin ≤ 3 g/dl
Bilirubin > 20 mg /dl
Alkaline phosphatase > 100 IU/l

Table 1.5: Risk factors associated with increased postoperative mortality in patients with benign and malignant biliary obstruction [247].

Dixon *et al* examined 373 cases of patients with obstructive jaundice and using univariate and multivariate analyses found that an initial haematocrit of 30% or less, an initial plasma bilirubin of greater than 200 μmol/l and a malignant obstructing lesion were independent risk factors for post operative mortality. If two or three of these were present then postoperative mortality was in the region of 33% and for this reason the authors suggested considering preoperative biliary drainage in this group [131]. This study had a 9.1% postoperative mortality rate which was not associated with the type of surgery performed. Blamey *et al* also found obstructive

jaundice to be independently associated with postoperative mortality. In their series, which included both benign and malignant diseases, there were 89 jaundiced patients (1977-1981) and the operative mortality was 23.6% [248].

The three studies by Pitiakoudis, Pitt and Dixon all found that malignant disease, low haematocrit and elevated bilirubin were associated with increased rates of postoperative mortality.

Lacaine and Han *et al* both proposed complex formulae based on multivariate analyses of preoperative variables in malignant biliary obstruction. These formulae both resulted in either a 'positive' or 'negative' number and they proposed surgical or palliative treatment based around this figure [1, 249]. In the study by Han the duration of jaundice rather than the level was the discriminating factor although Su *et al* did not find that duration of jaundice (greater or less than one month) increased risks of surgery in malignant obstruction [250].

There are difficulties in extrapolating the studies described above to current clinical practice. Mortality following major surgical resection is low, ranging from 1-5.8% for pancreaticoduodenectomy [13, 251-253], which reflects improvements in perioperative care. In addition there is lack of uniformity across studies with arbitrary cut off values being applied to the studied variables.

While the above studies focus principally on extrahepatic biliary conditions, postoperative mortality is also increased following hepatic resection for conditions such as colorectal metastases, cholangiocarcinomas and hepatocellular carcinomas in

patients with preoperative jaundice [2, 254]. In liver transplantation a history of preoperative jaundice is associated with an increased postoperative mortality on univariate analysis. However after multivariate analysis only the date of surgery was significant i.e. surgery performed most recently had a better outcome than those performed 10 years ago, supporting the theory that with improved supportive techniques operative survival increases regardless of premorbid conditions [255].

In a study of 295 pancreaticoduodenectomies between 1999 and 2005, Cheng *et al* did not find a correlation between obstructive jaundice and postoperative mortality [256], although they routinely performed biliary drainage when bilirubin levels were greater than 10mg/dL.

Summary

Surgical mortality in jaundiced patients is increased in the presence of higher levels of bilirubin, a low haematocrit or the presence of malignant disease.

1.6: Methods of biliary drainage

The use of preoperative biliary drainage is attractive for both its theoretical physiological advantages and logistical advantages. Drainage may allow recovery of the pathological and pathophysiological changes described in previous sections and may improve postoperative recovery. Biliary drainage also allows for greater flexibility in the logistical task of planning the timing of definitive surgery in the jaundiced patient. Two main approaches are employed to achieve drainage namely internal and external biliary drainage.

1.6.i: Internal biliary drainage

Internal biliary drainage restores continuity of the enterohepatic circulation by drainage of the occluded biliary system into the duodenum. Endoscopic placement of a plastic biliary stent at ERCP is the most common method although metal stents can also be placed endoscopically or percutaneously. Plastic stents are generally used for benign disease or malignant disease when surgical resection remains an option, with metallic stents reserved for palliation of malignant obstruction. The use of covered metallic stents has been described as an option for benign biliary obstruction [257] as an alternative to surgical management. Placement of any type of biliary stent can also be achieved in an antegrade fashion by the transhepatic route, particularly when endoscopic placement is not possible due to duodenal stenosis or ampullary distortion due to tumour. Finally, combinations of percutaneous and endoscopic approaches (PTC/ERCP) can be used for the placement of plastic stents to reduce the size of catheter required for the transhepatic component.

Complications seen with endoscopic drainage are not uncommon and include those seen with any diagnostic or therapeutic ERCP. These include problems occurring at the time of the procedure, such as haemorrhage and more rarely perforation of the duodenum [258]. Pancreatitis is perhaps the most common complication and occurs in 3.9-7% of diagnostic and therapeutic procedures [259, 260] although the differences in definition of pancreatitis makes comparisons between studies difficult. Between 25-40% of patients will have elevated serum amylase following ERCP [261, 262], although opacification of the pancreatic duct has been shown to cause hyeramylaseaemia in 75% of patients in one study [263]. However elevated amylase levels in the absence of clinical signs of pancreatitis are not generally considered significant [263].

Instrumentation of an obstructed biliary tree may cause cholangitis in up to 5% of patients [264]. Sedation is usually required in order to undertake ERCP and this can precipitate cardiorespiratory complications, particularly in the elderly and frail. Mortality rates of 1% have been reported in larger series of ERCP [264] which were attributed to haemorrhage, perforation, pancreatitis and cardiorespiratory complications.

Other complications seen with biliary stents include stent occlusion and migration. Complications such as intestinal obstruction and perforation have been reported as a result of such stent migration [265]. Rates of occlusion of plastic stents of up to 30% at 3 months have been reported [266], while metal stents have median patency rates of up to 9 months [267]. In a study of malignant hilar obstruction, metallic stents were associated with fewer complications such as cholangitis,

migration, perforation or the need for reintervention compared to plastic stents [268]. Metallic stents are either 'uncovered' where the metallic mesh is in contact with the lumen or 'covered' where a coating such as silicone is used in order to try to prevent tumour ingrowth. A randomised controlled trial of covered versus uncovered stents in malignant obstruction demonstrated significantly reduced rates of tumour ingrowth in covered stents but no overall difference in survival between patients treated with a covered or uncovered stent. Other authors however have not found improved patency with covered stents versus uncovered stents for malignant biliary obstruction [269]. Cholecystitis was seen in the covered stent group and the authors postulate this may be a complication peculiar to covered stents [270].

Expandable metal stents are by their nature difficult to remove, although the easier removal of covered stents endoscopically has been reported [271]. In this study all covered stents could be removed quickly at ERCP but no uncovered stents could be removed. Treatment of occluded stents with insertion of a second stent (stent-in-stent) can be performed with either a second covered or uncovered stent or with a plastic stent, although longer term patency may again be seen with covered stents [272].

The placement of an internal biliary stent creates structural changes in the common bile duct. Wall thickness is increased and histological changes of fibrosis, submucosal gland hypertrophy and inflammatory cell infiltration are seen [273, 274]. These changes can make biliary dissection more challenging and also makes clinical assessment of what is tumour or inflammation essentially impossible. However this aspect is difficult to define and quantify experimentally.

1.6.ii: External biliary drainage

Endoscopic drainage may be difficult near the confluence of the hepatic ducts, with duodenal compression or where large ampullary cancers compress the ampulla [275]. External biliary drainage using percutaneous transhepatic cholangiography (PTC) achieves biliary decompression with external collection of bile. This approach can be used for the antegrade insertion of an internal stent with or without simultaneous endoscopic assistance. Sedation is again usually required as dilatation of intrahepatic biliary tracts can be extremely painful.

Procedural complications include those seen with internal drainage, including haemorrhage, visceral perforation and haemobilia [276]. Infective complications of cholangitis and bacteraemia are seen in up to 13% of patients even with the use of prophylactic antibiotics [277]. Complications such as pancreatitis, cholecystitis and pleural effusion are also reported with mortality rates related to PTC of 2% [278]. Pneumoperitoneum has been described and may be treated conservatively [279].

Catheter dislodgement is a serious complication as this can result in bile leak and biliary peritonitis. As the biliary system is then collapsed, treatment of this complication by percutaneous reinsertion of a second catheter is difficult if the intrahepatic ducts are not dilated and ERCP may not be possible. In this situation bile will continue to leak into the peritoneum and laparotomy may be required. Implantation of cutaneous metastases and intrahepatic implantation along the catheter tract have also been reported [280].

1.6.iii: The relative merits of internal and external biliary drainage

Sewnath *et al* [19] published a meta-analysis of 23 papers which had been published between 1982 and 2001. These papers compared internal and external biliary drainage compared to no drainage prior to operative management. The authors concluded that there was no difference between mortality and post operative hospital stay between drained and undrained patients but also that preoperative biliary drainage was associated with significant increase in complications from the drainage procedure itself.

Five papers were randomised controlled trials [14-17, 281] but the total number of patients across these studies were relatively small with 157 undergoing biliary drainage and 155 having no biliary drainage. Eighteen papers were non randomised, eleven of which were prospective cohort trials and the remaining seven were retrospective cohort studies. In total 3365 patients were included across all studies with 1845 undergoing some form of preoperative drainage and the remainder proceeding directly to surgery. The papers used in this study are discussed further below.

1.6.iii.a: Randomised trials

Randomised controlled trials considered both internal and external drainage methods compared with no preoperative drainage. Internal drainage was carried out in 41% of drained patients and 59% had external biliary drainage. Only one study by Smith *et al* [14] found that external biliary drainage reduced postoperative complications but significant morbidity from the drainage procedure itself was seen.

McPherson *et al* terminated the study due to a high number of complications related to external drainage [16].

The drainage group had a 27.4% complication rate as a direct result of the drainage procedure and 33.8% developed stent dysfunction leading to cholangitis in 9% of patients. The total length of stay was naturally increased in those undergoing preoperative biliary drainage but the postoperative length of stay was the same.

After excluding complications related to drainage procedures, postoperative mortality was no different between the drained (internal and external combined) and undrained patients (10.8% drainage vs 13.5% no drainage). The main difference seen between the two groups was that the rate of postoperative complications (excluding those not related to the drainage procedure) was significantly higher in the non drainage group compared to those patients who did undergo preoperative biliary drainage (41.9% vs 29.9%).

1.6.iii.b: Non randomised trials

As might be expected this group had a far greater number of patients with 2853 in total and 1688 (59%) having preoperative biliary drainage. Internal drainage was performed in 66%, external in 29% and a combined approach in 5%. Complications related to the drainage procedure were seen in 10% with a reported stent dysfunction rate of 26%, which is lower than the randomised trials.

The meta-analysis of this group showed no difference in either postoperative complications, overall death rate, or post operative death rates between the drained and non drained groups.

Within these studies, Sohn *et al* [282] published one of the largest retrospective studies involving 567 patients undergoing pancreaticoduodenectomy. Patients were drained either externally or internally but further analysis grouped both types together. Overall postoperative morbidity and mortality was the same between those having preoperative drainage and those having no drainage, although preoperative biliary drainage did increase the rates of wound infection and postoperative pancreatic fistulae (both 10% vs 4%, p=0.02).

In comparison however, Povoski *et al* [283], in a study of 240 patients using both internal and external drainage found that preoperative drainage was associated with increases in all complications, infectious complications, intraabdominal abscesses and post operative death. Postoperative mortality in patients having undergone stenting was 7.9% vs 1.8% in those who proceeded directly to surgery.

1.6.iii.c: Studies since 2001

Since 2001 a further eight papers comparing preoperative biliary drainage with no drainage have been published and are summarised in Table 1.6. No further randomised controlled trials examining preoperative biliary drainage have been published.

Authors	N=	Number drained (%)	Study type/duration	Drainage Methods	Difference in postoperative mortality	Post operative morbidity (Significant difference only)	Drainage complication
Bhati [284]	48	21 (43.5)	Retro 10 years	Internal	NS	Increased sepsis, wound infection and minor bile leak in biliary drainage	None stated
Tsai [253]	313	210 (67)	Retro 13 years	Internal	NS	No difference	5.7% drainage related complications
Barnett [285]	104	50 (48)	Prosp 12 years	Internal	NS	No difference	None stated
Gerke [286]	72	58 (80.5)	Prosp 7 years	Internal (n=53) External (n=5)	NS	No difference	None stated
Hodul [13]	212	154 (72.6)	Retro 6 years	Internal	NS	Increased wound infection post drainage	Longer operative times and blood loss with stent
Pisters [251]	300	172 (57.3)	Prosp 9 years	Internal	NS	Increased wound infection post drainage	None stated
Srivasta [287]	121	54 (44.6)	Retro 9 years	Internal (n=50) External (n=4)	NS	Drainage – increase in general septic complications, intraabdominal abscesses, wound infection and pancreatic leak No drainage – higher relaparotomy rate and increased bleeding	None stated
Martignoni [252]	257	99 (38.5)	Retro 9 years	Internal	NS	No difference	None stated

Table 1.6: Published studies since 2001 comparing drainage vs no drainage in patients undergoing Whipple's procedures.
NS= no significant difference. Retro= retrospective study. Prosp= prospective study

One of the limitations when comparing studies is that only a few have documented protocols for preoperative biliary stenting, except for the occurrence of sepsis or cholangitis. Sano *et al* published a series of 102 hepatobiliary resections for perihilar cholangiocarcinoma and directly stated their criteria for preoperative drainage [288]. The criteria for biliary drainage required bilirubin levels >3mg/dl, the presence of dilated ducts (>7mm) in the future liver remnant and treatment of segmental cholangitis. While their overall postoperative mortality rate was zero and the only variable related to postoperative complications was the presence of preoperative cholecystitis or cholangitis the authors do not specifically state whether those patients being drained had higher complication rates compared to those not drained.

As can be seen the Table 1.6, most patients in recent studies have been drained internally preoperatively. The percentage of patients in these studies undergoing preoperative biliary stenting ranges from 38.5% [26] to 80.5% [22]. No differences were seen in postoperative mortality in any study; however in some no mortality at all was recorded and in others was too low to allow meaningful statistical analysis. This compares quite differently with many of the studies discussed by Sewnath *et al* where average mortality ranged across all studies from 1.8-13.5%. Again the main difference seen is that several studies reported increases in minor postoperative complications such as wound infection [13, 251, 284, 287].

The study by Srivasta *et al* report increased postoperative complication rates in preoperatively drained patients compared to non drainage. However the complication rates seem high compared to other studies with biliary drainage having

higher complication rates from wound infections (43% vs 24%), intra-abdominal abscesses (28% vs 15%), pancreatic anastomotic leak (20% vs 4%) and relaparotomy rates (27% vs 13%). Hodul *et al* [13] found that perioperative bleeding and operative times were longer with preoperative drainage.

1.6.iii.d: Discussion of published papers

In the meta-analysis by Sewnath *et al* there was no distinction made between type of drainage in terms of assessing overall morbidity and mortality. Only Sohn *et al* looked at whether internal or external was superior and concluded there was no difference [282]. Variations in study design, site of pathology, reporting of complications and small group sizes (particularly in the randomised studies) can make conclusions difficult to draw. Care should be taken to separate curative resections and palliative procedures as direct comparison between the two conditions is fraught with difficulty. The age of many of the studies may also not reflect the modern approach to pancreaticobiliary surgery with perioperative care now such that morbidity and mortality is expected to be lower than range of 10.8-13.5% seen in the randomised trials considered in Sewnath's meta-analysis [289]. In terms of the method of drainage it is clear looking at the more recent publications that internal drainage is the most common modality and is used when feasible.

While Sewnath *et al* helped to summarise the available data the authors acknowledge that conclusions are difficult to draw from their data and that no high quality randomised trials have been published to look at preoperative biliary drainage. The DROP trial (DRainage vs OPeration) [290], is a multicentre randomised controlled trial currently aiming to recruit 210 patients to evaluate

preoperative internal drainage versus no drainage. Patients with peri ampullary cancers who are suitable for curative resection will be randomised to early surgery (within one week) or four weeks of preoperative biliary drainage. The primary outcome measure will be the number of severe complications within 90 days of surgery. Others factors that will be considered include length of stay, number of invasive procedures, cost and quality of life.

Summary

Morbidity and mortality following major resection for pancreatic adenocarcinoma with or without preoperative biliary drainage remains low. Currently there is no evidence that preoperative biliary drainage prevents postoperative complications.

1.7: Pathophysiological changes associated with biliary drainage

The pathophysiological effects of biliary drainage on immunity, biliary bacterial colonisation, gut mucosal barrier, albumin and changes in hepatic parenchyma are discussed below.

1.7.i: Changes in immune function

Depression of T cell function [88] and its recovery after biliary drainage has been demonstrated in both animal [9, 87, 89] and human [91, 291] studies.

Conflicting results have been found in recovery of T lymphocyte function between internal and external biliary drainage in the rat model. Mizuguchi *et al* [91] found that after 14 days of biliary obstruction T cell function improved more quickly after 7 days of external drainage compared to internal drainage. Thompson *et al* [89] however showed that after 21 days of obstructive jaundice, 14 days of internal drainage was superior to external biliary drainage in respect of the recovery of T cell function, but also that 28 days of internal drainage was needed to restore this to normal.

This finding may be due to the longer duration of jaundice used in the study by Thompson *et al* and this finding is confirmed by another study with rats by Mok *et al* [9]. They showed that after two weeks of jaundice, T cell function recovered to normal levels after two weeks of either internal or external biliary drainage. After 3 weeks of jaundice however T cell function did not return to normal after two weeks

of drainage, although internal drainage produced a greater response compared to external drainage.

However in contrast to animal studies in a human study of 59 patients with malignant biliary obstruction, Fan *et al* [291] did not find a similar improvement in T cell function in patients having preoperative biliary drainage. In fact after 14 days postoperatively more patients had deteriorating T cell function than those showing improvement, and this deterioration was associated with increased post operative sepsis.

Kupffer cell function is improved in rats after 21 days of biliary drainage and this effect is greater in internal drainage compared to external biliary drainage [292]. Increased Kupffer cell production of superoxide [293] in addition to iNos mediated production of nitric oxide in rats is completely reversed with internal drainage but only partially so in external biliary drainage [294].

1.7.ii: Renal function

Improvements in renal function following both internal [295] and external [296, 297] biliary drainage in patients has been well described.

McPherson *et al* cautioned that this improvement may be due to routine administration of fluids in jaundiced patients and not due to the drainage procedure itself [295]. Padillo *et al* showed that administration of 3 litres of saline in the 24 hours prior to internal biliary drainage increased both urine output and creatinine clearance in the first 24 hours [298]. However this effect was lost after 24 hours at

which point creatinine clearance fell and then took a week to recover to baseline levels. They stated that restoration of intestinal bile was necessary for recovery of renal function although this cannot account for the improvement in renal function seen in external biliary drainage.

Recovery of the extracellular fluid compartments and a fall in ANP levels is seen in internal biliary drainage [299].

1.7.iii: Endotoxin

Changes in endotoxin in obstructive jaundice and at the time of biliary drainage vary between studies. In rodent models elevations in portal and systemic endotoxaemia seen after biliary obstruction can be reduced with both internal and external drainage [300]. However in this study intestinal mucosal changes, such as loss of villous height, were more marked in the external drainage group, perhaps predisposing to further endotoxaemia. Others have not found similar advantages of internal drainage in reducing levels of portal endotoxin in animal models [301].

Parks *et al* found no change in peripheral endotoxin levels between jaundiced patients and controls, either before or after operation or drainage (internal and external), or at any time point after intervention [170]. In other human studies of malignant biliary obstruction, systemic endotoxin levels were seen to be significantly lower 10 days after a biliary bypass, compared to external biliary drainage [302]. Kimmings *et al* also found no elevations in systemic endotoxin levels prior to drainage, or any change in this after internal drainage and suggested animal and human models were not comparable. They did however find a large increase in

endotoxin binding proteins in jaundice, levels of which reduced with internal drainage [303].

1.7.iv: Cytokines

Kimura *et al* found increased levels of IL-6 in patients with obstructive jaundice that were decreased with external biliary drainage. They also found a correlation between high levels of IL-6 and hypoalbuminaemia [304]. Padillo *et al* found that in a small number of patients with a combination of benign and malignant disease elevated levels of IL-6 and TNF alpha were reduced in patients following internal drainage but these levels increased again at day seven, perhaps reflecting bile reflux and biliary sepsis [305].

Kimmings *et al* conversely found no elevations of IL-6, TNF or IL-10 but did find elevations of IL-8 which decreased with internal drainage [303]. Rodent models have consistently demonstrated superiority of internal drainage over external biliary drainage in reduction of serum levels of endotoxin, TNF alpha and IL-6 [115].

1.7.v: Changes in bacterial culture of bile/ infection

As discussed in previous sections, preoperative biliary drainage has been associated with a trend towards increased post operative infection. Biliary sepsis occurs more frequently where biliary instrumentation or a drainage procedure has taken place. Bile may also become infected even when no biliary drainage takes place with bacteria such as *Enterococcus* and *Escherichia coli*, but instrumentation may increase the types of bacteria seen with multiple organisms isolated [306]. Up to 85% of patients with preoperative ERCP and stent have been found to have

bacterobilia, compared with 40% of those proceeding directly to surgery. Fungal infection is also increased with 34% of drained patients being infected compared to 8% proceeding directly to surgery [307].

1.7.vi: Changes in gut barrier function

One marker of gut permeability is the lactulose:mannitol ratio where urinary excretion ratios of lactulose and mannitol are measured. Lactulose is absorbed via the intercellular route and mannitol via the transcellular route. Therefore a higher lactulose:mannitol ratio suggests increased permeability and is seen in obstructive jaundice. Following drainage the ratio falls to normal by 28 days whether the obstruction is treated with definitive surgical management, or internal or external drainage [170]. However in the post operative period, these authors found that the ratio was significantly increased from day 1 to 7, suggesting increased permeability related to surgical stress.

Within the rats small bowel, reduced levels of T cells are seen within the lamina propria in obstructive jaundice. With internal drainage for one week, T cells increase towards the normal range but this increase is not seen with external drainage [308]. This study also found a fall in levels of T cell adhesion molecules within the venules of the lamina propria, and also that levels only increased after internal drainage. This suggests that bile may be required for the necessary expression of adhesion molecules to allow lymphocyte adhesion within the intestine. Refeeding of bile in patients having PTC drainage has been shown in the clinical setting to restore normal intestinal permeability [309].

1.7.vii: Albumin

In rat models the duration of obstructive jaundice is related to the degree of hypoalbuminaemia. Two weeks of internal drainage returned levels to normal in rats that were previously jaundiced for either two or three weeks. External drainage for two weeks only returned albumin to normal in rats with a duration of jaundice for less than two weeks [9].

Protein calorie malnutrition is common in humans with obstructive jaundice and while evidence for recovery of albumin after drainage is less abundant than animal models [10, 310] Padillo *et al* did find improvements in levels of visceral prealbumin with internal drainage [310]. Fan *et al* found that following internal drainage, recovery of T cell function in rats is directly correlated with increased albumin levels [291]. Isla *et al* found that biliary infection was related to the presence of hypoalbuminaemia in patients undergoing internal biliary drainage [311].

1.7.viii: Changes in hepatic parenchyma

Histological changes associated with biliary obstruction include cholestasis, intracytoplasmic inclusion bodies and loss of cannalicular microvilli with cannalicular dilatation [312]. Some authors found that in patients with pancreatic cancer, 14 days of biliary drainage did not significantly alter these changes [312]. However Mok *et al* found that after 2 weeks of bile duct ligation liver parenchyma returned to normal after 14 days of either internal or external drainage. After 3 weeks of bile duct obstruction however, the liver still showed evidence of widened portal tracts and bile duct proliferation and variable inflammatory cells. Following two weeks of drainage changes were reversed and there were differences between those

drained internally or externally [9]. This suggests that the period of jaundice prior to drainage is important in recovery of hepatic histology.

Mizuguchi *et al* used the histological activity index to evaluate internal and external drainage in rats. After 7 days of drainage the index was much lower with external drainage compared to internal drainage (0 vs 6 out of a total possible of 18), reflecting reduced levels of fibrosis, necrosis and portal inflammation in the external drainage group [91]. The authors postulate that this marked difference may be due to problems with blockage and poor tube drainage in the internal drainage group. However Knodell's scoring system as used in this paper is for the classification of chronic active hepatitis [313] and not changes due to biliary obstruction. In fact no dedicated histological scoring system is available to compare changes seen in biliary obstruction.

Following hepatectomy, Mizuto *et al* and Suzuki *et al* demonstrated that hepatic regeneration was more rapid in rats undergoing preoperative internal biliary drainage compared to those having external or no biliary drainage and postulated that an intact enterohepatic circulation was important for optimal hepatic regeneration [314, 315]

1.7.ix: Recovery of hepatic function

Standard biochemical measurement of liver function tests do return to normal after biliary drainage but may not always reflect the recovery of hepatic cellular metabolism and function.

Koyama, in 1981, suggested that due to the prolonged time needed for recovery of hepatic mitochondrial function, drainage should be performed for at least 4-6 weeks prior to surgery and for longer than this where obstruction has been present for 12 weeks or more [316]. Other authors have similarly found a correlation between duration of obstruction and time needed for hepatic mitochondrial recovery [317]. 31-phosphorus magnetic resonance spectroscopy can be used as a measure of the use of phosphorous in cells and therefore organ energy metabolism. In patients with obstructive jaundice, hepatic energy measurements are reduced and improve after drainage, with no difference between the internal and external routes in one study [318].

1.8: Albumin and operative mortality

The negative impact of preoperative hypoalbuminaemia on postoperative morbidity and mortality in biliary tract surgery has been demonstrated in many studies [134, 247, 248, 250, 319, 320]. Su *et al* even suggest increasing serum albumin to over 3g/dL is the most important factor in reducing postoperative mortality in patients undergoing resection for hilar cholangiocarcinoma [250].

Hypoalbuminaemia is also associated with increased post operative mortality and morbidity in benign and malignant colorectal disease [321, 322], major lower limb amputations [323] and oesophagectomy [324]. Very low albumin levels (<20g/l) on the first postoperative day following oesophagectomy is associated with a longer operating time and mortality and increased rates of further surgery and return to ITU [325]. Hypoalbuminaemia prior to lung resection has been shown to have either no effect [326] or increased risk of postoperative complications [327]. Similar conflicting results are reported in patients undergoing resection for gastric cancer with some authors finding no effect of low BMI or hypoalbuminaemia on mortality and morbidity [328] while others have demonstrated a significant correlation [329].

Hypoalbuminaemia prior to cardiac surgery is associated with increased rates of mortality in some studies [330], although Rich *et al* found no increase in mortality in elderly cardiac surgery patients but did show that hypoalbuminaemia was associated with an increased risk of postoperative complications and a prolonged hospital stay

[331]. This association has also been described following surgery for hip fractures [332, 333].

As stated previously, hypoalbuminaemia may develop as part of the development of a systemic inflammatory response. The Glasgow Prognostic Score (GPS) is a measure of inflammation using a combination of elevated CRP (>10 mg/l) and hypoalbuminaemia (<35g/l) to give a score of 0 to 2 [334]. Higher scores have been shown to have a negative correlation with mortality in patients with inoperable [335, 336] and metastatic [337, 338] cancer, but it also has a correlation with lower median survival in patients following elective colorectal surgery [339]. In patients undergoing a palliative bypass procedure for inoperable pancreatic cancer a high GPS is associated with increased mortality independent of TNM classification [340].

Hypoalbuminaemia is associated also with increased mortality following pancreaticoduodenectomy [341, 342]. Shirahatti *et al* suggested that in those patients requiring palliative bypass procedures the presence of hypoalbuminaemia, anaemia and obstructive jaundice should prompt the use of non operative measures due to the higher risk in these patients [343]. Patients with potentially resectable pancreatic cancer and preoperative cachexia associated with hypoalbuminaemia have higher mortality rates and a higher prevalence of previously undetected metastatic disease at the time of resection [344], compared to those with normal preoperative albumin levels. The presence of hypoalbuminaemia one month after a potentially curative pancreaticoduodenectomy is associated with a longer operative time and length of hospital stay in addition to increased mortality 5 years following resection [345].

Hypoalbuminaemia in patients undergoing liver resection for benign and malignant disease is also associated with an increased rate of postoperative mortality [346] and morbidity [347]. Long term survival after liver resection for hepatocellular cancer is inversely associated with preoperative albumin levels [348, 349].

Foschi *et al* studied malnutrition in jaundiced patients with both benign and malignant conditions. Measures used to assess malnutrition included the degree of weight loss, serum transferrin, albumin, triceps skinfold thickness and impairment of delayed hypersensitivity skin tests. Triceps skin fold thickness and delayed skin hypersensitivity were present in 70% of patients but only 40% of patients had hypoalbuminaemia. Hyperalimentation using either TPN or enteral nutrition reduced both post operative morbidity (47% to 19%) and mortality (12.5%-3.5%) but did not objectively raise serum albumin levels [350]. In hypoalbuminaemic patients following aortic aneurysm or aortoiliac repair, albumin replacement did not hasten the return of normal gastrointestinal function [351].

Albumin levels have also been shown to be predictive of long term cardiovascular risk. The British Regional Heart Study followed 7735 men over ten years and found lower levels of albumin to be associated with increased mortality from cardiovascular disease as well as cancer and that this was independent of age, social class and premorbid conditions [352]. The inclusion of albumin in the APACHE (Acute Physiology and Chronic Health Evaluation) scoring system only occurred in the third revision (APACHE III), which is felt to be a more sensitive predictive of outcome [353] in comparison to previous versions.

Summary

Hypoalbuminaemia is a significant factor in the development of postoperative morbidity and mortality not only in hepatobiliary surgery but also following surgery for a wide range of conditions.

1.9: Animal models of obstructive jaundice

The rat model of obstructive jaundice is the one which has been the most extensively used. Other models have been described but in significantly smaller numbers. Small animals are used almost exclusively with few published large animal studies. It has been suggested that the reason large animal models are not used is due to a higher rate of fistulisation and recanalization [354].

A Medline search for the number of citations using animal models of obstructive jaundice showed the most commonly used animal is the rat, followed by dogs, rabbits, mice and non-human primates.

The use of animal models is always fraught with difficulties, especially when attempting to draw comparisons with the situation in man. Indeed, variations between animal species and even between different breeds of animals make interpretation difficult. Study designs themselves can also alter the outcome, a good example being the effect of anaesthesia on renal [355] and hepatic function.

1.9.i: Rats

The rat is the most widely used model of obstructive jaundice. The rat does not have a gallbladder, whereas mice, rabbits, dogs and primates do. This means that gallbladder complications arising from the creation of obstructive jaundice (such as empyema or perforation) do not occur.

Several methods of creating jaundice have been described, namely simple ligation, ligation and division of the common bile duct and ligation and excision of a segment of common bile duct. Internal drainage of jaundice has been achieved by choledochojejunostomy [356]**,** suture of a cannula between the bile duct and duodenum [357] or reversible kinking of the common bile duct [358]. External drainage has been achieved with cutaneously tunnelled catheters [357] or via choledochovesical cannulae [359]. Survival after the production of obstructive jaundice has been documented for up to six weeks, at which point the animals were euthanased [360]. The majority of studies do not require the animals to be jaundiced for this length of time.

It has been suggested that the method of causing bile duct obstruction is relevant in experimental outcome and in the interpretation and comparison of different studies using different techniques. Some authors believed that ligation and division of the common bile duct produces a more profound level of obstructive jaundice compared to simple ligation, which produces a more protracted course of jaundice with fewer fatalities in the experimental population [361]. Guidry *et al* found that a high ligation of the bile duct compared to a low ligation produced higher levels of bilirubin but no change in other biochemical parameters or change in liver histology [362].

Thompson *et al* found that after 35 days of biliary obstruction mortality was up to 35% and beyond this mortality increased even further [89].

Recanalisation of the common bile duct and the subsequent restoration of bile flow has been reported, and as expected this occurs more frequently where the bile duct

has been simply ligated and lower where a segment of bile duct is excised. Following transposition of the distal end of the common bile duct behind the stomach and duodenum a zero percent recanalisation rate has been described [363]. In terms of interpretation of data from the rat model it is known that rats do have species specific liver enzymes such as 7α-hydroxylase of taurodeoxycholate or 6β-hydroxylase of taurodeoxycholic acid and lithocholic acid [364]. In addition there are also specific differences seen between different strain of rat such as the different responses to endothelin blockade seen in Wistar-Kyoto and Long-Evans rats [365].

1.9.ii: Dogs

After rats, dogs are the most frequently cited animals used in experimental obstructive jaundice. Methods of producing jaundice most commonly involve simple ligation and division of the common bile duct and cholecystectomy [183, 366], but bile duct ligation with exteriorisation of bile via tunnelled occluded cannulae has also been described [367]. Duration of obstructive jaundice of up to 15 [183] and 28 days has been reported with no mortality in the study group. In a review of animal models it has been suggested that in comparison to rats, the rate of salt and water retention is greatly increased in dogs with obstructive jaundice. In addition dogs develop systemic hypotension, whereas rats become normotensive after a period of hypotension and baboons do not show a hypotensive response [361].

1.9.iii: Rabbits

In the few studies of obstructive jaundice in the rabbit, the predominant method of producing jaundice is with bile duct ligation or bile duct ligation and transection.

There are very few studies showing long term mortality with jaundice although in one study the survival was very poor as the greatest length of survival ranged only from 4-8 days [368], however in another study the animals survived up to three weeks after which obstruction was relieved [90]. One group of authors used a complex system of exteriorisation of cannulae to enable the creation of obstructive jaundice, followed by restoration of the normal flow into the duodenum and also the creation of a direct bilio-venous fistula [369]

Rabbits have been found to have similar bile acids compared to humans [309]. In comparison to bile duct ligated rats, the rise in serum bile acids in the rabbit are lower [364]. Cholesterol 7 alpha hydroxylase is the enzyme responsible for the first stage of synthesis of bile acids from cholesterol. Levels are seen to increase in bile duct ligated rats but decrease in bile duct ligated rabbits [364]. It may therefore be that the rabbit is a more suitable model than the rat to study bile acid synthesis as in the humans the levels are also seen to decrease in biliary obstruction.

1.9.iv: Mice

Fewer studies are available using mice as a model of obstructive jaundice and bile duct obstruction is mainly created by double ligation [370], ligation and division [371] or the use of a removable surgical clip [372] . Injection of parvovirus into neonate mice has been shown to create biliary atresia and intra and extrahepatic atresias and cholestasis [373]. After four days of obstruction using bile duct ligation alone, mortality rates of 35% have been reported [374] while others however showed no fatalities in mice after 10 days of bile duct ligation [375].

1.9.v: Primates

Published studies on the effects of obstructive jaundice in the primate are rare. Methods of inducing jaundice primarily use the bile duct ligation and transection method but with additional ligation of the cystic duct [376]. The few published studies were mainly in the 1970s and include investigations into renal blood flow and effect of dopamine on baboons [377]. Similar studies demonstrated decreased renal cortical blood flow but with preservation of total renal blood flow in obstructive jaundice [145] and altered cerebral vascular perfusion in response to noradrenalin in the jaundiced baboon [378].

Another study with rhesus and cynomolgus monkeys used distal common bile duct transection to simulate a distal obstructing lesion, found that massive extrahepatic bile duct dilatation was not always accompanied by intrahepatic duct dilatation. For this reason they therefore used high ligation to create obstruction with intrahepatic duct dilatation. They also reported spontaneous perforation of the bile duct and concluded the greater distensibility of the common bile duct may be the reason for lack of raised intrahepatic pressure. This study noted that very jaundiced monkeys, some with massive lobar infarct and intrahepatic bileomas remained clinically well throughout the study [379].

1.9.vi: Other animals

Pigs have not been widely used as models for obstructive jaundice, but have been used more often to evaluate pathophysiology of biliary stent occlusion [380]. In one study using only 5 animals, survival was seen to 32 days with no evidence of

recanalisation of the bile duct as evidenced by maintenance of elevated bilirubin levels [381]. However another study using 22 large white pigs found that no animal survived beyond 27 days due to a combination of gastro-oesophageal haemorrhagic ulceration and bilateral pulmonary abscesses [382]. There are no references to use of sheep, guinea pigs or cats for evaluation of common bile duct ligation.

2: Experimental Aims

The aim of this study is to compare internal and external biliary drainage procedures in the relief of pre-operative jaundice using a rodent model.

Outcome measures

In this study the specific relationships between internal and external biliary drainage and the effect of a major surgical resection on mortality are considered. The specific physiological and biochemical parameters considered are albumin, weight, haemoglobin, total protein, globulin, bilirubin, ALP, ALT and GGT.

2.1: Hypothesis

The null hypothesis states “different methods of preoperative biliary drainage will not affect either post operative outcome or albumin levels”.

3: Experimental methods

3.1: Experimental overview

Male Wistar rats weighing 350-450g were used in this model, for reasons outlined in the previous discussion. Obstructive jaundice was created using bile duct ligation and division. After one week these jaundiced animals were then allocated to one of three groups: internal drainage, external drainage or no biliary drainage, prior to undergoing a major visceral resection.

Internal drainage was achieved using a choledochoduodenal cannula and external biliary drainage by placement of a choledochocutaneous cannula.

Major visceral resection consisted of a left nephrectomy, distal pancreatectomy, splenectomy and small bowel resection. Those animals undergoing biliary drainage underwent major visceral resection three weeks after the drainage procedure while the no drainage group had the major visceral resection after one week of biliary obstruction.

One further group was allocated to two sham laparotomies at the same interval as the rats undergoing bile duct ligation and then biliary drainage, followed by major visceral resection to control for the effects of laparotomy alone.

All animals in the study were euthanased two weeks after the major visceral resection. Blood samples were taken at the commencement of the experimental

process and weekly thereafter. The experimental outline is summarised in Figure 3.1 below.

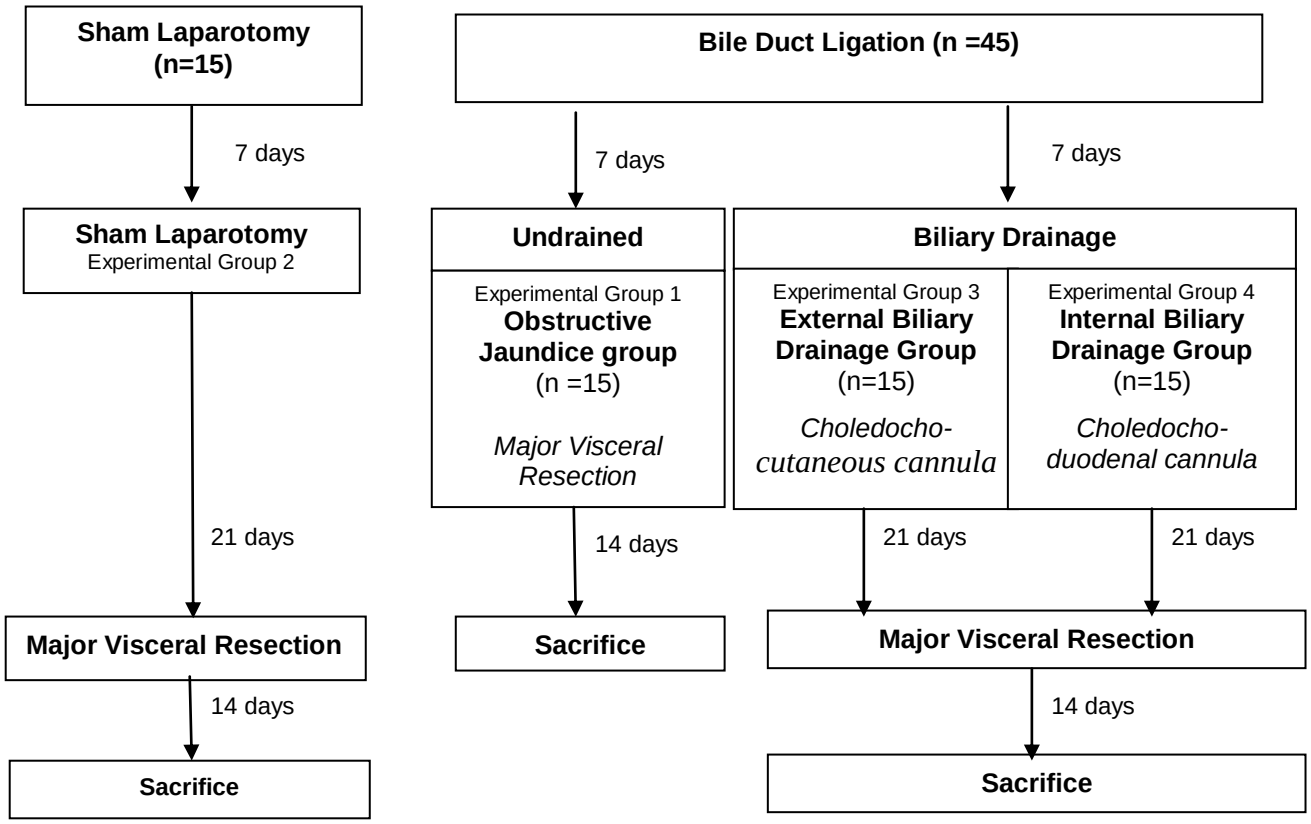


Figure 3.1: – Flow Chart of planned experimental outline.

3.2: Care of animals/husbandry

Ethical approval was obtained for this study from the University of Adelaide Animal Ethics Committee and The Queen Elizabeth Hospital Animal Ethics Committee in accordance with the South Australian Prevention of Cruelty to Animals Act 1985 and the Australian code of practice for the care and use of animals for scientific purposes - 6th Edition 1997. The rats arrived in the animal house at least 2 days prior to the commencement of the project in order to acclimatise to the

surroundings. Animals were housed individually and were allowed free access to standard food and water *ad libitum*, except prior to operative procedures when they were fasted for 12 hours.

3.3: Operative procedures

3.3.i: Induction and maintenance of anaesthesia/operative monitoring

Animals were placed supine on an insulating mat under a warming lamp. Core temperature was monitored using rectal temperature monitoring. All limbs were taped to the insulating mat to ensure the rat did not become disconnected from the anaesthetic equipment.

General anaesthesia was induced using an isoflurane / oxygen mixture via an induction box (Figure 3.2). Anaesthesia was maintained using inhalation of 2-2.5% isoflurane and 1.5l oxygen per minute via a face mask (Figure 3.3), which incorporated an active gas scavenging system.

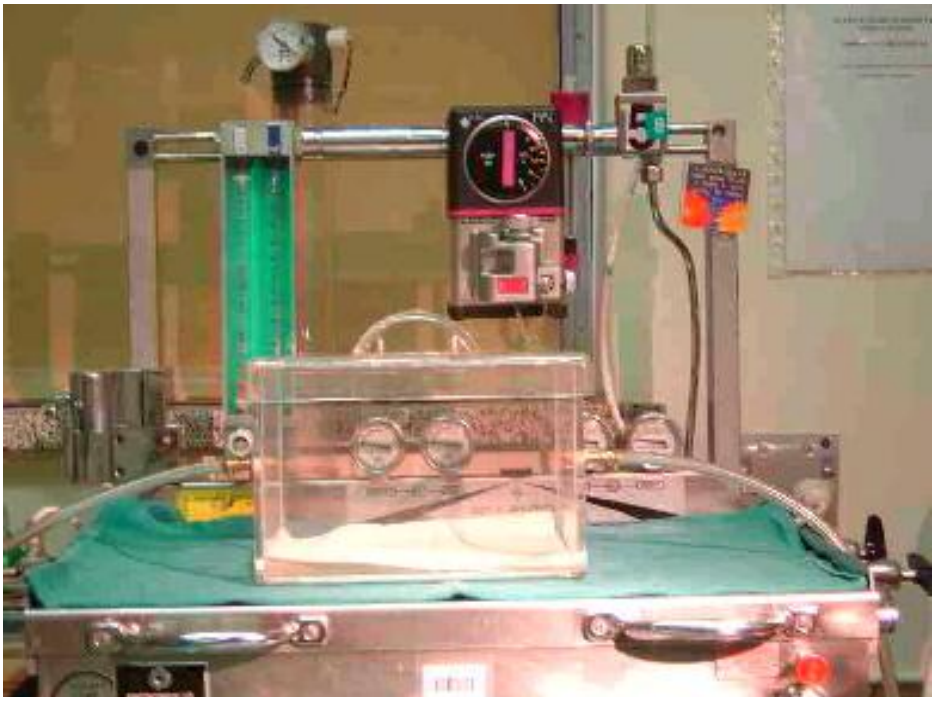


Figure 3.2: Anaesthetic equipment and gas induction box

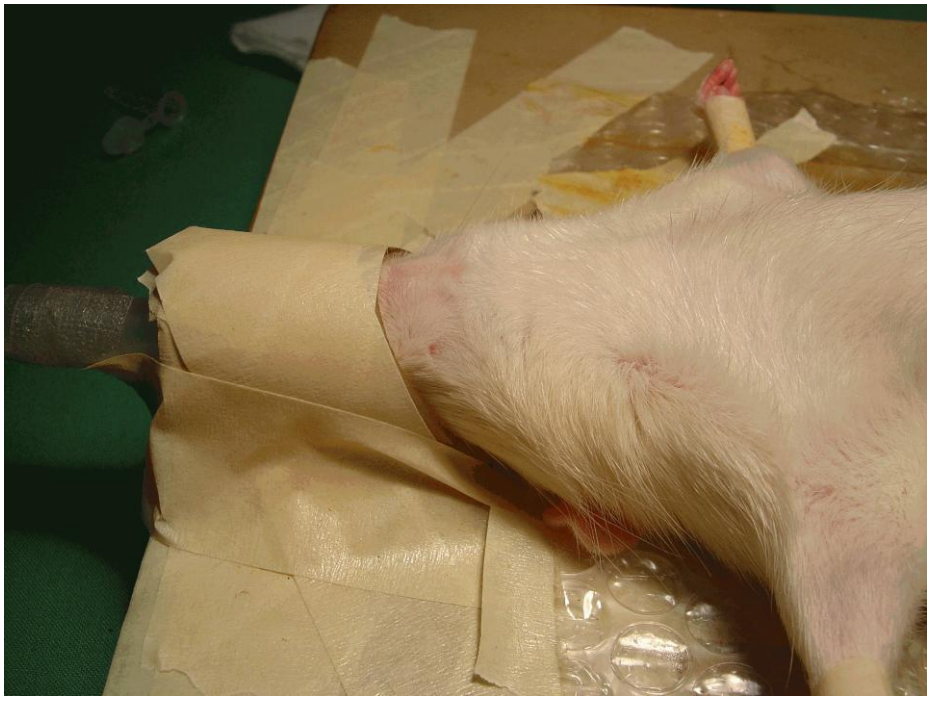


Figure 3.3: The use of a facemask during anaesthesia

Laparotomies were performed under standard sterile conditions. Prior to surgery the rats were shaved from xiphisternum to the lower abdomen. For skin preparation a mixture of 0.05% chlorhexidine, 0.5% cetrumide in 70% alcohol was used followed by application of povidone iodine 70% w/v. Standard sterile surgical drapes were used to isolate the abdomen (Figure 3.4). Preoperatively a dose of amoxicillin (150mg/kg) was administered subcutaneously, made up to a volume of 3ml with 0.9% sodium chloride.

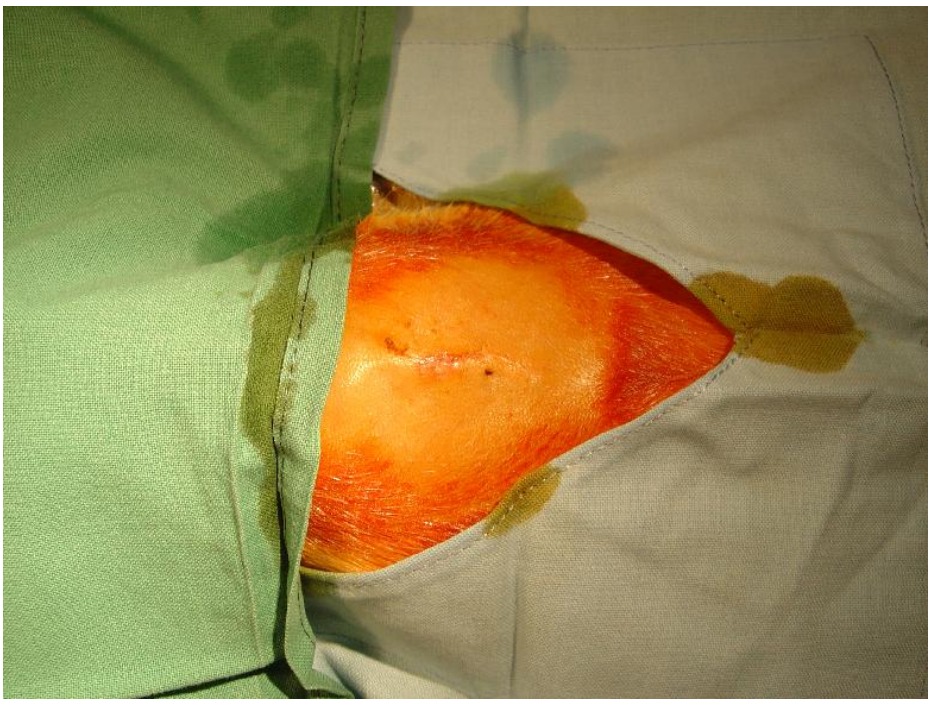


Figure 3.4: Skin preparation and sterile draping prior to laparotomy

3.3.ii: Bile duct ligation and division

Prior to establishing the method described below several problems were encountered in achieving the technique for biliary obstruction and these are detailed further in Appendix A.

A midline laparotomy was performed through a 2.5-3cm upper midline incision and a self-retaining retractor used for access. Through this incision the abdominal cavity and contents were inspected. The anatomy of the biliary tree in the rat is demonstrated in Figure 3.5.

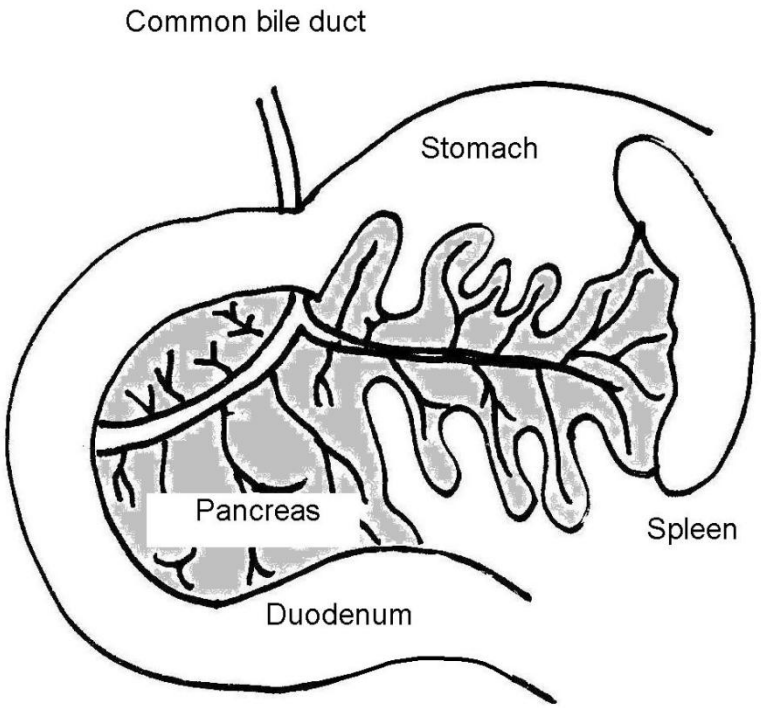


Figure 3.5: Anatomy of the rat biliary tree

The liver, common bile duct, pancreas and duodenum were identified. The liver was packed out of the operative field using wet packs. Using an operative microscope (x12 magnification) the duodenum and pancreas were gently retracted and the common bile duct located and its path through the pancreas identified (Figure 3.6). A point on the common bile duct distal to the porta hepatis and proximal to the commencement of pancreatic tissue was identified. The surrounding fat and loose

areolar tissue was dissected free, ensuring no damage to the adjacent pancreas or pancreatic ducts. Care was also taken to protect the portal vein and hepatic artery and to ensure the portion of common bile duct to be ligated was below the confluence of main right and left hepatic ducts.

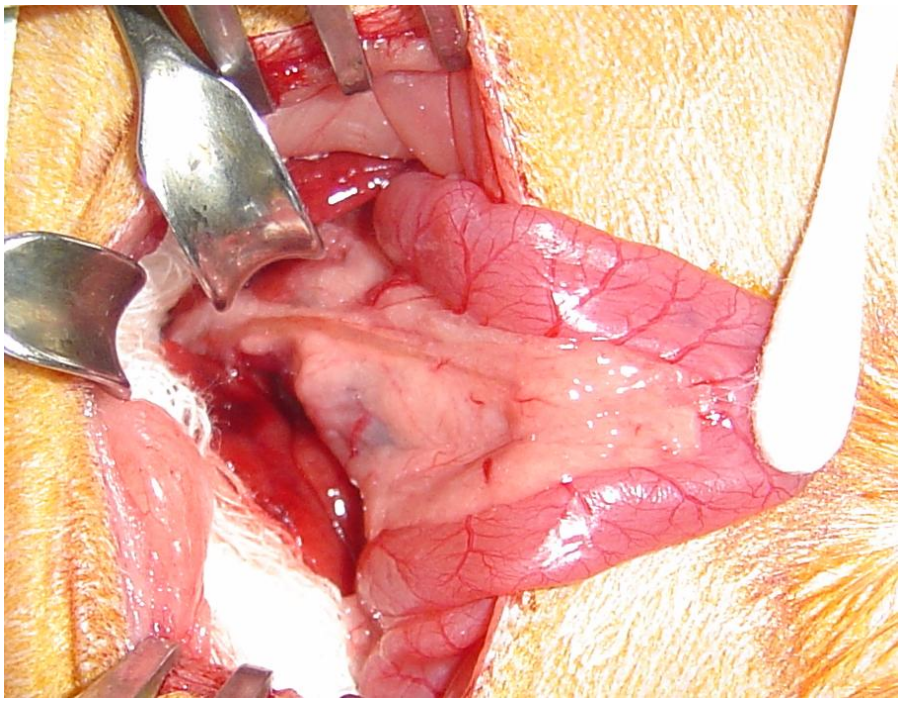


Figure 3.6: Common bile duct and its course through the pancreas

Once a few millimetres length of bile duct was isolated it was ligated and divided using 4/0 silk ties. Two ties were placed proximally and one distally and the duct divided between them (Figure 3.7). The packs were then removed and the abdomen inspected prior to abdominal closure. A two-layer approach was employed for closure of the laparotomy wounds. A continuous suture of 4/0 Vicryl was used for abdominal muscle closure and the skin was closed using a 5/0 Vicryl subcuticular stitch (Figure 3.8).

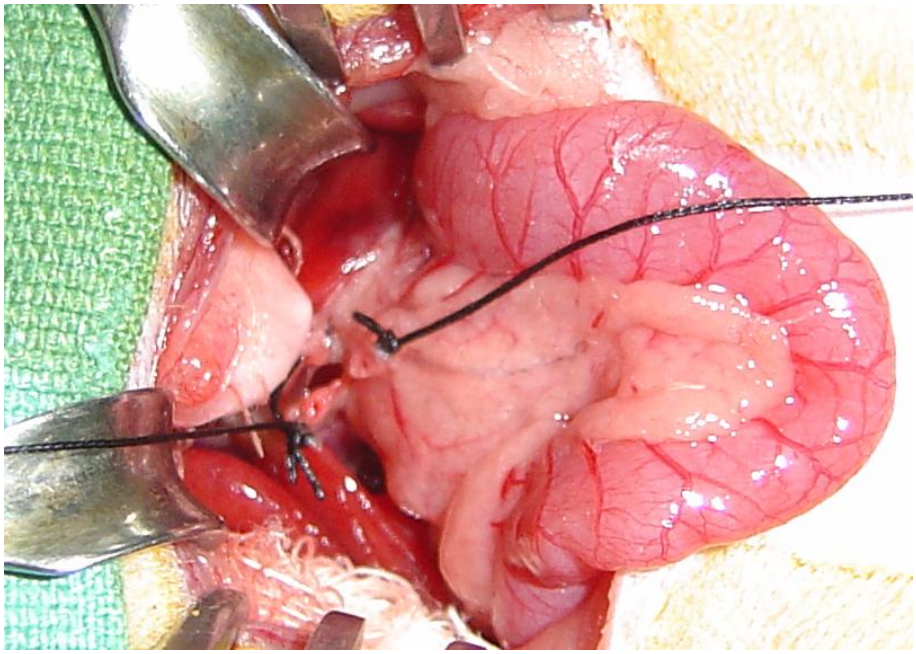


Figure 3.7: Ligated and divided common bile duct

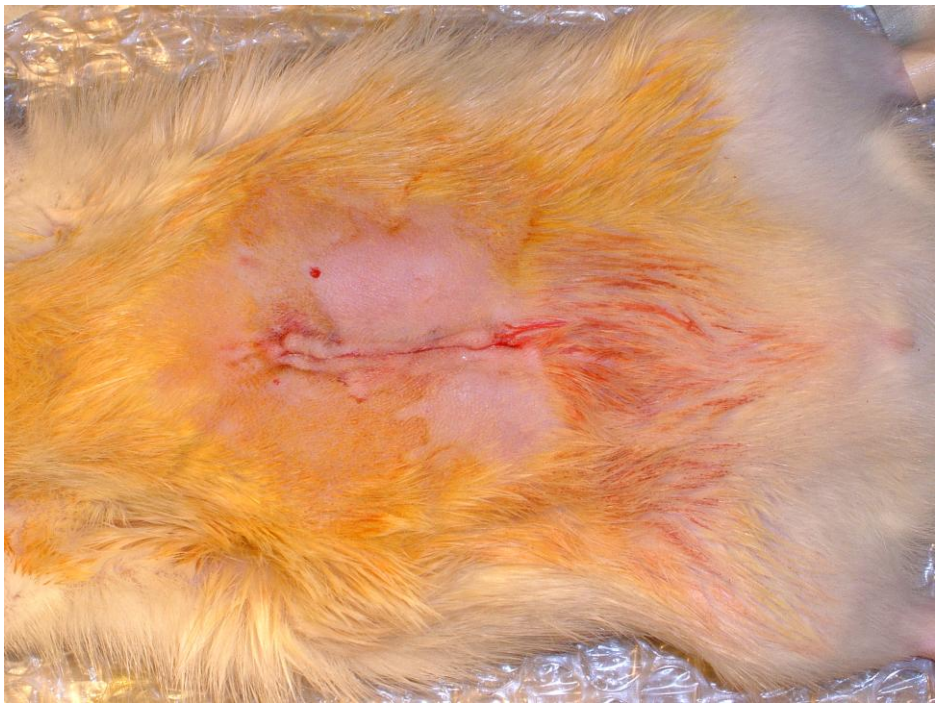


Figure 3.8: Final appearance following abdominal closure

The appearance of the common bile duct after one week of bile duct ligation is shown in Figure 3.9 where a significant degree of dilatation is apparent.

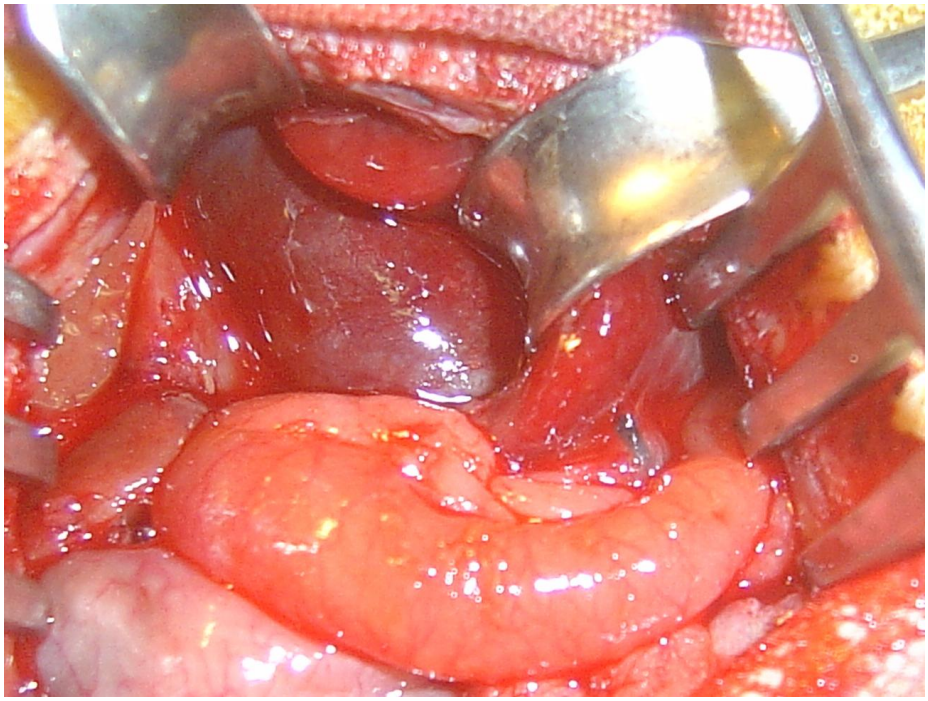


Figure 3.9: Distended common bile duct after one week of biliary obstruction.

3.3.iii: Internal biliary drainage

Internal biliary drainage was performed one week after bile duct ligation. Repeat laparotomy through the same incision was performed and inspection of the abdominal cavity performed after placement of a self-retainer. The dilated bile duct was then identified. While in the majority of cases the bile duct was very dilated with minimal adhesions and therefore easily accessible, in some cases extensive adhesions of the omentum, stomach, duodenum and pancreas were encountered. These adhesions tended to be highly vascular, compounded by the clotting derangements, therefore gentle, blunt dissection was used wherever possible and ligation of dense adhesion bands where necessary prior to division with scissors. Before continuing, haemostasis was secured using abdominal packs and gentle pressure if necessary.

Once a satisfactory length of bile duct had been identified further wet packs were placed to retract the liver and small bowel from the operating field. At this point the cannula to be used was prepared prior to proceeding. The cannula used was a modified 5 Fr paediatric feeding tube (Maersk Medical, Sydney) initially cut to a length of approximately 5cm. The tube was modified by placement of two silastic rubber cuffs at each end which were approximately 1mm in length and 1mm apart (Figure 3.10).

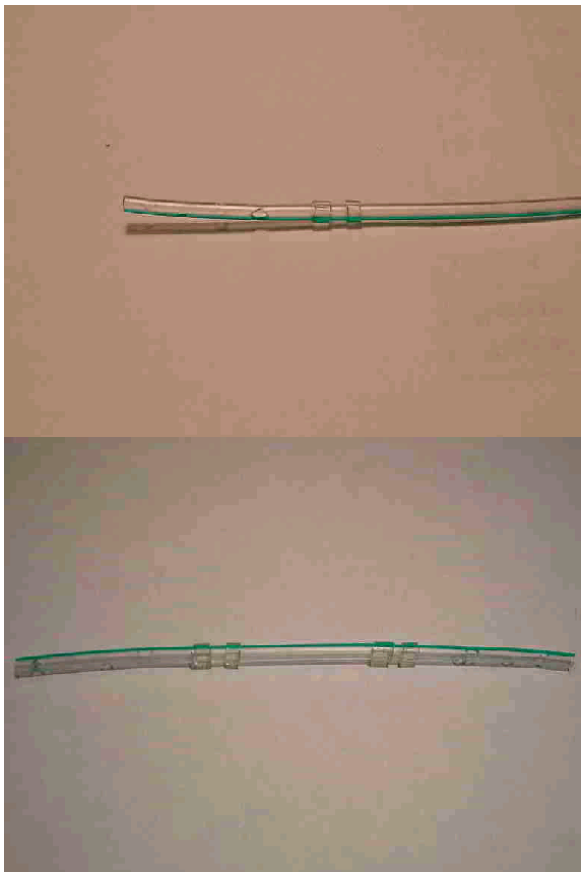


Figure 3.10: Close up of adapted infant feeding tubes for use as biliary drainage cannulae. Drainage holes have been cut and silicone cuffs placed for securing into the bile duct and duodenum.

The cuffs were placed approximately 5mm from each end of the tube and fenestrations cut in order to facilitate drainage from the common bile duct into the duodenum. The purpose of these cuffs was to prevent slippage of the tube and

provide improved anchorage for the suture. A 6/0 Prolene purse string suture was placed on the anterior surface of the bile duct and a small choledochotomy made with fine scissors, through which the cannula was passed. The bile duct normally decompressed rapidly during placement of this suture and care needed to be taken, as the walls of the bile duct were extremely fragile and liable to trauma and tearing. The purse string was then tied with the knot laid to rest between the two cuffs to prevent slippage. A further purse string suture using 6/0 Prolene was then placed in the common bile duct around the proximal end of the cannula for extra security.

The appearance of bile draining freely from the end of the cannula (Figure 3.11) confirmed that the tube was placed correctly. If bile was not seen moving spontaneously up the tube this indicated that either a leak at the site of cannula insertion or damage elsewhere in the bile duct had occurred. Repair of the leak by insertion of further sutures was therefore needed.

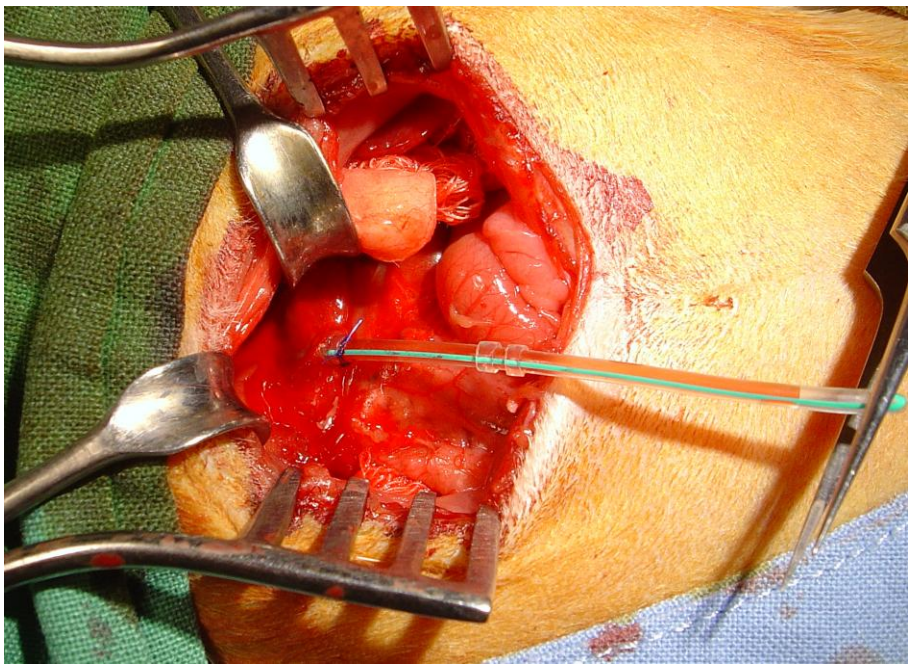


Figure 3.11: Cannula in common bile duct. Bile can be seen draining through the tube

When the tube was securely placed in the common bile duct a point was selected on the duodenum approximately 1.5cm from the pylorus for insertion of the cannula. Using 6/0 Vicryl another purse string suture was placed on the anterior wall, close to the mesentery. At this point the length of tubing needed to intubate the duodenum with minimal tension or kinking of the tube was assessed. The two distal cuffs were then moved to a suitable position, the tube cut to the suitable length and fenestrations created. A small duodenotomy was then created and curved forceps were used to confirm entry into the duodenal lumen. The cannula was then threaded into the duodenum and the purse string tied securely between two cuffs (Figure 3.12).

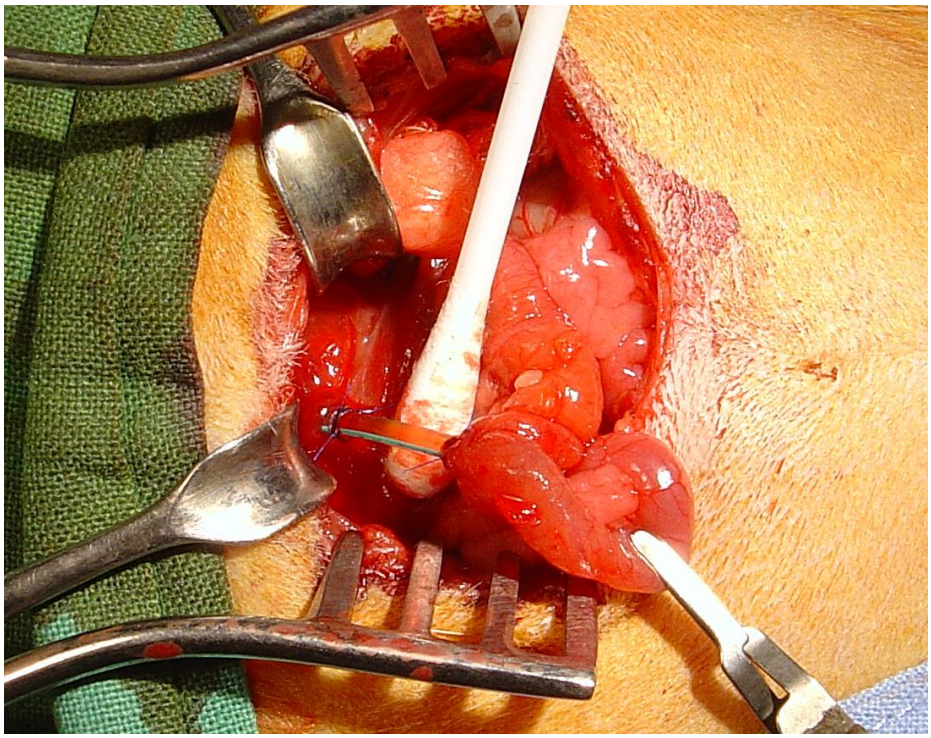


Figure 3.12: Cannula with both ends in CBD and duodenum. Bile is seen draining through the cannula.

Haemostasis was checked and the abdomen lavaged with 0.9% sodium chloride. Once the abdominal cavity was dry both ends of the tube were again

inspected to confirm correct placement with no leakage of bile. The abdomen was then closed as described above and the rat recovered.

3.3.iv: External biliary drainage

External biliary drainage was performed one week after bile duct ligation. The anaesthetised rat was prepared initially for external drainage by placing it prone and shaving the hair in an area from the base of the skull to a point below the scapulae. The midline and position of the scapulae were marked with permanent marker to assist in identification of the mid-scapular point for exteriorization of the drainage tube later in the procedure (Figure 3.13). The skin was prepped with chlorhexidine, cetrumide and povidone iodine as above and left covered with an iodine soaked swab and the animal turned supine and secured to the operating table.

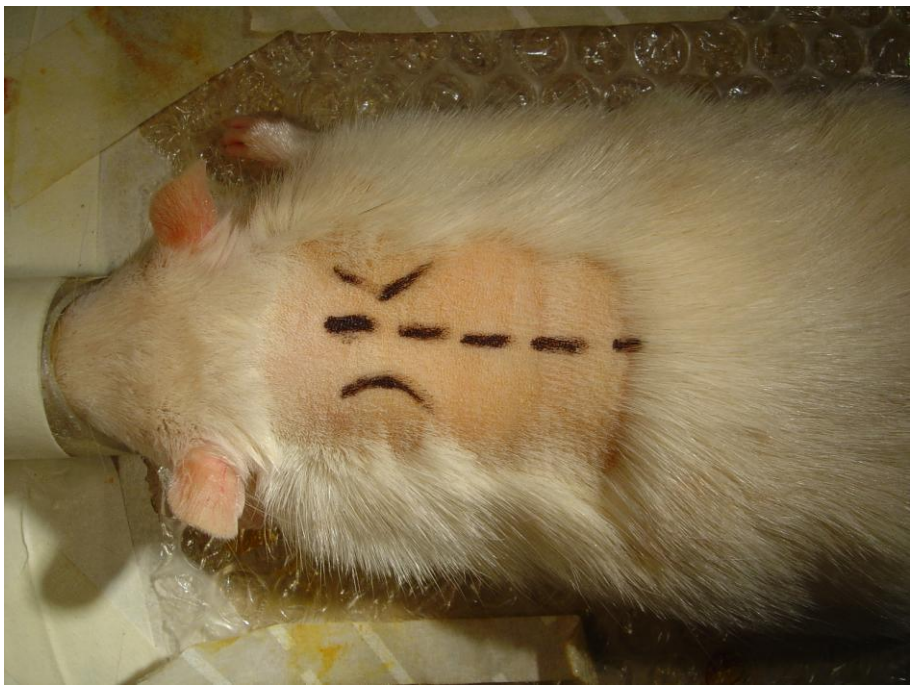


Figure 3.13: Preoperative marking of site for exit of external drainage cannula

The same 5Fr paediatric feeding tube was used for external biliary drainage and was prepared proximally in the same manner as above. Distally a single 4mm silastic cuff was placed to assist fixation to the skin and avoid slippage of the tube and the overall length increased to allow the tube to reach the skin.

Following skin preparation and draping, a midline laparotomy was again performed. The dilated common bile duct was identified and proximal cannulation carried out in the manner as described above for internal drainage.

After proximal cannulation of the bile duct, the skin was dissected off the abdominal wall muscle laterally and the distal end of the cannula passed through the lateral part of the anterior abdominal wall under direct vision. The tube was then secured to the muscle using 4/0 silk, ensuring no tension occurred at the site of common bile duct cannulation. At this point the midline muscles were closed using 4/0 Vicryl but leaving the skin open. A povidone iodine soaked swab was placed over the wound and the rat was turned to a left lateral position in order to gain access to the interscapular region. A small incision was made at a point just distal to the interscapular point. A curved artery clip was then passed (Figure 3.14) along the subcutaneous plane toward the midline and brought out at the point where the drainage cannula was emerging from the abdomen.

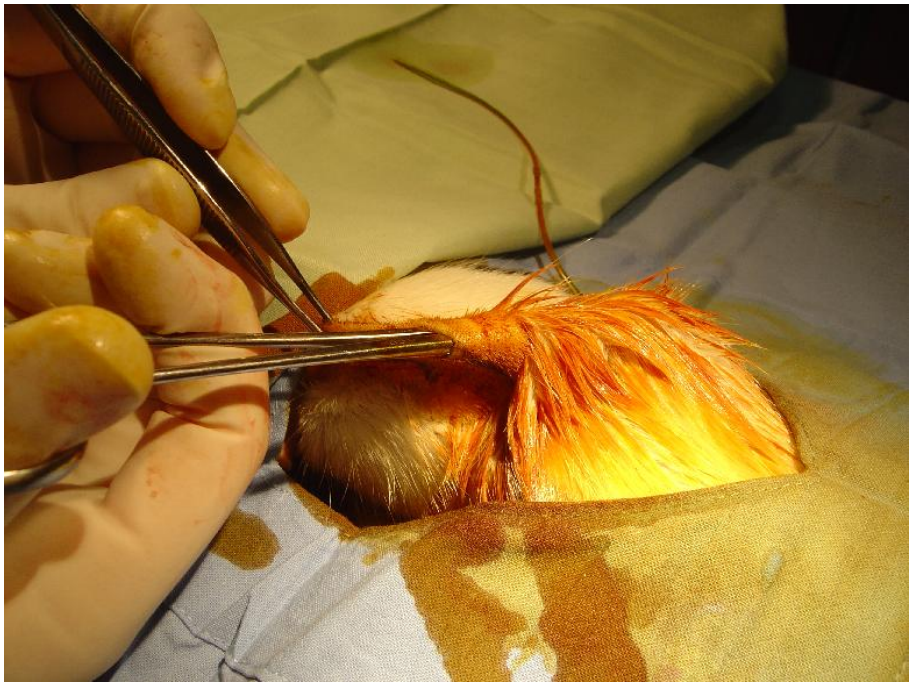


Figure 3.14: Creation of subcutaneous skin tunnel from interscapular region toward site where the cannula has been delivered through the anterior abdominal wall

The cannula was then cleaned with povidone iodine, grasped and pulled out through the interscapular skin incision (Figure 3.15). The 4mm cuff was moved along the tube to a point whereby it could be secured at the skin with no underlying tension on the tube and secured in place with a 4/0 silk suture by tying knots above and below the cuff. The exit site skin was closed at the same time using subcuticular vicryl. The tube was then trimmed to a length of 4cm from the skin surface (Figure 3.16).

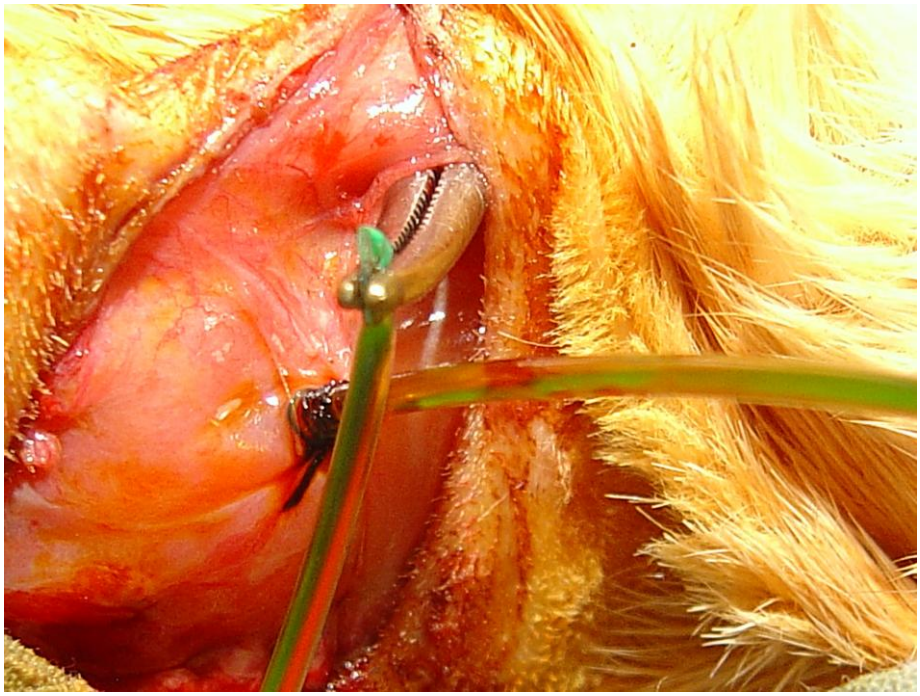


Figure 3.15: The cannula is grasped at the point at which it emerges through the closed anterior abdominal wall (bottom of picture) and delivered through subcutaneous tunnel to the interscapular drainage point

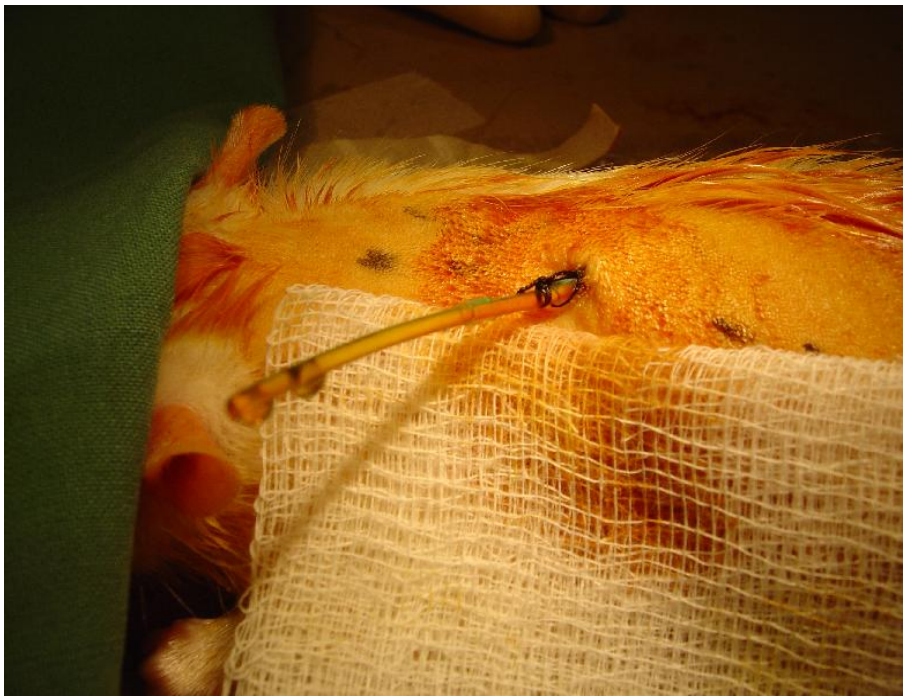


Figure 3.16: The final appearance of the external drainage cannula emerging from interscapular point

The rat was then turned supine again and the abdominal incision skin was closed with a 5/0 Vicryl continuous subcuticular stitch.

3.3.v: Major visceral resection

Three weeks after either internal or external biliary drainage or one week after bile duct ligation in the control group, the rats underwent major visceral resection. The major visceral resection entailed splenectomy, left nephrectomy, distal pancreatectomy and small bowel resection.

A repeat midline laparotomy was performed, extended caudally as necessary for access, although usually only a further few millimetres of extension was required. Evidence of free fluid or bowel obstruction was noted and the area of the common bile duct and drainage tube examined. While some adhesions were inevitably encountered these were not divided unless access to the spleen, pancreas, kidney or small bowel was impeded.

Splenectomy and distal pancreatectomy were performed en-block as the tail of the pancreas is closely associated with the spleen, which is a long tongue-like structure in the rat (Figure 3.17).

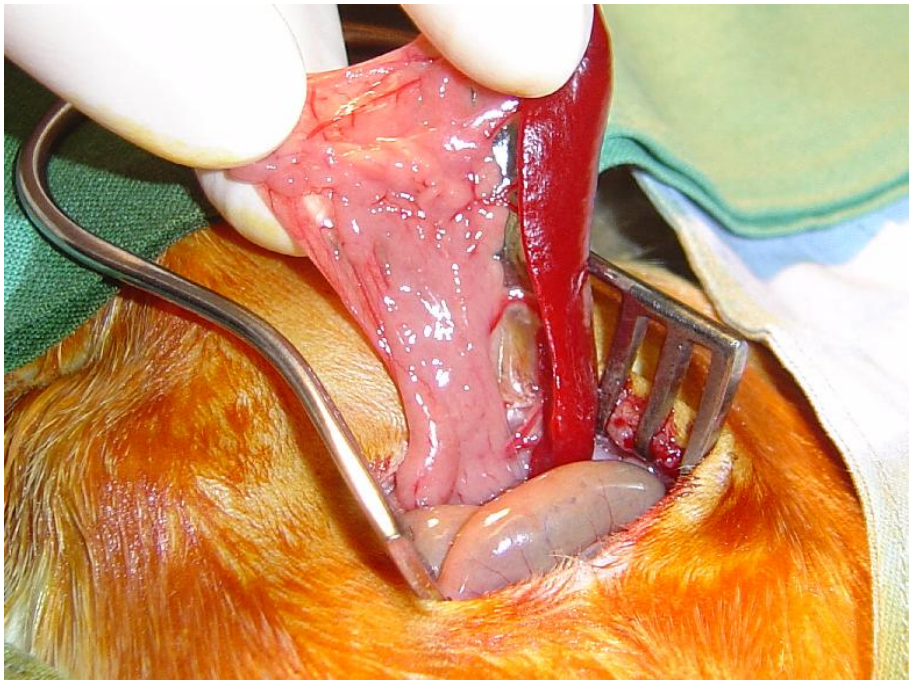


Figure 3.17: Demonstration of the spleen and pancreas en-bloc prior to resection

The pancreas and spleen were identified and delivered into the wound, dividing any filmy peritoneal or mesenteric attachments. A window was created between the spleen and pancreas at a point midway along the pancreas. The pancreas was then clamped at this point ligated with 3/0 silk and divided, the pancreatic tail remained attached to the spleen. The spleen was then mobilised to the splenic vessels, which were clamped and divided and ligated using 3/0 vicryl. Following this the left kidney was identified and dissected free from the perinephric fascia using a combination of sharp and blunt dissection, taking care not to puncture the renal capsule. Dissection was continued to the hilum, where the renal artery, vein and ureter were clipped en-mass and the kidney excised. The pedicle was ligated with 3/0 Vicryl (Figure 3.18).

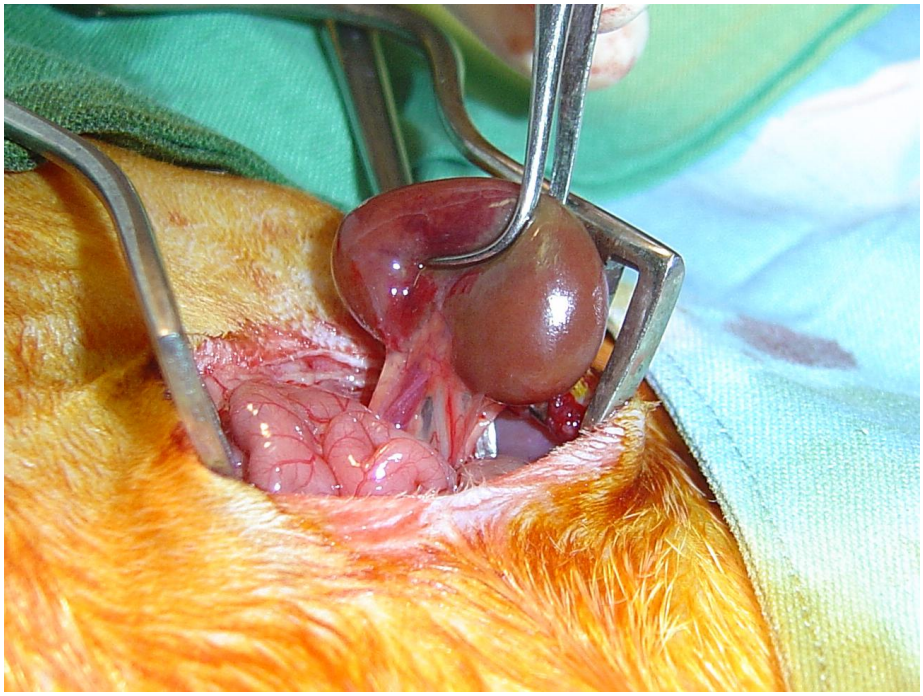


Figure 3.18: Left kidney prior to resection

For the small bowel resection a segment of jejunum was identified and delivered out of the wound onto a wet swab (Figure 3.19). A segment of bowel 5cm long was identified and the mesenteric vessels supplying that section identified and ligated. In most cases only one mesenteric vessel needed to be ligated. The segment of bowel between vessels was then excised; ensuring a good blood supply to the cut ends remained (Figure 3.20).

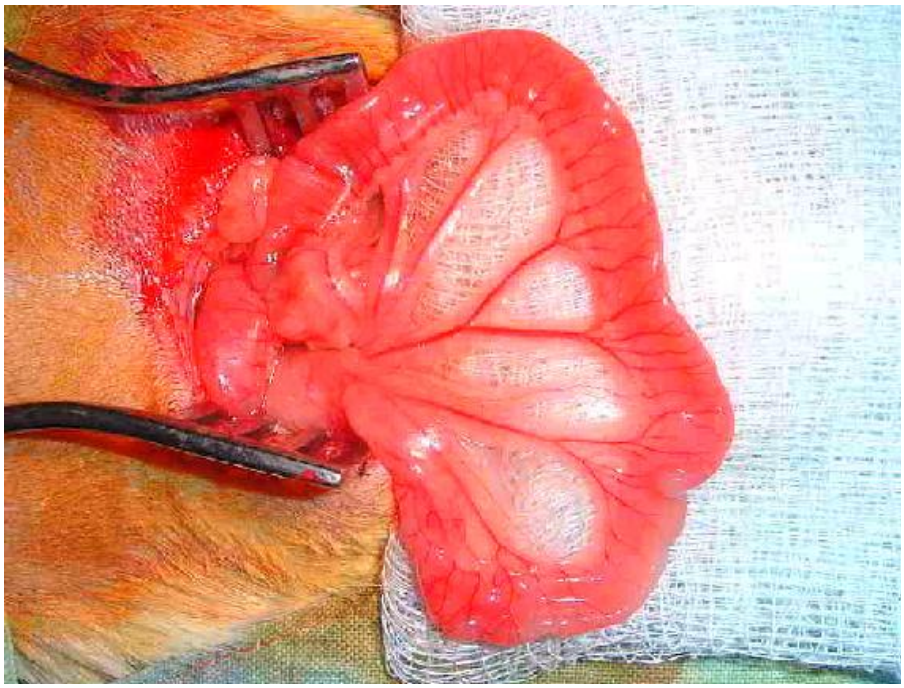


Figure 3.19: Small bowel and mesentery demonstrating vascular arcades

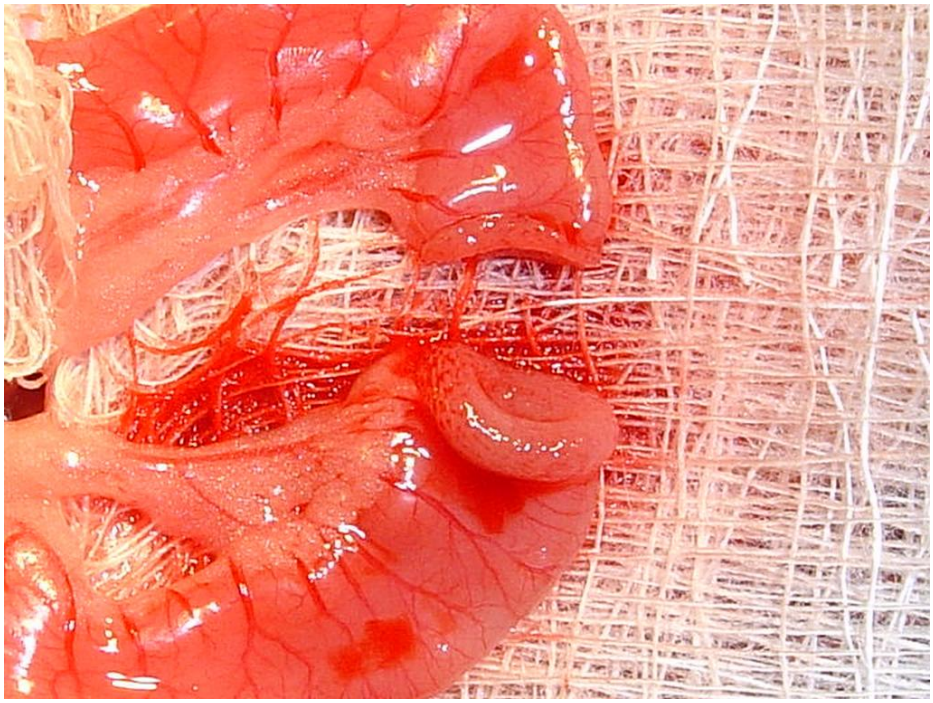


Figure 3.20: Cut ends of bowel after resection prior to anastomosis

Then an end-end anastomosis was performed with the assistance of an operating microscope. Using 6/0 prolene two stay sutures were placed at the mesenteric and antimesenteric borders of the bowel, which were clipped and placed under mild tension. First the anterior border of the bowel was anastomosed using full thickness single layer interrupted 6/0 prolene sutures. Once the anterior wall had been completed the posterior wall was closed, ensuring the anterior wall was not caught up in the closure. Once the anastomosis was completed (Figure 3.21) it was leak tested using saline injected into the lumen through a 27-gauge insulin needle that was inserted obliquely through the wall. Atraumatic clips were used to occlude the bowel and the bowel inflated to a moderate tension. If any evidence of saline leakage was apparent further stitches were placed.

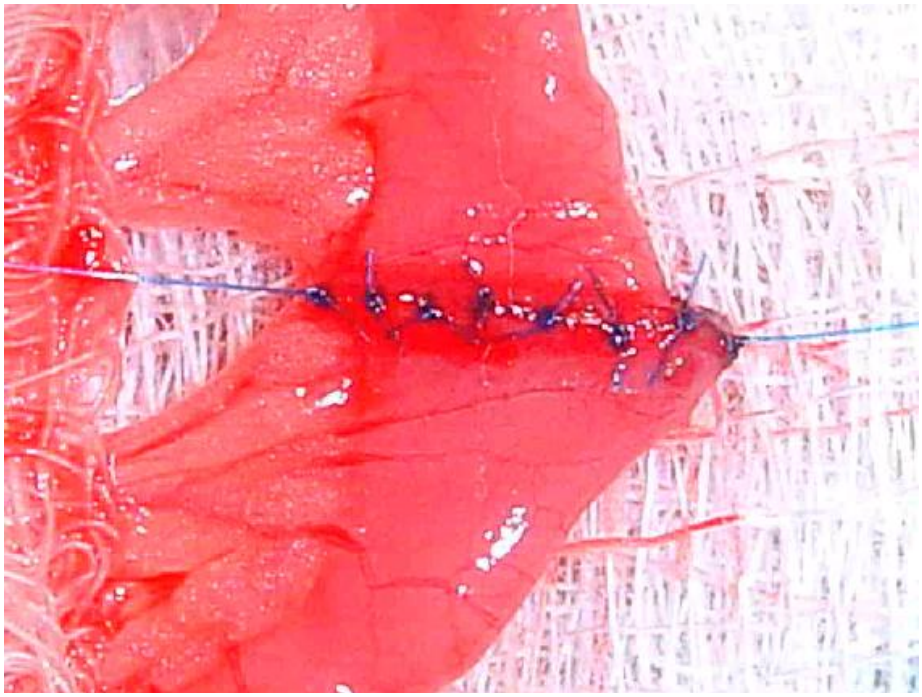


Figure 3.21: Small bowel anastomosis completed (stay sutures still in place)

Following this the abdomen was again inspected for haemorrhage and washed out with saline. The abdomen was then closed and the rat recovered from anaesthesia.

3.3.vi: Sham laparotomy

One group of rats underwent two sham laparotomies followed by major visceral resection. The sham laparotomies were performed via the same midline laparotomy as the other groups. In the first sham laparotomy the common bile duct was isolated and dissected free from surrounding tissue but no bile duct ligation was performed. For the second laparotomy the common bile duct was again isolated, adhesions dissected free and the duodenum mobilised. The animals remained under general anaesthesia for a similar length of time as those groups that underwent bile duct ligation and biliary drainage procedures respectively.

3.4: Postoperative monitoring and support

At the termination of the procedure all wounds were infiltrated with 0.5% bupivacaine (2-3mg/kg) diluted with 0.9% sodium chloride. Buprenorphine 0.01-0.05mg/kg was administered subcutaneously as the rat recovered from anaesthesia. Once the isoflurane inhalation had been discontinued supplemental oxygen was administered until the animals had fully recovered. Warming lamps were used during recovery until the animal was awake. Animals were only returned to their cages once they were alert and able to mobilise. Food and water was provided immediately following surgery.

Postoperatively, the rats were monitored for a period of six hours to ensure there were no immediate complications. A once daily dose of amoxicillin was continued for a further 48 hours at a dose of 150mg/kg. Buprenorphine 0.01-0.05mg/kg s.c. was administered twice daily for 48 hours.

Subsequently the rats were examined on a daily basis and weighed together with monitoring of their general condition to guide the need for further analgesia, fluids or euthanasia. Clinical record sheets were maintained and daily distress scores calculated for each animal to ensure animal welfare (Appendix C). Any animal meeting the predefined criteria was automatically euthanased.

3.5: Blood sampling

Blood samples were taken under general anaesthesia to minimise distress. Under anaesthesia warm packs were applied to the base of the tail to facilitate vasodilation. Using a 25g 16mm long needle and syringe, 1mm of blood was aspirated from the ventral tail artery (Figure 3.22). Pressure was applied after withdrawal of the needle to stop further bleeding. A drop of blood was placed onto a microcuvette for haemoglobin analysis as described below. Replacement fluid in the form of 3ml subcutaneous sodium chloride was administered prior to waking the rat up.

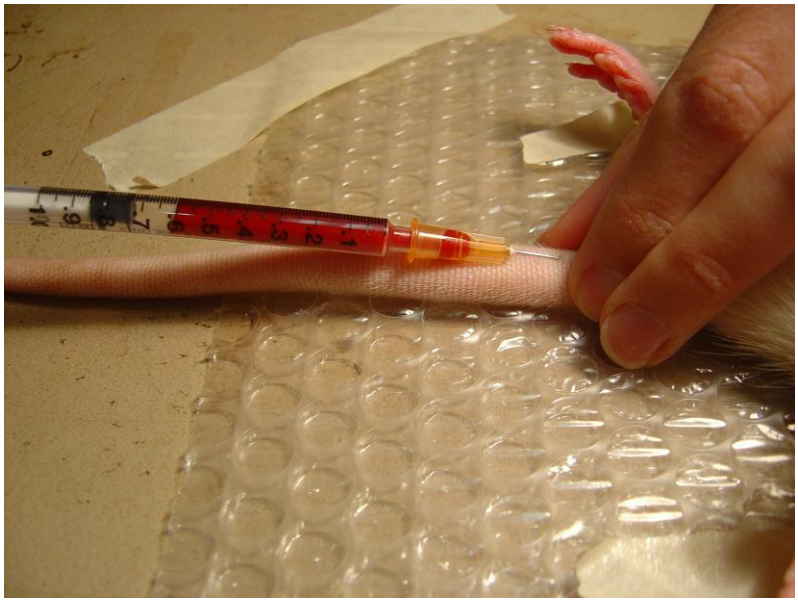


Figure 3.22: Technique of tail artery bleeding using ventral artery of the tail

3.6: Euthanasia

At the termination of the experiment the rats were euthanased. Initially they were anaesthetised as described above following which blood was taken from either the ventral tail artery or via cardiac puncture. The rat was then euthanased with an intracardiac injection of sodium pentabarbitone 1mg/2kg bodyweight. A post mortem was then performed through the laparotomy incision. The abdomen was inspected with particular attention to the position of the drainage tubes, size of the common bile duct and patency of the intestinal anastomosis. A segment of the liver was then taken for histological analysis.

3.7: Sample analysis

Blood samples were taken for measurement of haemoglobin, bilirubin, alkaline phosphatase, alanine transaminase, γGT, total protein, albumin and globulin

when the study was commenced and at weekly intervals thereafter until the termination of the study.

3.8: Statistical methods

The results from the experimental work in this thesis consist of serial measurements of biochemical and biometric indices, and survival rates of various groups of animals over time. This generated a large volume of data and inevitable challenges in ensuring the correct statistical interpretation of this data was used to allow meaningful conclusions to be drawn. The comparisons made in examining this data are as follows:

- Intra-group variability (section 4.1 to 4.4)
    - the changes in a measured factor in one group of animals over time

- Inter-group variability (section 4.5)
    - the changes in any particular factor in one group of animals compared with another group of animals

- Differences in survival between the various animal groups (survival).

Because of the relatively small numbers of animals available for comparison in each group and Gaussian data distributions (i.e. normal distribution) could not be assured non-parametric tests were used throughout except in a few exceptions (*vide infra*).

3.8.i: Intra-group variability over time

Where only two time points existed for comparison, for example bilirubin levels between the time of bile duct ligation and internal drainage (group 4), a Wilcoxon

signed-rank test was applied. In the cases where multiple time points existed, a Friedman (Chi-squared) test was applied which is the non-parametric analogy of a one-way repeated measures ANOVA. In the case of the Friedman test a significant result implies that there is significant variation over time between all of the measures but it does not inform of where this variation occurs. The point of variation can be determined either using a Dunn's test (but only if the comparisons are limited to the significant ones) or more simply, and the method adopted for this thesis, by directly comparing values at each of the time points using a Wilcoxon signed-rank test. Since this makes no adjustment for multiple hypothesis testing, precise P-values for each of these comparisons have been quoted.

The analysis described above has been applied to sections 4.1 to 4.4 in the results chapter.

3.8.ii: Inter-group variability over time

The comparison of the variability in a particular factor over time between experimental groups was more complex.

For these results it was necessary to adjust for any differences in the baseline values at the first time point in the series, since the different experimental groups would have undergone different interventions/experimental protocols before reaching this stage. If the differences between the values at the time points were compared directly, this would risk finding significant results not as a result of the intervention being

compared between the groups but as a result of their prior interventions. The statistical test used for this analysis was a two-way ANOVA which, whilst primarily a parametric method contains a test to detect for non-parametric data and an appropriate adjustment. A significant result in these analyses implies that the way in which each value being examined varies over time is different in each group, *not* that there are differences in the values over time. Examples are given below to demonstrate these points.

In Figure 3.23, although the two hypothetical datasets differ greatly in value, because they vary over time in precisely the same way (i.e. both sets increase in value in similar increments) there would be no significant difference demonstrated if a two-way ANOVA were applied.

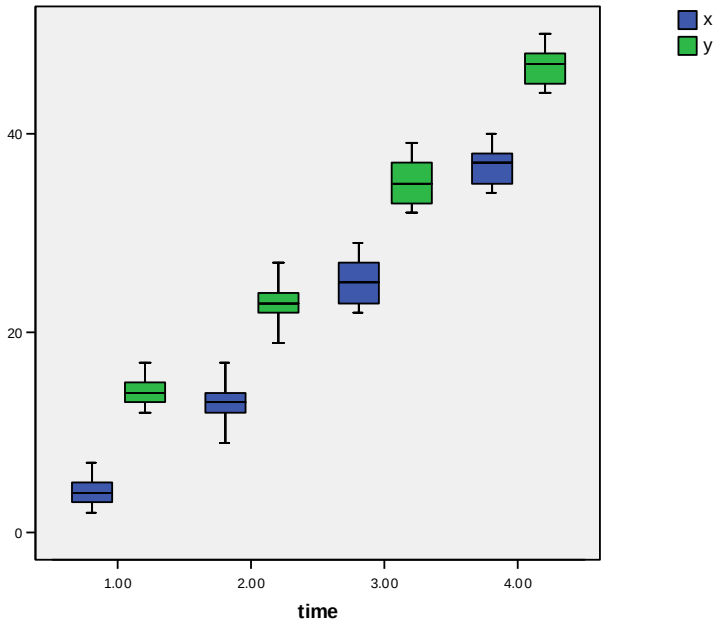


Figure 3.23: Example of a negative ANOVA for variation over time despite significant changes between the two groups at each timepoint.

The following figures demonstrate a positive ANOVA for variation over time.

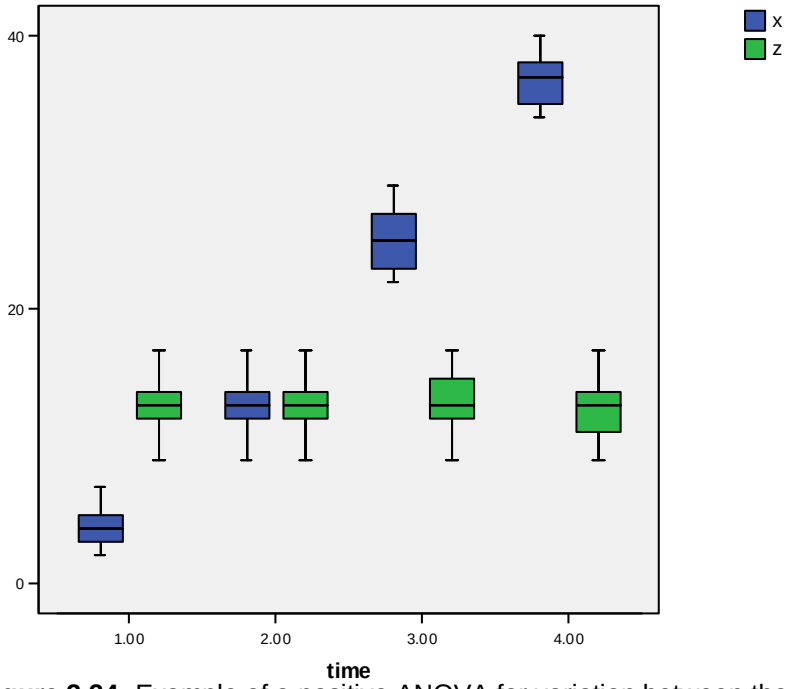


Figure 3.24: Example of a positive ANOVA for variation between the two groups over time.

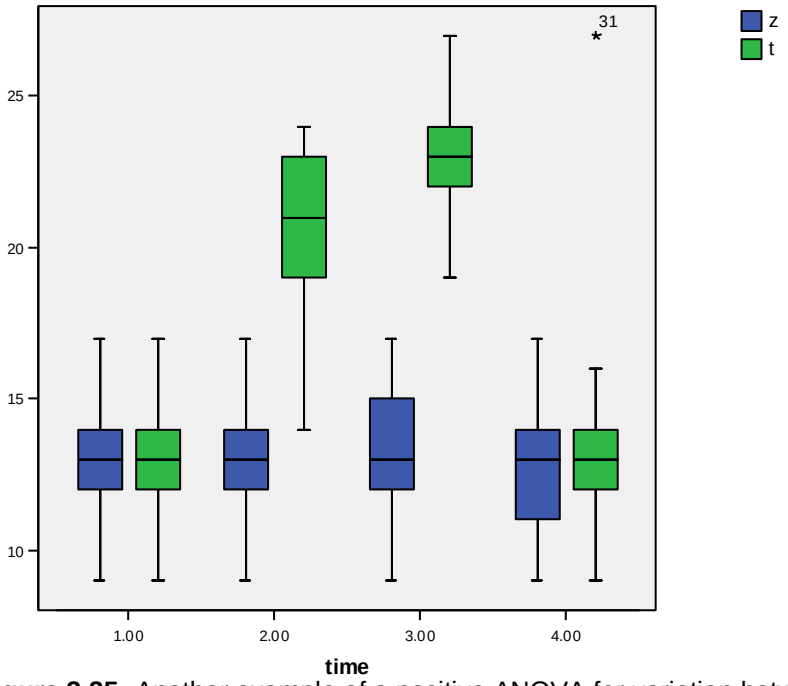


Figure 3.25: Another example of a positive ANOVA for variation between the two groups over time.

In Figures 3.24 and 3.25 because the two datasets differ in the way they vary over time a significant result would be generated.

The results from this analysis consist of an F-statistic (the ratio of the sample variances) which in turn is used to calculate the P-values. The higher the value of F the greater the difference in variance between the two samples and therefore the results for these analyses have been presented in order of descending values of F. These analyses also include a test that is analogous to testing for parametric data but is referred to as a test for 'sphericity' (Mauchley's test). Where this test reveals that sphericity cannot be assumed an approximation of the results from the test is used – the Greenhouse-Geisser estimate. Two values for the degrees of freedom for this test are quoted with the results, where the Greenhouse-Geisser estimate is used these become decimalized as a result.

This analysis described above is applied to section 4.5 in the results chapter.

3.8.iii: Survival

Standard survival analysis was performed using log-rank tests to determine any differences in survival between groups.

4: Results

This chapter describes the biochemical and physical measurements taken in each group and then the analysis of mortality. Mortality figures include both the animals that died and also those that were euthanased after falling ill and meeting the criteria for euthanasia (Appendix C).

The results are presented and analysed to explore the following areas:

4.7. The effect of bile duct ligation

4.8. The effect of sham laparotomy and the recovery period following two sham laparotomies

4.9. The effect of external and internal biliary drainage

4.10. The effect of major visceral resection (MVR) in all experimental groups

4.11. Direct comparison of variability between groups

4.12. Survival differences

Group numbers

Due to the difficulties in establishing the model the number of animals in each group did not total 15 at the start of the experiment. The following numbers of animals that were included from the start of the experiment in each group was as follows:

Group One	No biliary drain	11
Group Two	Sham laparotomies	18
Group Three	External biliary drainage	12
Group Four	Internal biliary drainage	14

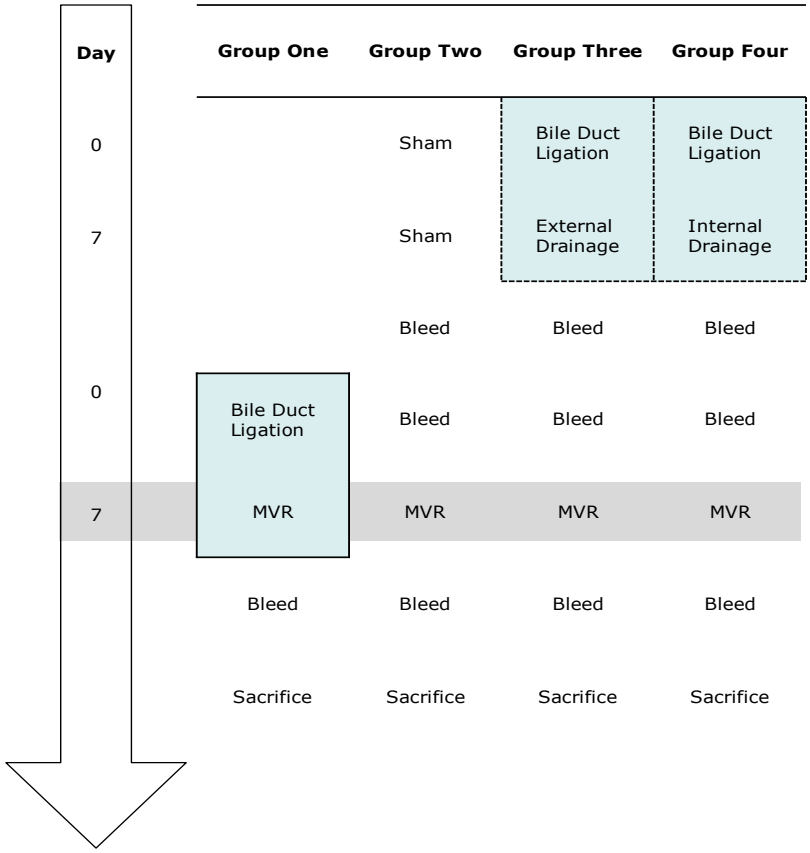
Three extra animals were added to the sham group in order to obtain liver histology for later use. Mortality figures during the experiment are discussed at the end of this chapter.

4.1: The effect of bile duct ligation

In order to assess the variation in biometric variables as a result of bile duct ligation the levels of these variables were compared at the time of bile duct ligation and 7 days following bile duct ligation. The data from all three groups was combined and analysed together. The following figures (Figure 4.1 – 4.9) show the values of each variable measured after bile duct ligation for all of the groups in which this was performed (groups 1, 3 and 4) at day 0 (at the time of ligation) and day 7 after ligation. A summary of these changes is shown after the graphs in Table 4.1.

In all figures the boxes represent the interquartile ranges (between 25-75%). The bar represents the median point and the whiskers demonstrate the range of data points excluding outliers. Outliers are represented by ° and extreme outliers by *. Comparison between the two time points was performed using a Wilcoxon-signed rank test. This also applies to all graphs from section 4.1 to 4.4 unless otherwise specifically stated.

The following chart graphically represents the groups being analysed. The area of the chart shaded in light blue represents the groups that are presented below.



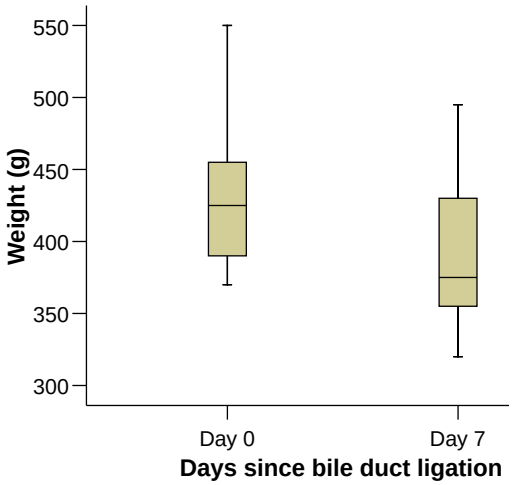


Figure 4.1: Weight at baseline and after 7 days of bile duct ligation (n=37, p<0.001)

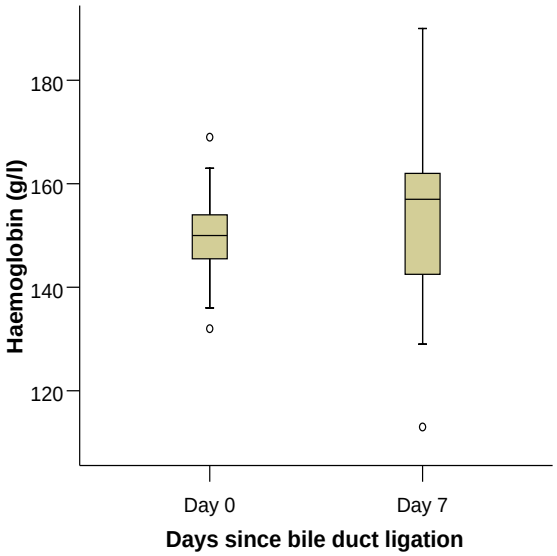


Figure 4.2: Haemoglobin at baseline and after 7 days of bile duct ligation (n=35, p=0.143)

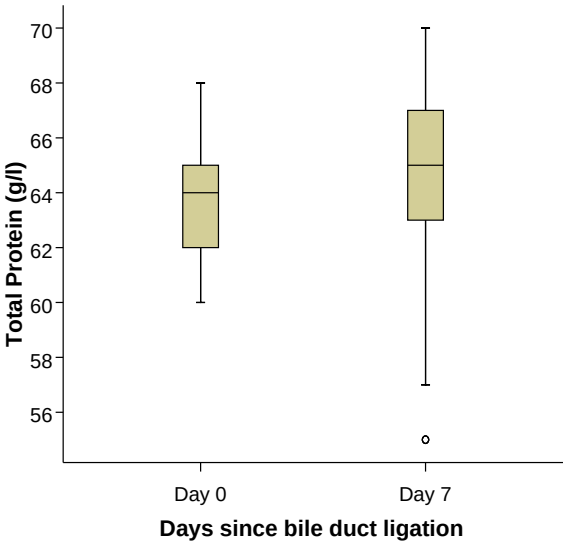


Figure 4.3: Total protein at baseline and after 7 days of bile duct ligation (n=37, p= 0.167)

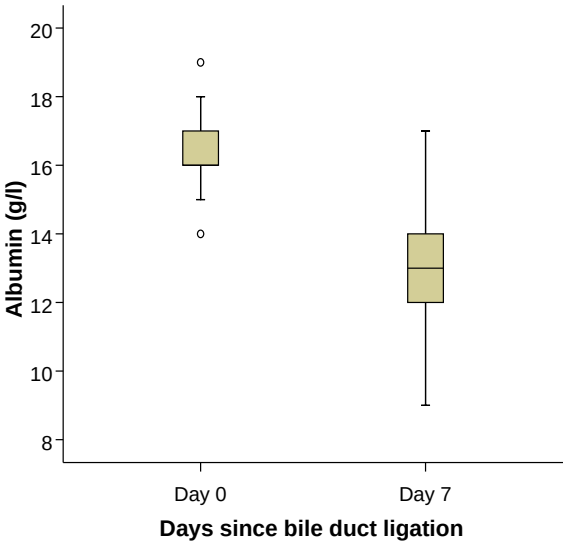


Figure 4.4: Albumin at baseline and after 7 days of bile duct ligation (n=37, p<0.001)

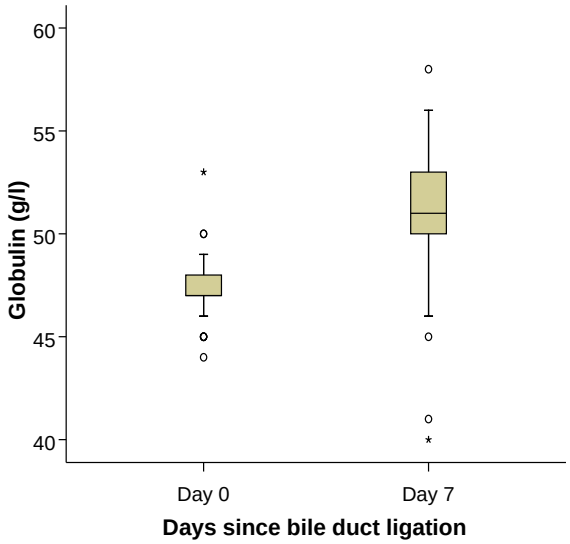


Figure 4.5: Globulin at baseline and after 7 days of bile duct ligation (n=37, p<0.001)

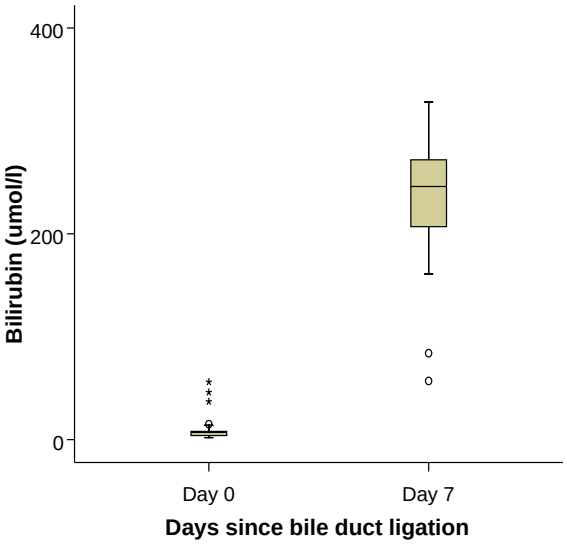


Figure 4.6: Bilirubin at baseline and after 7 days of bile duct ligation (n=37, p<0.001)

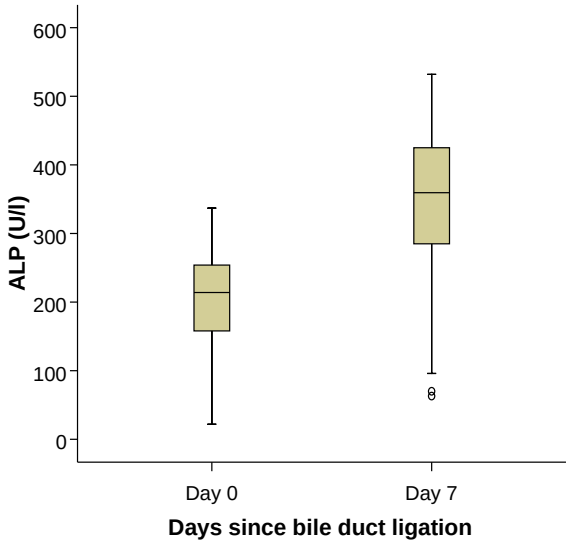


Figure 4.7: ALP at baseline and after 7 days of duct ligation (n=36, p<0.001)

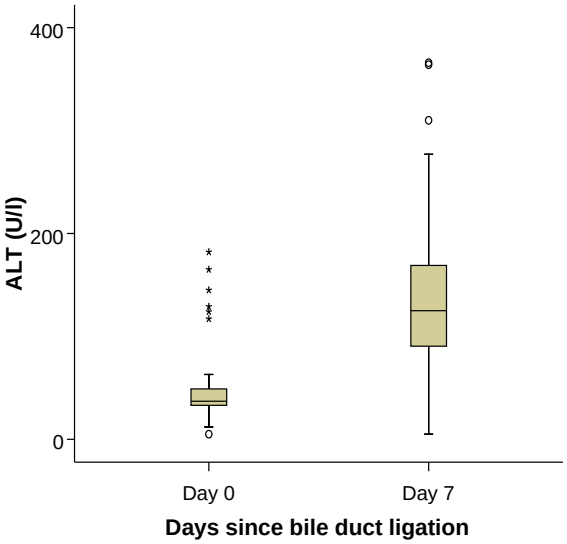


Figure 4.8: ALT at baseline and after 7 days of bile duct ligation (n=35, p<0.001)

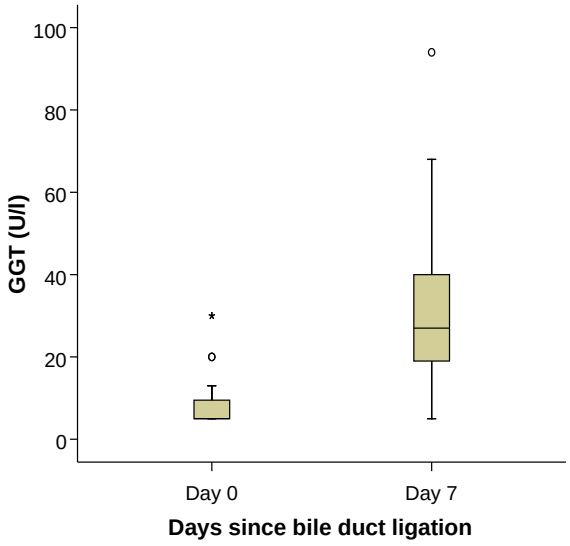


Figure 4.9: GGT at baseline and after 7 days of bile duct ligation (n=28, p<0.001)

Summary of variations observed following bile duct ligation

Table 4.1 (below) summarises the significant differences observed in the variables presented above between the time immediately before bile duct ligation and 7 days after bile duct ligation.

	Overall trend in variable after bile duct ligation
Weight	Decrease ($p<0.001$)
Haemoglobin	None ($p=0.143$)
T Protein	None ($p=0.167$)
Albumin	Decrease ($p<0.001$)
Globulin	Increase ($p<0.001$)
Bilirubin	Increase ($p<0.001$)
ALP	Increase ($p<0.001$)
ALT	Increase ($p<0.001$)
GGT	Increase ($p<0.001$)

Table 4.1: Summary of changes in all animals after seven days of bile duct obstruction.

As one would expect this data shows that after seven days of bile duct ligation there are significant rises in bilirubin, ALP, ALT and GGT. Weight, albumin and globulin fall significantly but with no significant change in haemoglobin or total protein.

4.2: The effect of one sham laparotomy and the recovery period following two sham laparotomies

The following section presents the results of the biometric variables assessed in the sham laparotomy group over the period of their two sham laparotomies and the recovery period after the second sham laparotomy prior to major visceral resection. This data was examined in order to determine the effect of the sham laparotomies on the animals and to determine whether there were any significant changes in the variables being analysed as a result of this or over the period after the sham laparotomies.

4.2.i: The effect of the first sham laparotomy

Figures 4.11 to 4.18 present the data gathered from this group at the time of the first sham laparotomy (labelled "Day 0" in Figures 4.10 to 4.18) and at the time of the second sham laparotomy ("Day 7" in Figures 4.10 to 4.18). The differences seen in the figures are summarised in Table 4.2 below.

The first sham group and timepoints being assessed are demonstrated below. As in the previous section the following chart graphically represents the groups being analysed. The area of the chart shaded in light blue represents the groups that are presented in the following figures (Figure 4.10 to 4.18).

Day	Group One	Group Two	Group Three	Group Four
0		Sham	Bile Duct Ligation	Bile Duct Ligation
7		Sham	External Drainage	Internal Drainage
		Bleed	Bleed	Bleed
	Bile Duct Ligation	Bleed	Bleed	Bleed
	MVR	MVR	MVR	MVR
	Bleed	Bleed	Bleed	Bleed
	Sacrifice	Sacrifice	Sacrifice	Sacrifice

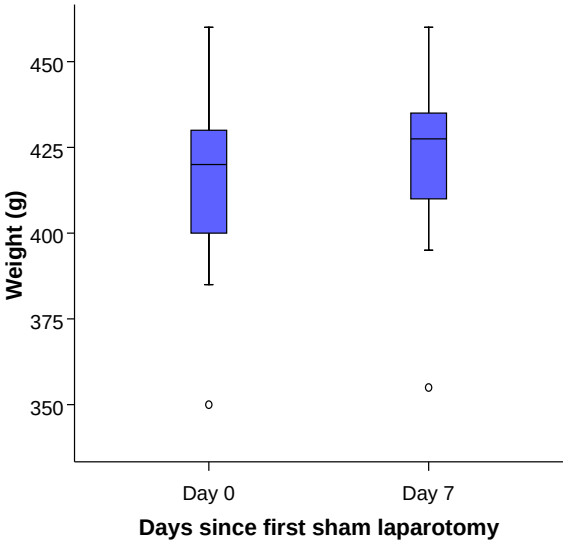


Figure 4.10: Weight at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.026)

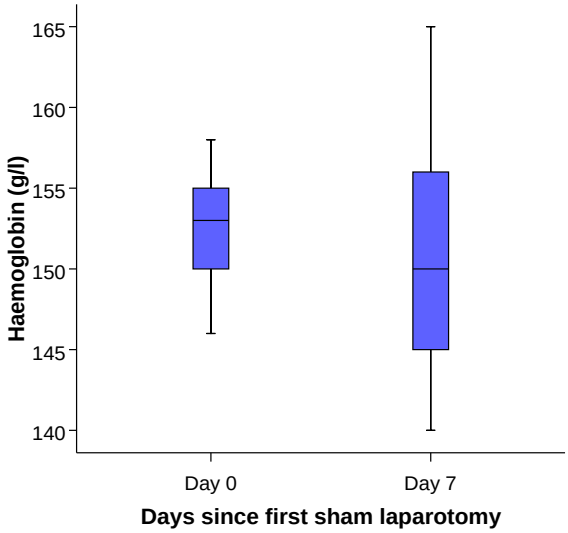


Figure 4.11: Haemoglobin at the time of the first (Day 0) and second (Day 7) sham laparotomy (n=17, p=0.191)

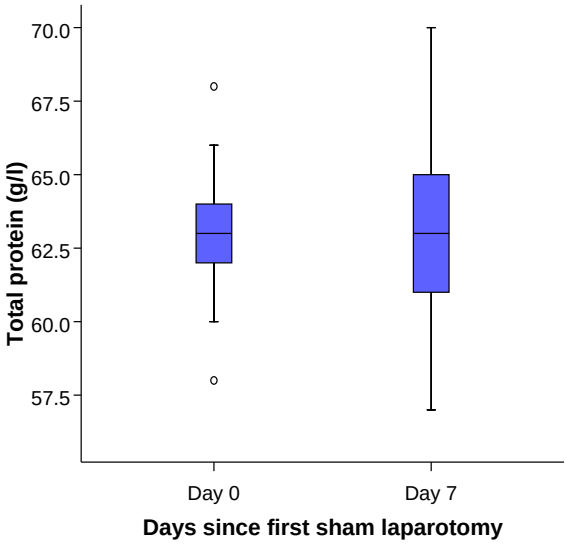


Figure 4.12: Total protein at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.841)

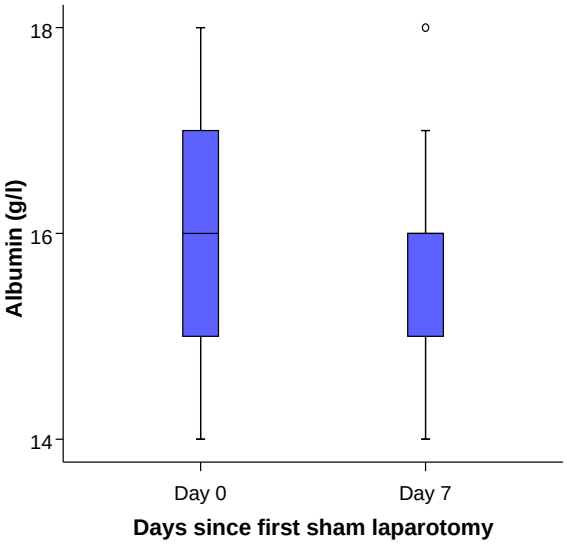


Figure 4.13: Albumin at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.713)

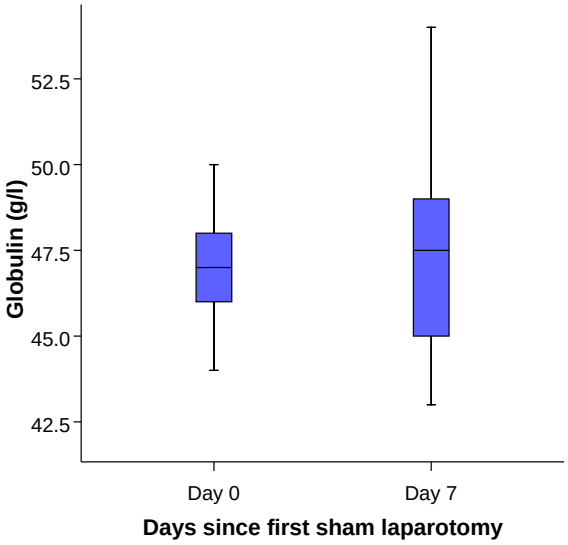


Figure 4.14: Globulin at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.854)

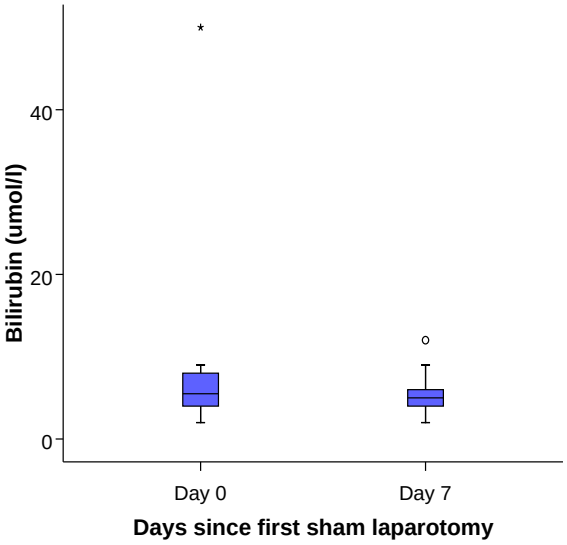


Figure 4.15: Bilirubin at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.291)

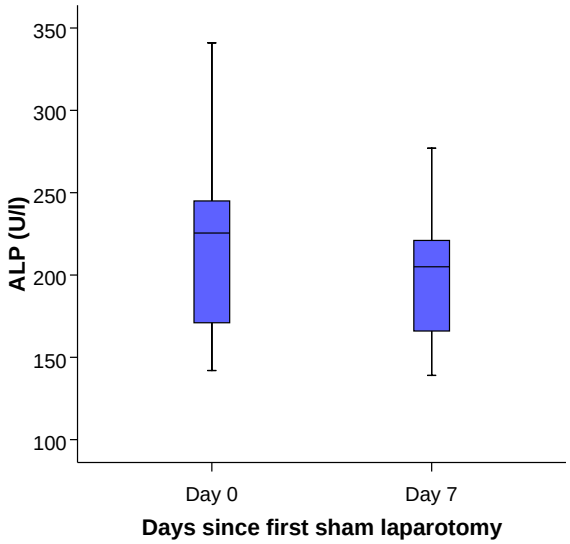


Figure 4.16: ALP at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.049)

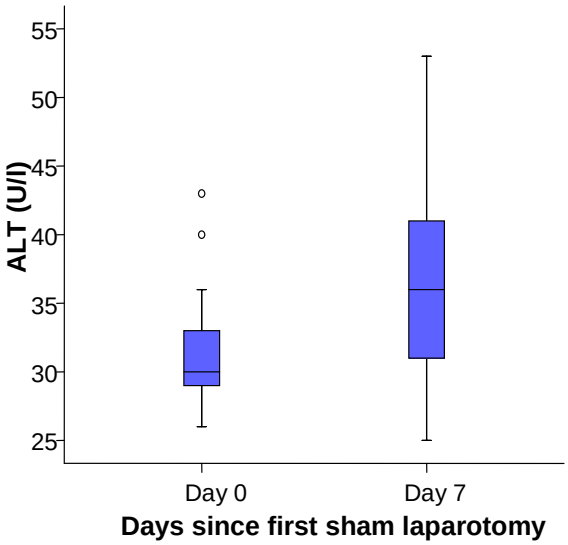


Figure 4.17: ALT at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.002)

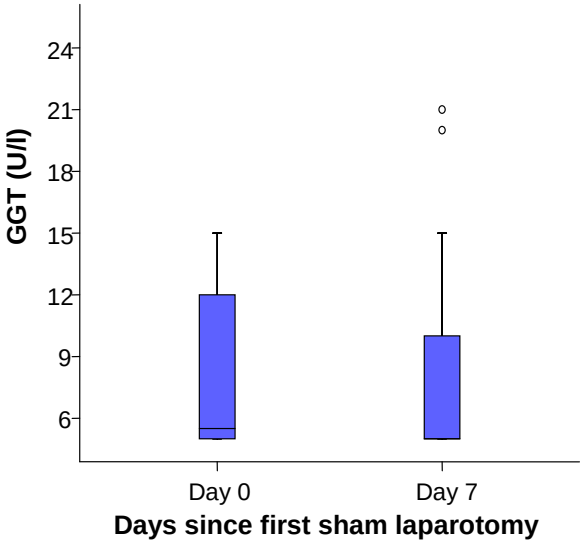


Figure 4.18: GGT at the time of the first (Day 0) and second (Day 7) sham laparotomies (n=18, p=0.959)

Summary of changes following first sham laparotomy

Table 4.2 (below) summarises the significant differences observed in the variables presented above (Figure 4.10–4.18) from the time of the first sham laparotomy and then 7 days later.

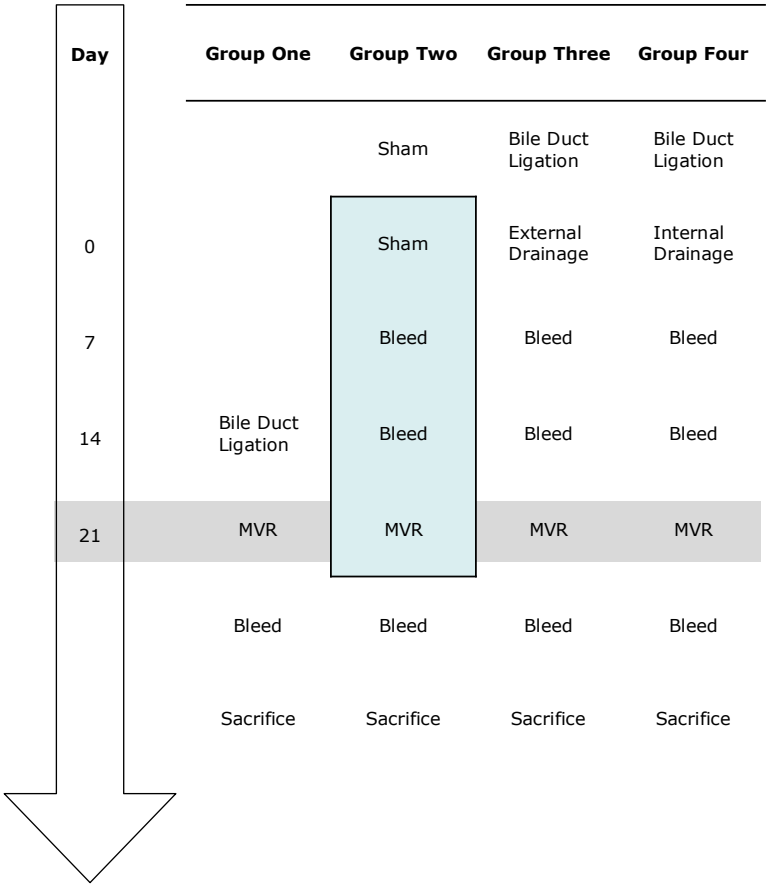
	Direction of change
Weight	Increase (p=0.026)
Haemoglobin	None (p=0.191)
T Protein	None (p=0.814)
Albumin	None (p=0.713)
Globulin	None (p=0.854)
Bilirubin	None (p=0.291)
ALP	Decrease (p=0.049)
ALT	Increase (p=0.002)
GGT	None (p=0.959)

Table 4.2: Summary of changes seven days after the first sham laparotomy in Group 2 (sham)

This data demonstrates that after a sham laparotomy the sham animals' weight continues to increase significantly. Although the rise in ALT and decrease in ALP are statistically significant the absolute values remained within normal parameters in all animals.

4.2.ii: The recovery period following two sham laparotomies

The second part of these results looks at the time between the two sham laparotomies until the MVR. Figures 4.19 to 4.27 present the values of the measured variables at four time points. The first time point (Day 0 in the figures below) is the day of the second sham laparotomy. The other time points are one-week intervals from the day of the second sham laparotomy to the day of major visceral resection (Day 21 in the figures below). Comparisons have been made to assess overall variation (Freidman test) and variation between each consecutive time point. The group and timepoints being measured are demonstrated graphically below. The area of the chart shaded in light blue represents the groups that are presented in the following figures (Figure 4.19 to 4.27). The changes shown are summarised below in table 4.3 below.



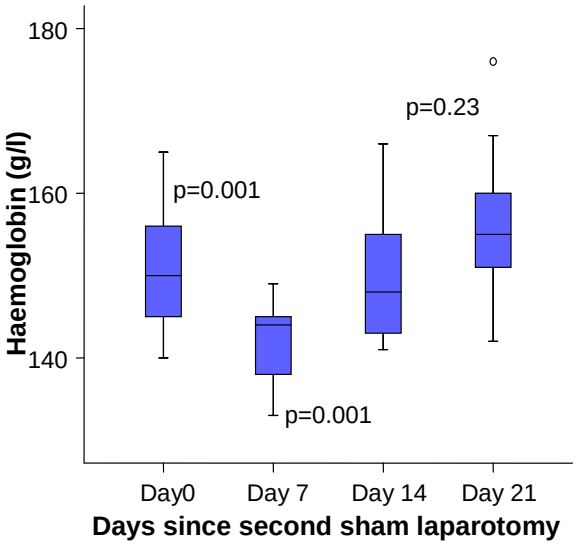


Figure 4.19: Haemoglobin levels from the time of second sham laparotomy until MVR. P values shown on figure (and those that follow) are for the comparisons between consecutive time points (n=18, Friedman Chi-Square =50.897, df=3, p<0.001)

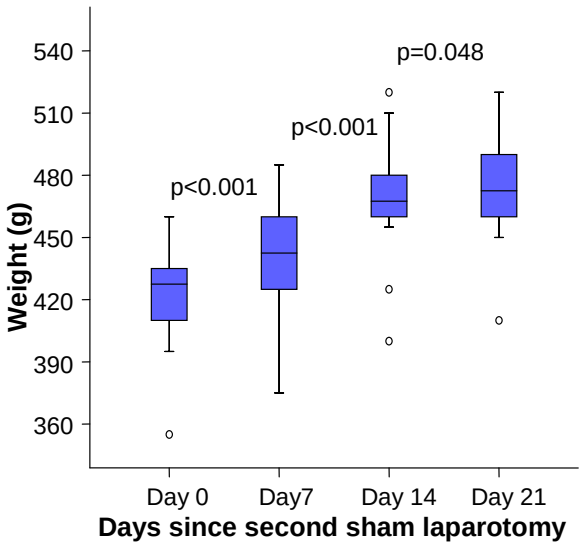


Figure 4.20: Weight from the time of second sham laparotomy until MVR (n=18, Friedman Chi-squared= 50.897, df=3, p<0.001)

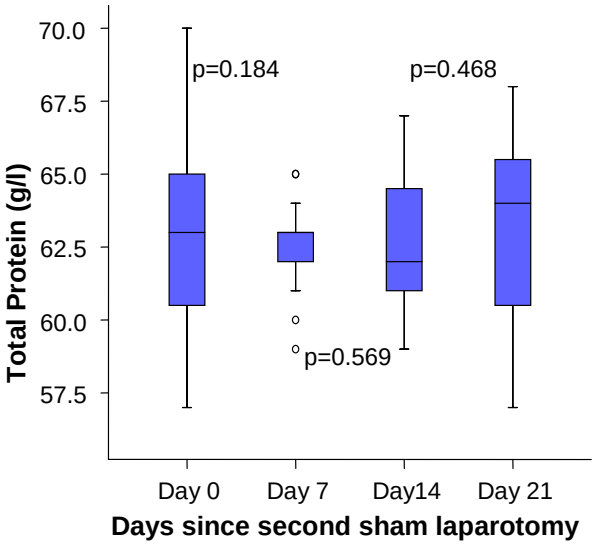


Figure 4.21: Total protein levels from the time of second sham laparotomy until MVR (n=17, Friedman Chi-Square=1.791, df=3, p=0.617)

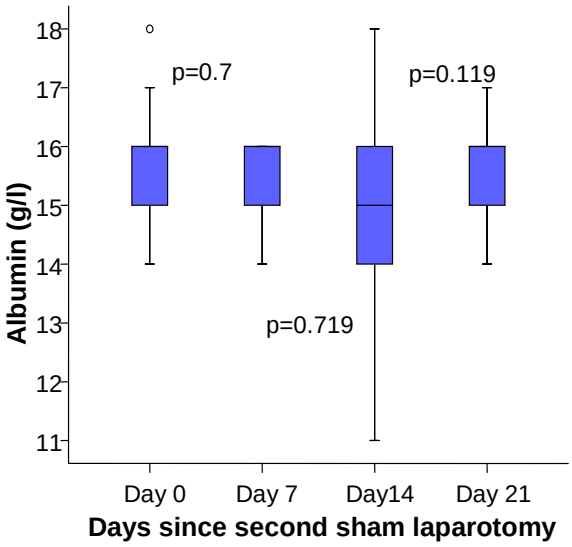


Figure 4.22: Albumin from the time of second sham laparotomy until MVR (n=15, Friedman Chi-Square=3.026, df=3, p=0.388)

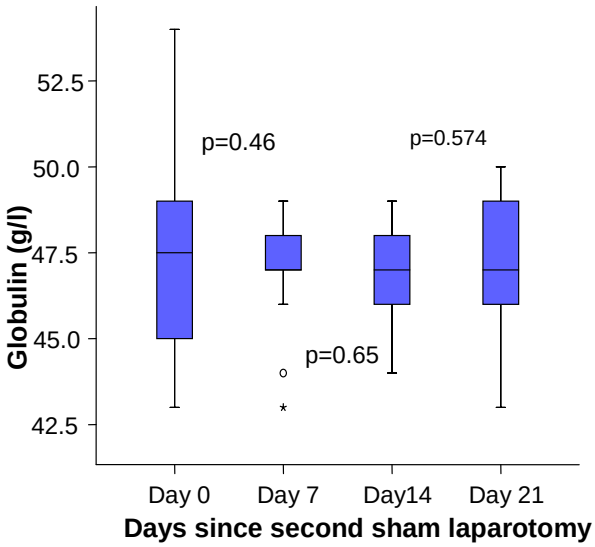


Figure 4.23: Globulin from the time of second sham laparotomy until MVR (n=14, Friedman Chi-Squared=0.471, df=3, p=0.925)

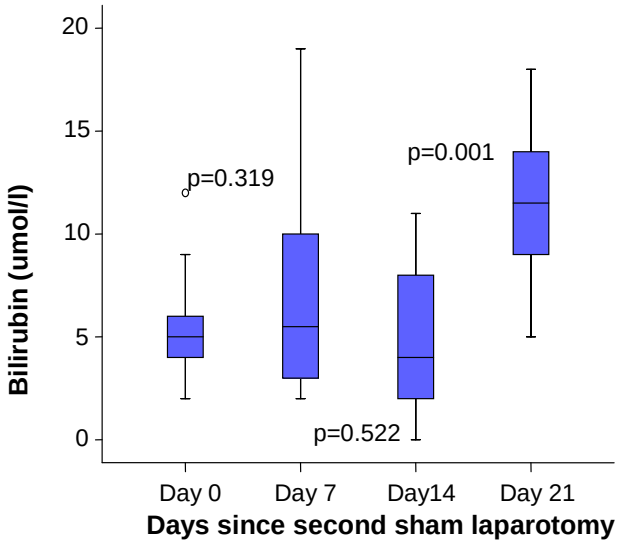


Figure 4.24: Bilirubin levels from the time of second sham laparotomy until MVR (n=18, Friedman Chi-Square=22.622, df=3, p<0.001)

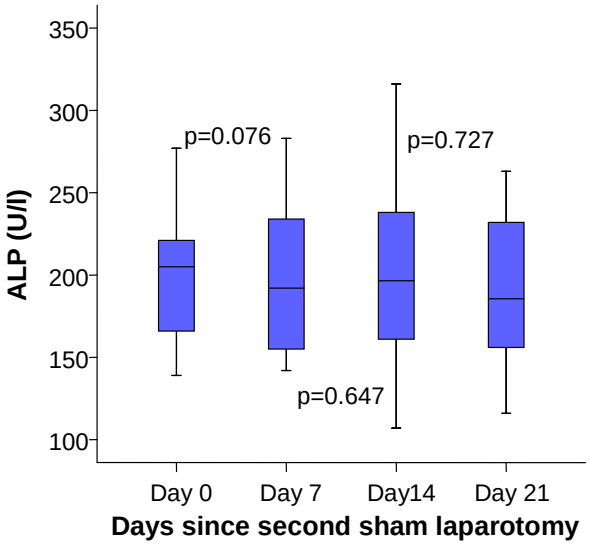


Figure 4.25: ALP from the time of second sham laparotomy until MVR (n=18, Friedman Chi-Square=2.832, df=3, p=0.418)

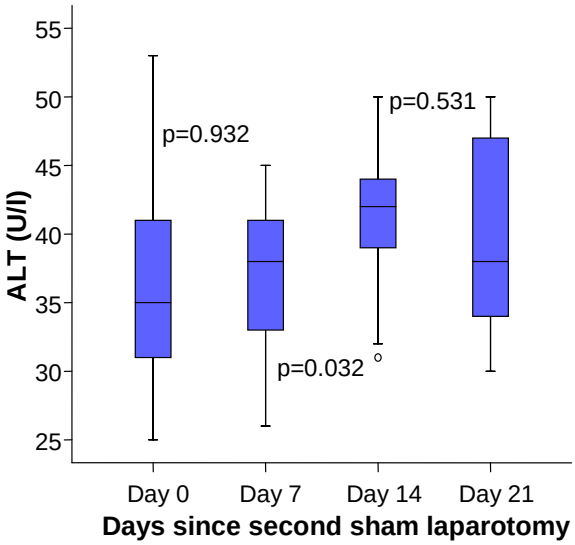


Figure 4.26: ALT from the time of second sham laparotomy until MVR (n=17, FriedmanChi-Square=7.785, df=3, p=0.051)

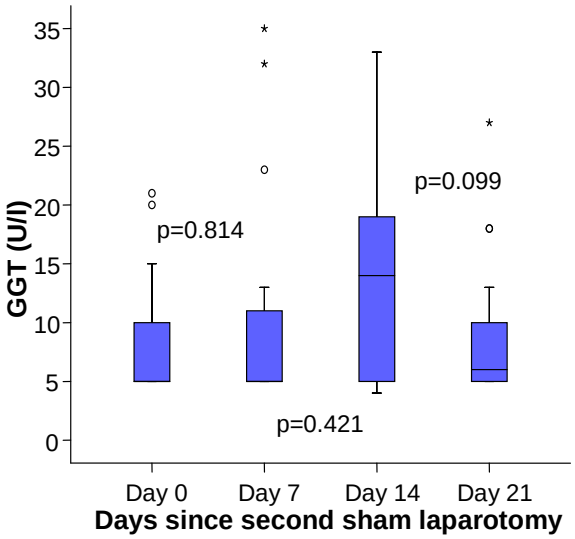


Figure 4.27: GGT from the time of second sham laparotomy until MVR (n=17, Friedman Chi-Square=2.750, df=3, p=0.432)

Summary of changes following second sham laparotomy

Table 4.3 below summarises the statistical analyses performed on the data presented in Figures 4.19 to 4.27 above from the time of the second sham laparotomy until the time of the major visceral resection (21 days after the second sham laparotomy).

Variable	Variability overall?	Time period (days) and direction of change		
		0-7	7-14	14-21
Weight	Yes (p<0.001)	Increase (p<0.001)	Increase (p<0.001)	Increase (p=0.048)
Haemoglobin	Yes (p<0.001)	Decrease (p=0.001)	Increase (p=0.001)	None (p=0.23)
Total Protein	No (p=0.617)	None (p=0.184)	None (p=0.569)	None (p=0.468)
Albumin	No (p=0.388)	None (p=0.7)	None (p=0.719)	None (p=0.119)
Globulin	No (p=0.925)	None (p=0.46)	None (p=0.65)	None (p=0.574)
Bilirubin	Yes (p<0.001)	None (p=0.319)	None (p=0.522)	Increase (p<0.001)
ALP	No (p=0.418)	None (p=0.076)	None (p=0.647)	None (p=0.727)
ALT	No (p=0.051)	None (p=0.932)	Increase (p=0.032)	None (p=0.531)
GGT	No (p=0.432)	None (p=0.814)	None (p=0.421)	None (p=0.099)

Table 4.3: Summary of changes between second sham laparotomy and major visceral resection

These results show that although there was significant variation in bilirubin in total over the 3 week period, this changed occurred only from day 14-21. The change in median bilirubin was from 4umol/l to 11.5umol/l which is still within the starting range of bilirubin

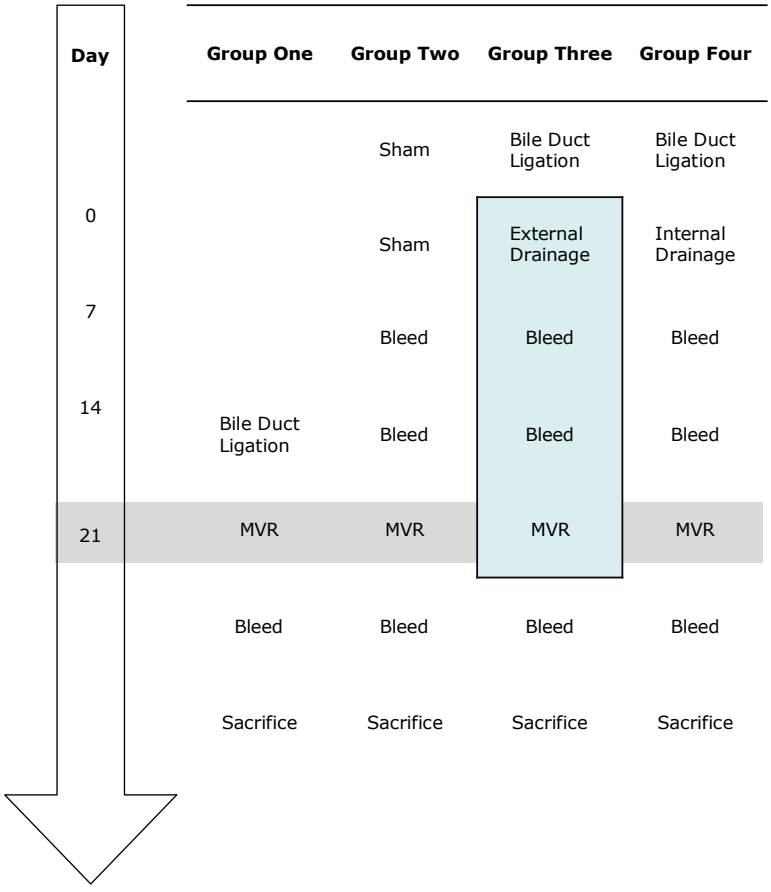
for all rats. Weight continued to rise in this group and haemoglobin initially fell for the first time before recovering again.

4.3: The effect of external and internal biliary drainage prior to MVR

4.3.i: External biliary drainage

In order to assess the effect of external drainage of the obstructed biliary system each variable was measured at the time of the drainage procedure and 7, 14 and 21 days following biliary drainage. The following graphs (Figures 4.28-4.36) show the values of each measured variable at these time points and comparisons have been made to assess overall variation (Freidman test) and variation between each consecutive time point.

The following chart demonstrates (in light blue) the group and time points being assessed in Figures.4.28 to 4.36, below. The differences seen in the figures are summarised in Table 4.3 below.



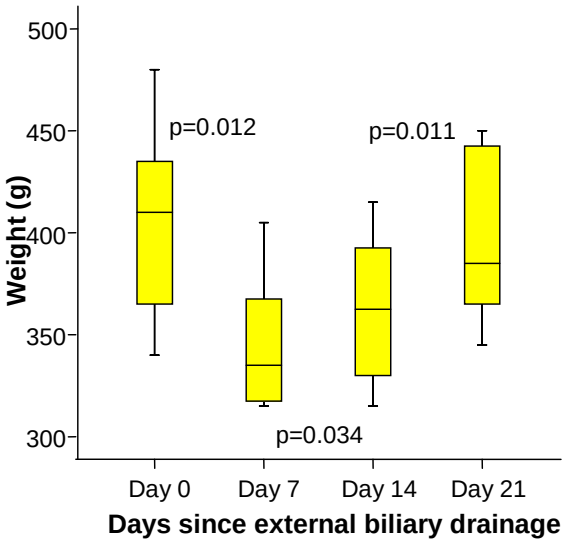


Figure 4.28: Weight following external biliary drainage (n=8, Friedman Chi-Square=17.808, df=3, p<0.001). P values shown on figure (and those that follow) are for the comparisons between consecutive time points.

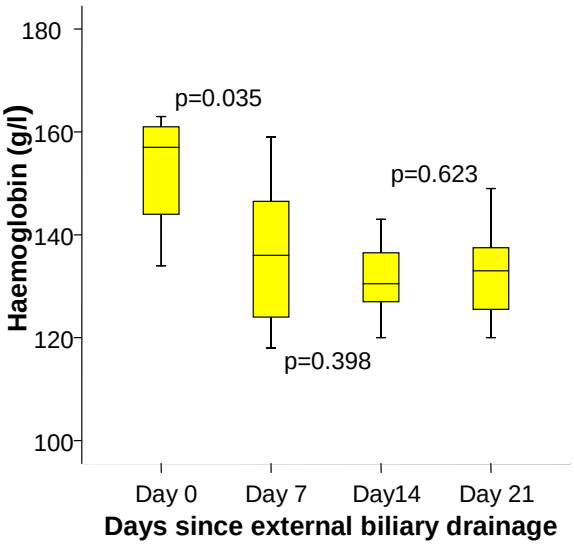


Figure 4.29: Haemoglobin following external biliary drainage (n=8, Friedman Chi-Square=8.241, df=3, p=0.041)

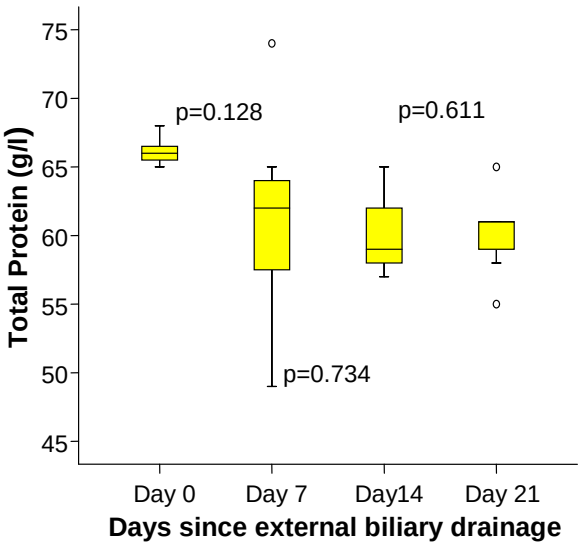


Figure 4.30: Total protein following external biliary drainage (n=7, Friedman Chi-Square=10.826, df=3, p=0.013)

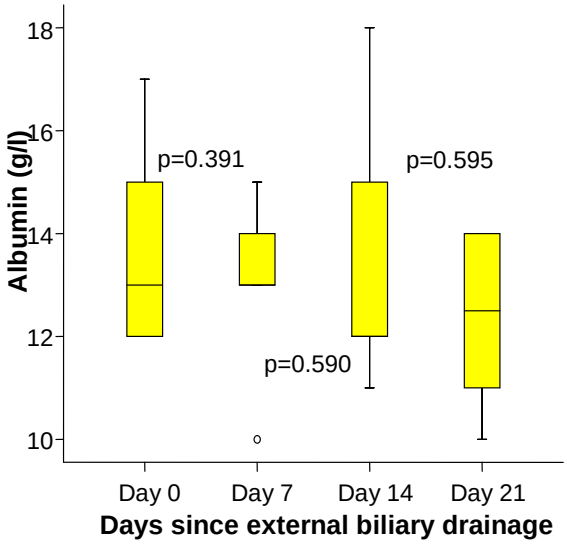


Figure 4.31: Albumin levels following external biliary drainage (n=6, Friedman Chi-Square=0.944, df=3, p=0.815)

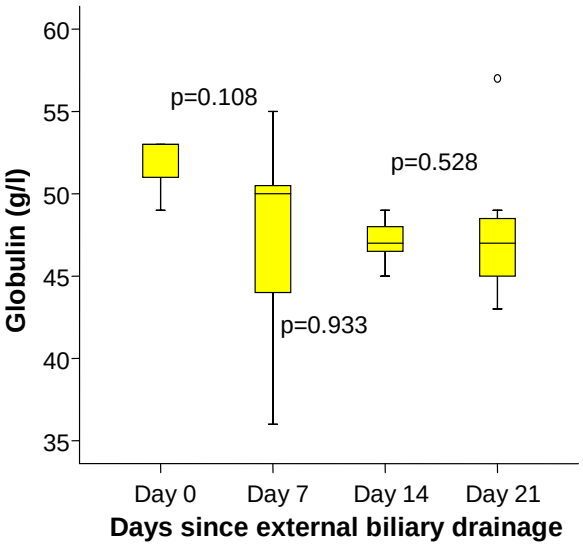


Figure 4.32: Globulin following external biliary drainage (n=7, Friedman Chi-Square =7.147, df=3, p=0.067)

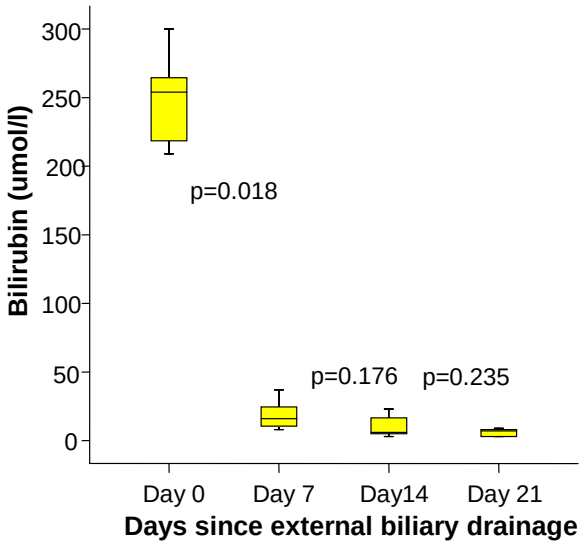


Figure 4.33: Bilirubin following external biliary drainage (n=7, Friedman Chi-Square=17.870, df=3, p<0.001)

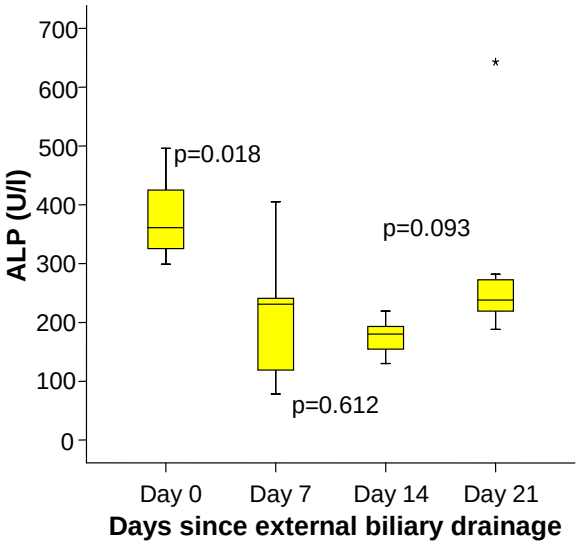


Figure 4.34: ALP following external biliary drainage (n=7, Friedman Chi-Square =16.20, df=3, p=0.001)

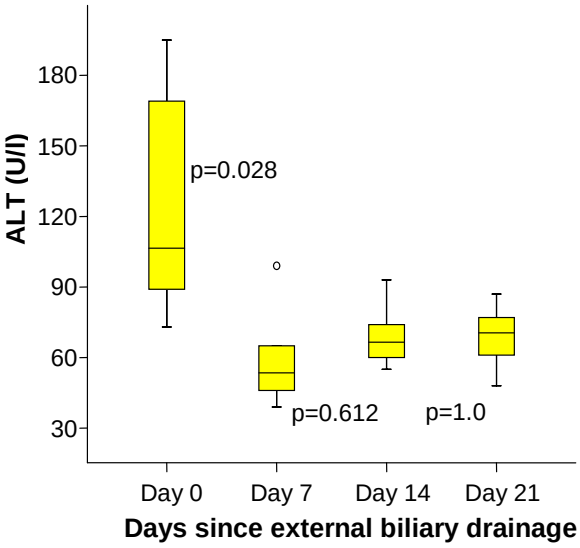


Figure 4.35: ALT following external biliary drainage (n=6, Friedman Chi-Square=7.80, df=3, p=0.050)

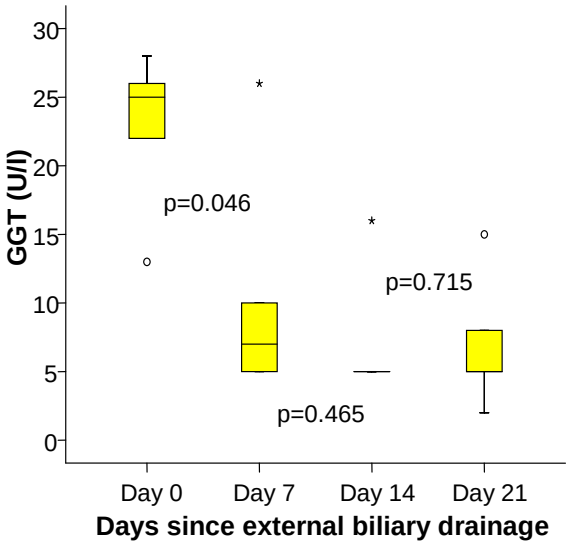


Figure 4.36: GGT following external biliary drainage (n=5, Friedman Chi-Square=9.00, df=3, p=0.029)

Summary of changes following external biliary drainage to day of MVR

Table 4.4 (below) summarises the results of the statistical analysis of the variation seen after external biliary drainage until the time of major visceral resection as shown in Figures 4.28-4.36.

Variable	Variability overall?	Time period (days) and direction of change		
		0-7	7-14	14-21
Weight	Yes (p<0.001)	Decrease (p=0.012)	Increase (p=0.034)	Increase (p=0.011)
Haemoglobin	Yes (p=0.042)	Decrease (p=0.035)	None (p=0.398)	None (p=0.623)
Total Protein	Yes (p=0.013)	None (p=0.128)	None (p=0.734)	None (p=0.611)
Albumin	No (p=0.815)	None (p=0.391)	None (p=0.590)	None (p=0.595)
Globulin	No (p=0.067)	None (p=0.108)	None (p=0.933)	None (p=0.528)
Bilirubin	Yes (p<0.001)	Decrease (p=0.018)	None (p=0.176)	None (p=0.235)
ALP	Yes (p=0.001)	Decrease (p=0.018)	None (p=0.612)	None (p=0.093)
ALT	No (p=0.050)	Decrease (p=0.028)	None (p=0.398)	None (p=1.0)
GGT	Yes (p=0.029)	Decrease (p=0.046)	None (p=0.465)	None (p=0.715)

Table 4.4: Summary of changes following external biliary drainage, prior to major visceral resection.

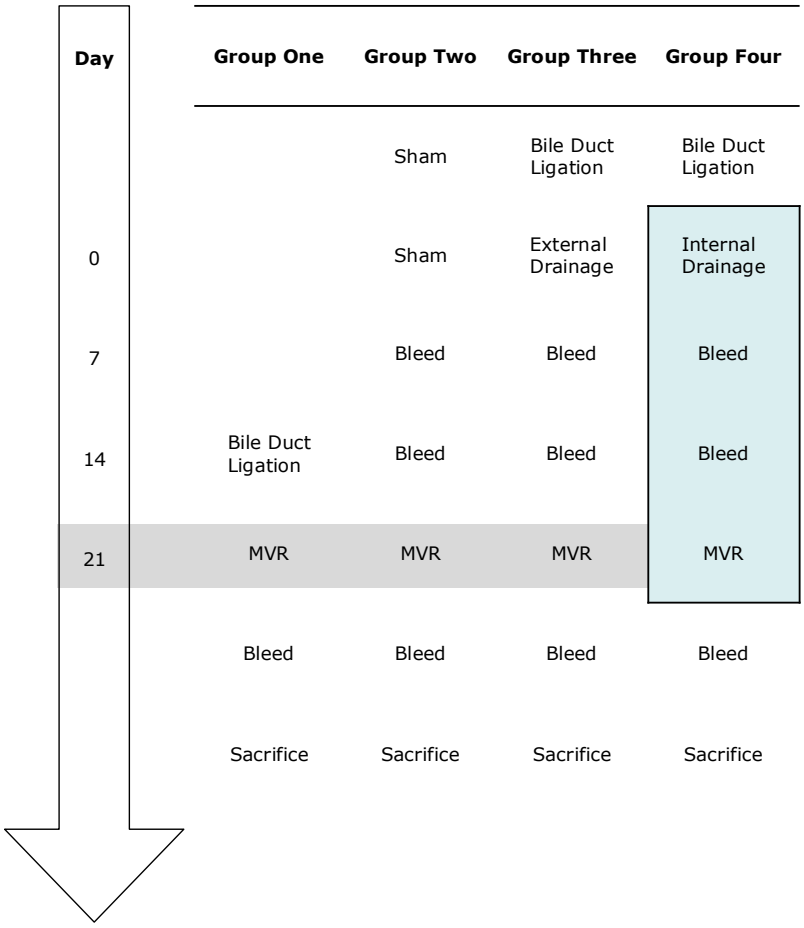
As expected there was a significant variation in the majority of the variables examined. Weight decreased significantly and then continued to rise at each measured timepoint thereafter until MVR. The LFTs all fell early and then no further significant

changes occurred after this over the three week drainage period. Albumin and globulin did not show any change at all over the three week drainage period i.e. they fell after bile duct ligation but external drainage for three weeks did not increase this. Total protein (Figure 4.30) did show overall variability over the 21 days although there was no significant change between each timepoint measured.

4.3.ii: Internal biliary drainage

In order to assess the effect of internal drainage of the obstructed biliary system each variable was measured at the time of drainage and 7, 14 and 21 days following biliary drainage. The following graphs (Figures 4.37-4.45) show the values of each measured variable at these time points. Comparisons have been made to assess overall variation (Freidman test) and variation between each consecutive time points.

The following chart demonstrates (in light blue) the group and time points being assessed in Figures.4.35-4.45, below. The differences shown are summarised in table 4.5 below.



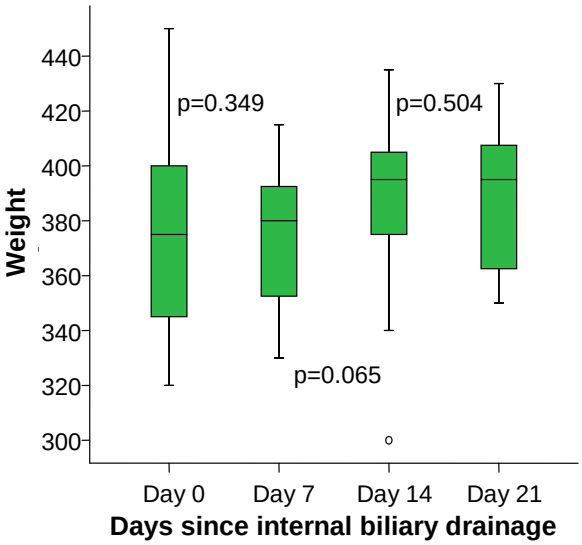


Figure 4.37: Weight following internal biliary drainage. P values shown on figure (and those that follow) are for the comparisons between consecutive time points (n=11, Friedman Chi-Square=8.525, df=3, p=0.036).

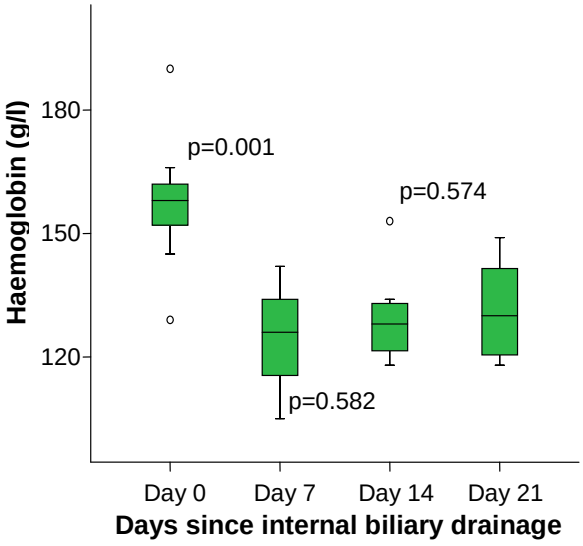


Figure 4.38: Haemoglobin following internal biliary drainage (n=11, Friedman Chi-Square=20.229, df=3, p<0.001).

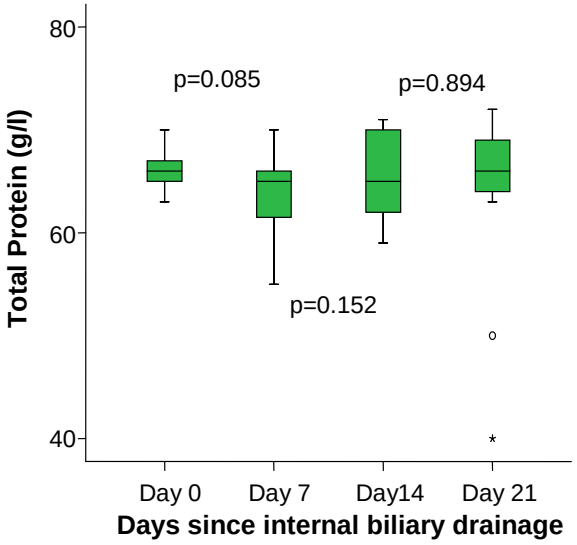


Figure 4.39: Total protein following internal biliary drainage (n=11, Friedman Chi-Square=2.349, df=3, p=0.503).

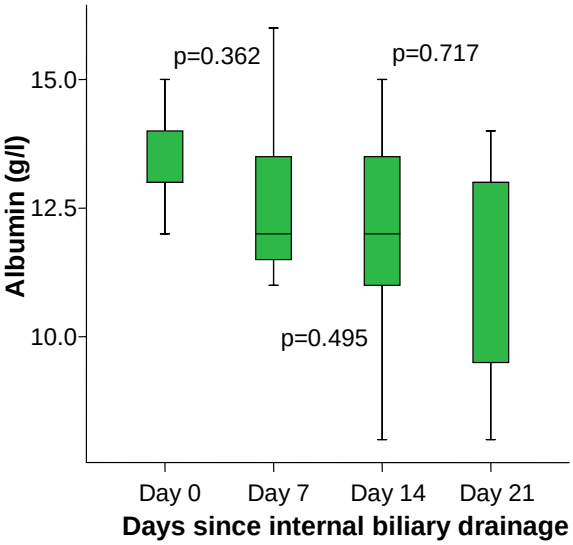


Figure 4.40: Albumin levels following internal biliary drainage (n=11, Friedman Chi-Square=4.129, df=3, p=0.248.

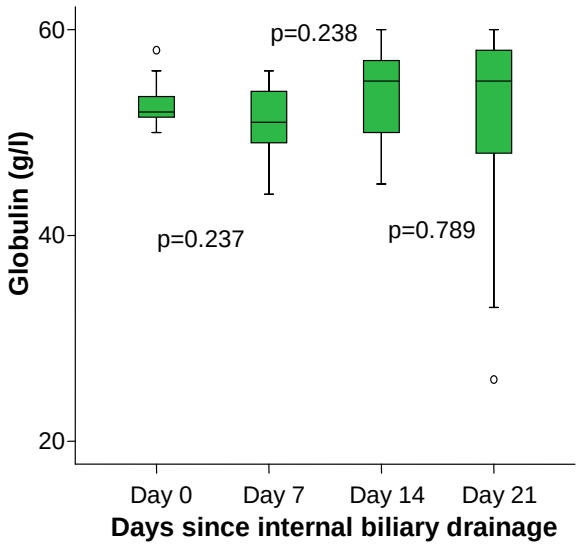


Figure 4.41: Globulin levels following internal biliary drainage (n=11, Friedman Chi-Square=1.679, df=3, p=0.642).

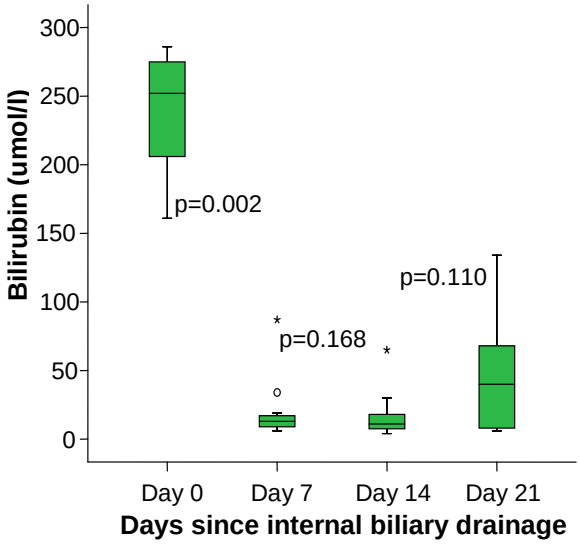


Figure 4.42: Bilirubin following internal biliary drainage (n=11, Friedman Chi Square=21.250, df=3, p<0.001).

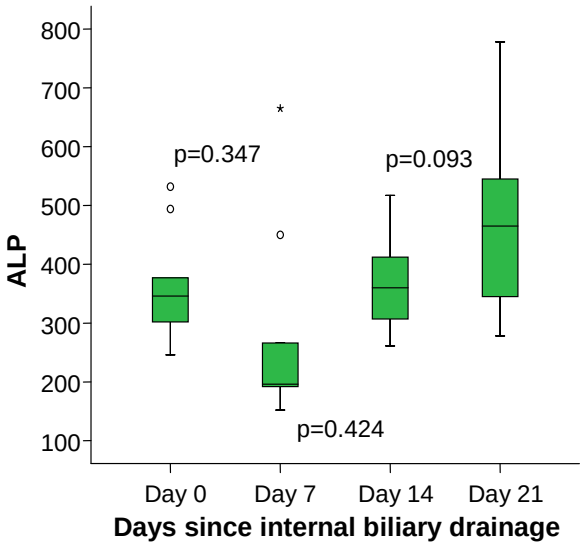


Figure 4.43: ALP following internal biliary drainage (n=9, Friedman Chi-Square=7.533, df=3, p=0.057).

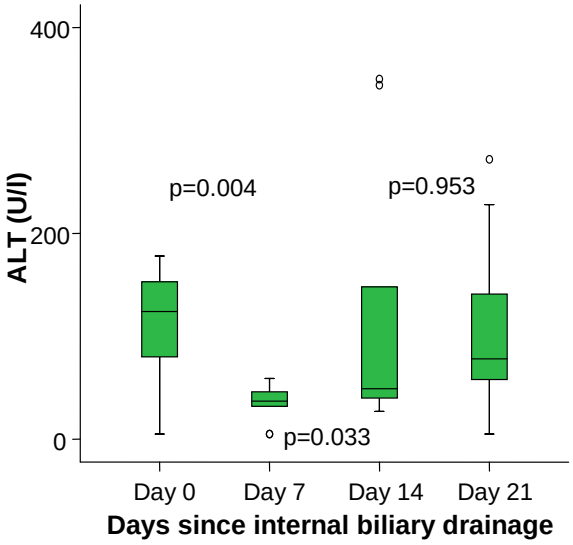


Figure 4.44: ALT following internal biliary drainage (n=9, Friedman Chi-Square=9.876, df=3, p=0.020).

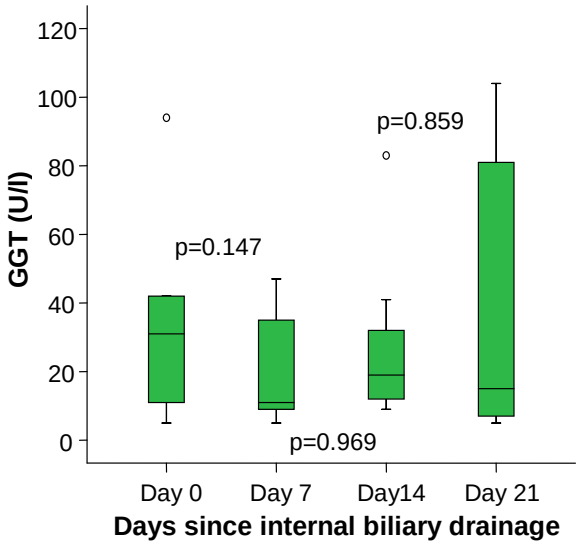


Figure 4.45: GGT following internal biliary drainage (n=9, Friedman Chi-Square=1.4, df=3, p=0.706).

Summary of changes following internal biliary drainage to day of MVR

Table 4.5 (below) summarises the results of the statistical analysis of the variations seen after internal biliary drainage until the time of major visceral resection as shown in Figures 4.37-4.45.

Variable	Variability overall?	Time period (days) and direction of change		
		0-7	7-14	14-21
Weight	No (p<0.36)	None (p=0.349)	None (p=0.065)	None (p=0.504)
Haemoglobin	Yes (p<0.001)	Decrease (p=0.001)	None (p=0.582)	None (p=0.574)
Total Protein	No (p=0.503)	None (p=0.085)	None (p=0.152)	None (p=0.894)
Albumin	No (p=0.248)	None (p=0.363)	None (p=0.495)	None (p=0.717)
Globulin	No (p=0.642)	None (p=0.237)	None (p=0.238)	None (p=0.789)
Bilirubin	Yes (p<0.001)	Decrease (p=0.002)	None (p=0.168)	None (p=0.110)
ALP	No (p=0.057)	None (p=0.347)	None (p=0.424)	None (p=0.093)
ALT	Yes (p=0.020)	Decrease (p=0.004)	Increase (p=0.033)	None (p=0.953)
GGT	No (p=0.706)	None (p=0.147)	None (p=0.969)	None (p=0.859)

Table 4.5: Summary of changes after internal biliary drainage and prior to major visceral resection.

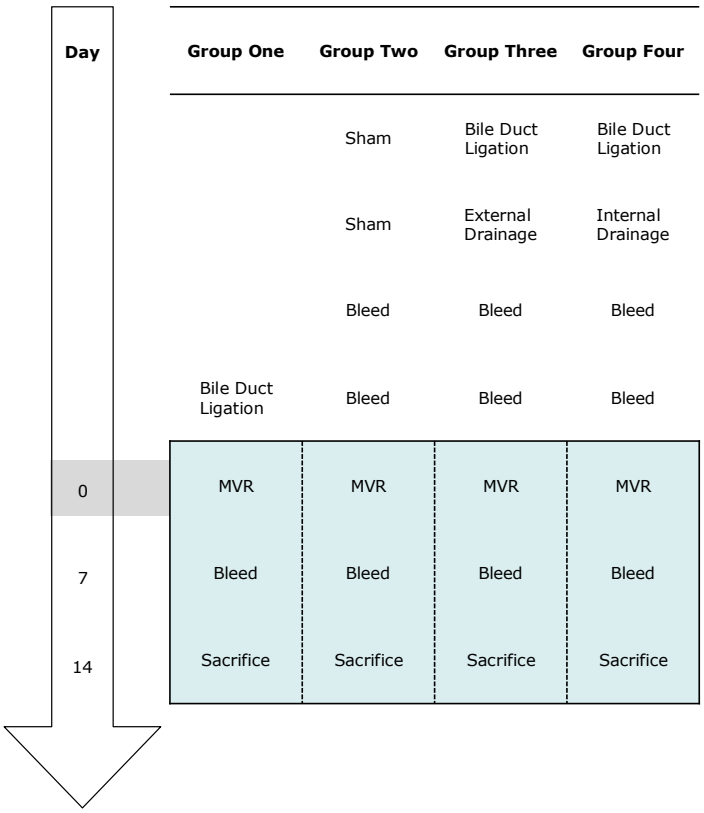
There was no initial fall seen in weight in internally drained rats (as seen in the externally drained rats) but then this group did not continue to gain weight over the three week drainage period. Similar to the external drainage group, significant changes are seen in

the liver function tests but to a lesser extent. There is significant overall variability in bilirubin (Figure 4.42) which fell and remained low (although visually this seems to start to rise at day 21 post drainage). ALT (Figure 4.44) initially fell but then rose again at day 14 and remained elevated. Neither ALP not GGT (Figures 4.43 and 4.45) had significant variation over time and in neither case was there a significant difference between the measured timepoints. This may suggest a degree of ongoing biliary obstruction that was not present in the externally drained group. No significant variation was seen in albumin, total protein or globulin in the internally drained rats which again was a similar finding to the externally drained rats.

4.4: The effect of major visceral resection in all experimental groups

The following graphs compare the changes in biochemical variables following the major visceral resection until the termination of the experiment (Day 14 post MVR). Each variable is compared in all 4 of the experimental groups and presented on a single page.

The timepoints being measured are demonstrated graphically below. The area of the chart shaded in light blue represents the groups that are presented below. These changes are summarised below in Table 4.6.



Group One	Group Two	Group Three	Group Four
	Sham	Bile Duct Ligation	Bile Duct Ligation
	Sham	External Drainage	Internal Drainage
	Bleed	Bleed	Bleed
Bile Duct Ligation	Bleed	Bleed	Bleed
MVR	MVR	MVR	MVR
Bleed	Bleed	Bleed	Bleed
Sacrifice	Sacrifice	Sacrifice	Sacrifice

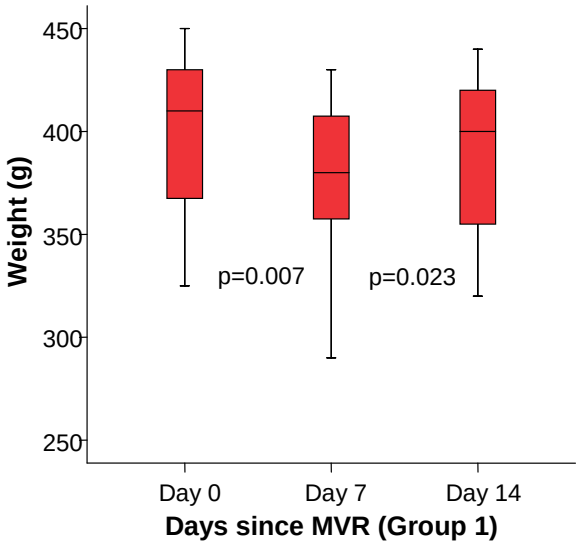


Figure 4.46: Weight following MVR in the undrained group (n=11, Friedman Chi-Square=10.585, df=2, p=0.005)

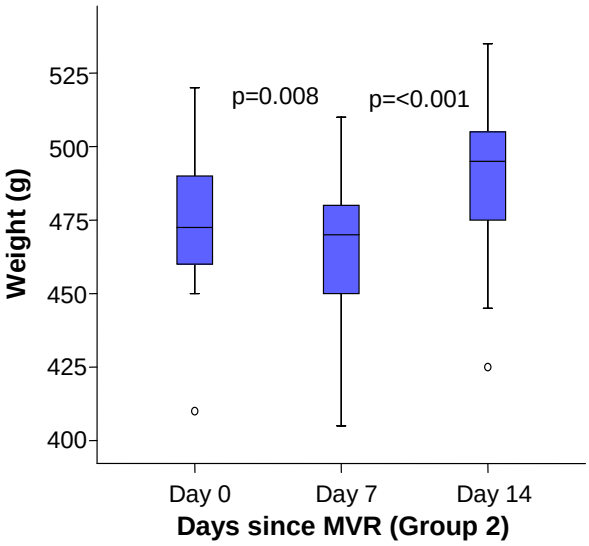


Figure 4.47: Weight following MVR in Sham Group (n=18, Friedman Chi-Square=23.121, df=2, p<0.001)

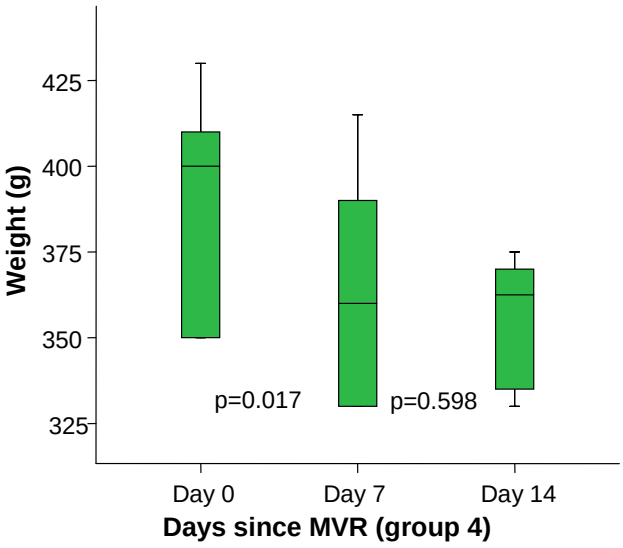


Figure 4.48: Weight following MVR in External Drainage group (n=7, Friedman Chi-Square=6.48, df=2, p=0.039)

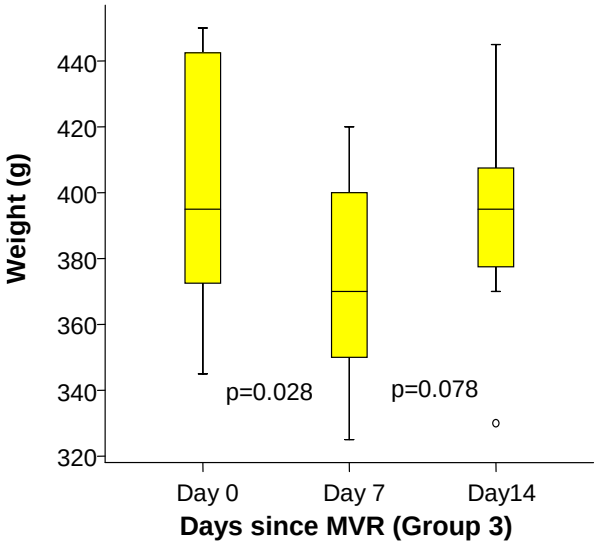


Figure 4.49: Weight following MVR in Internal Drainage group (n=6, Friedman Chi-Square=4.00, df=2, p=0.135)

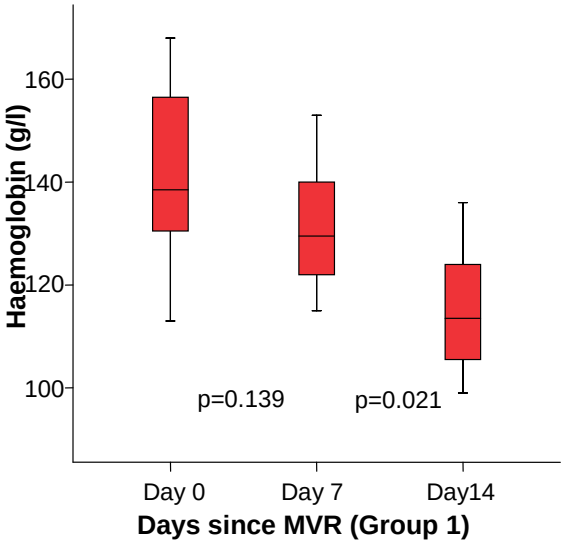


Figure 4.50: Hb following MVR in the undrained group (n=8, Friedman Chi-Square=6.645, df=2, p=0.036).

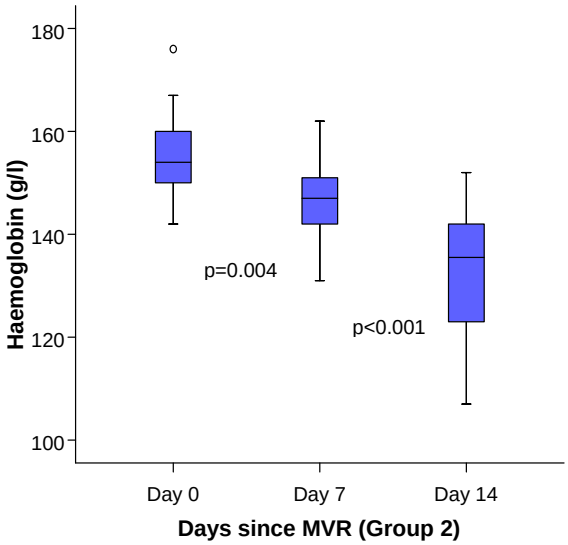


Figure 4.51: Hb following MVR in Sham group (n=18, Friedman Chi-Square=26.778, df=2, p<0.001)

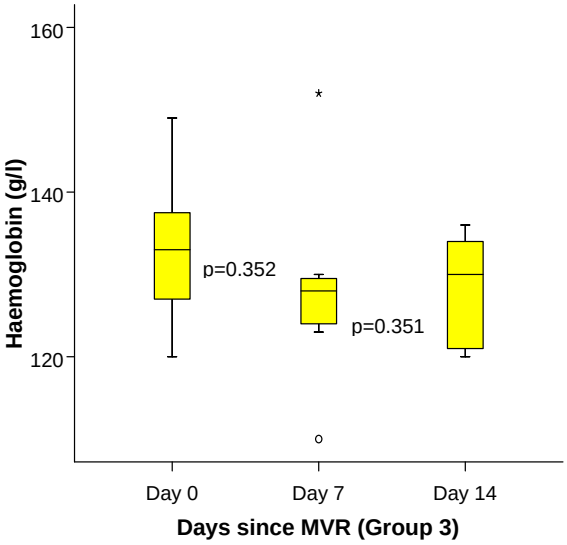


Figure 4.52: Hb following MVR in External Drainage group (n=7, Friedman Chi-Square=2.00, df=2, p=0.368).

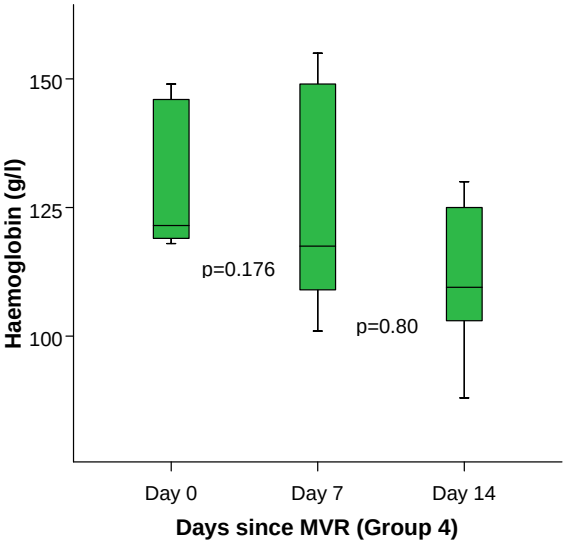


Figure 4.53: Hb following MVR in Internal Drainage group (n=6, Friedman Chi-Square=3.217, df=2, p=0.20).

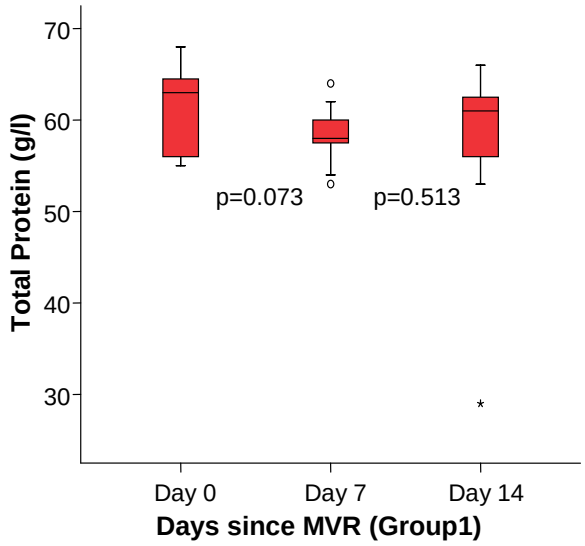


Figure 4.54: Total Protein following MVR in Undrained Group (n=11, Friedman Chi-Square =1.902, df=2, p=0.386).

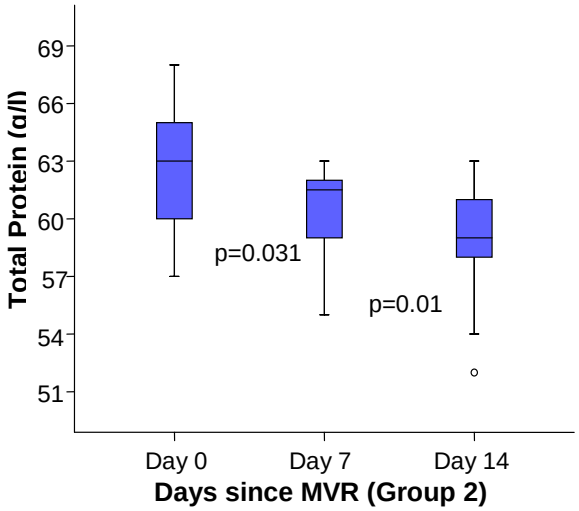


Figure 4.55: Total Protein following MVR in Sham group (n=18, Friedman Chi-Square=14.69, p=0.001).

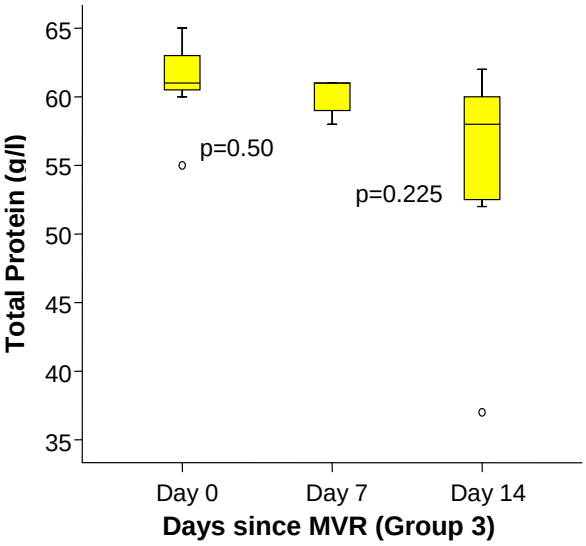


Figure 4.56: Total Protein following MVR in External Drainage group (n=7, Friedman Chi-Square=6.091, df=2, p=0.048)

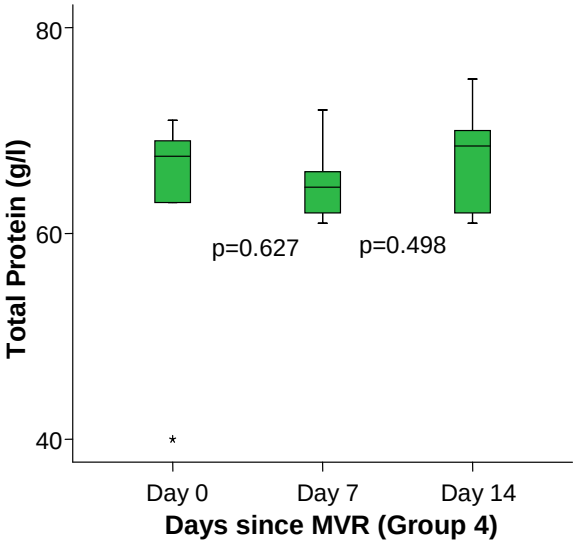


Figure 4.57: Total Protein following MVR in Internal Drainage group (n=6, Friedman Chi-Square= 0.273, df=2, p=0.873).

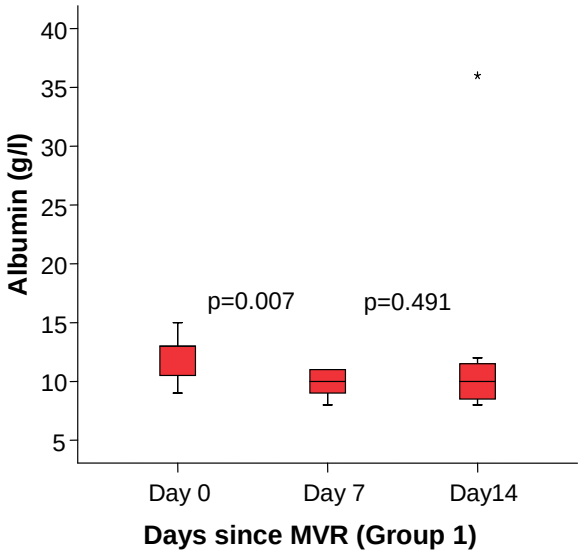


Figure 4.58: Albumin following MVR in Undrained group (n=11, Friedman Chi-Square=7.35, df=2, p=0.025).

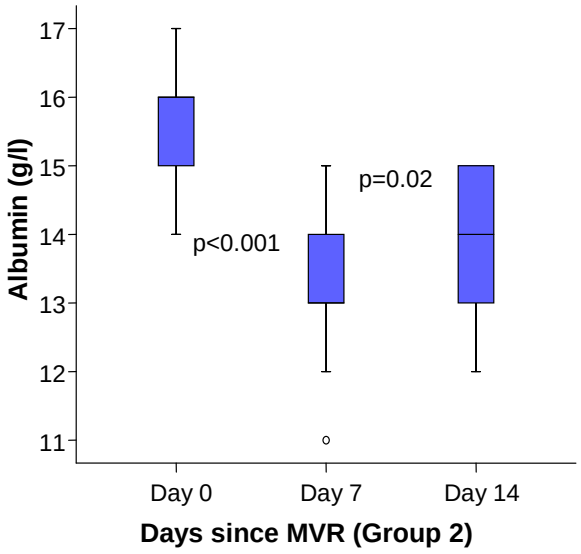


Figure 4.59: Albumin following MVR in Sham group (n=17, Friedman Chi-Square=25.483, df=2, p<0.001).

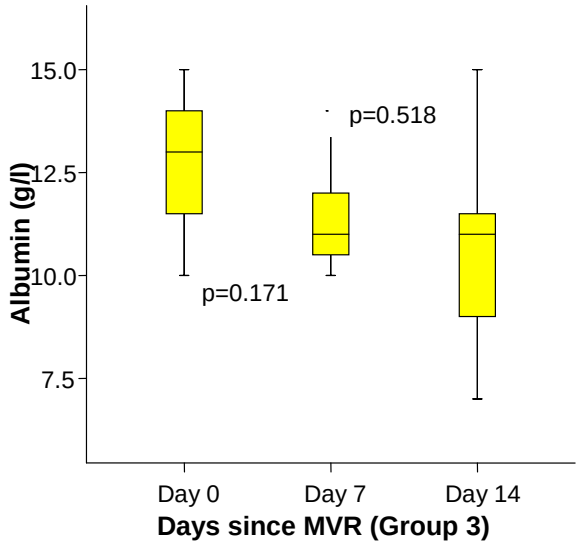


Figure 4.60: Albumin following MVR in External drainage group (n=7, Friedman Chi-Square=4.692, df=2, n=0.096).

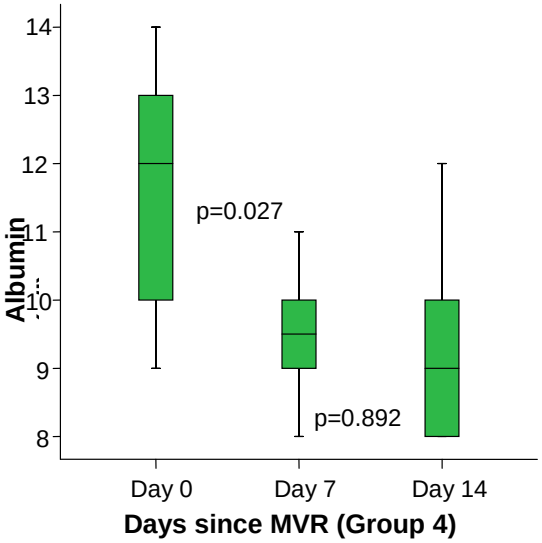


Figure 4.61: Albumin following MVR in Internal drainage group (n=6, Friedman Chi-Square=3.364, df=2, p=0.186).

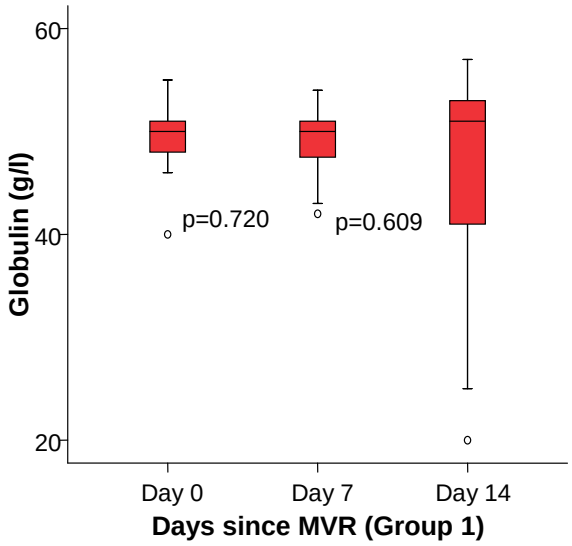


Figure 4.62: Globulin following MVR in the undrained group (n=11, Friedman Chi-Square=2.450, df=2, p=0.294)

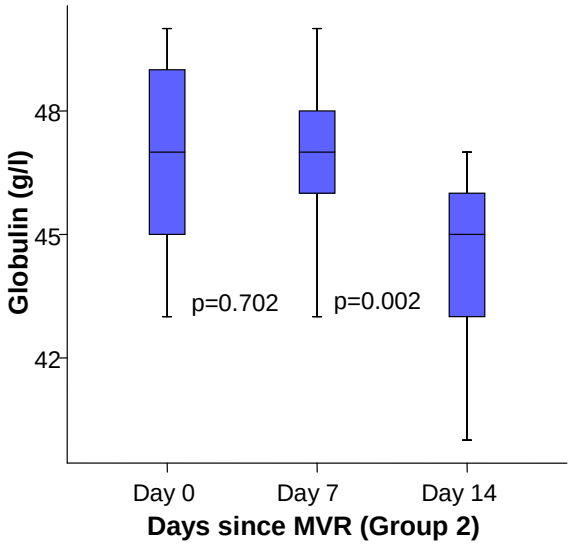


Figure 4.63: Globulin following MVR in the Sham group (n=17, Friedman Chi-Square=13.364, df=2, p=0.001)

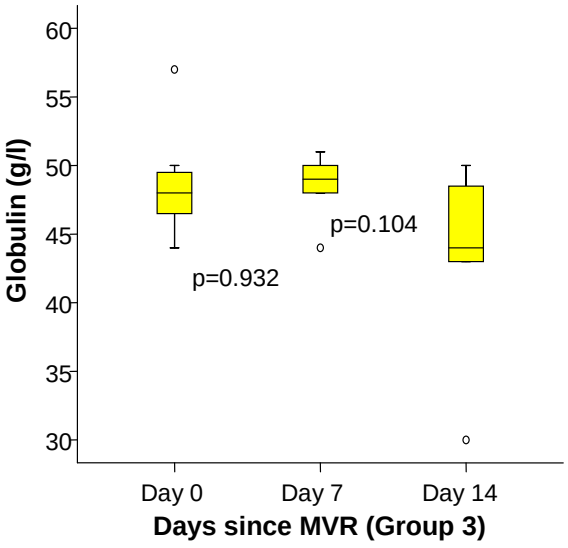


Figure 4.64: Globulin following MVR in External drainage group (n=7, Friedman Chi-(Square=1.68, df=2, p=0.432)

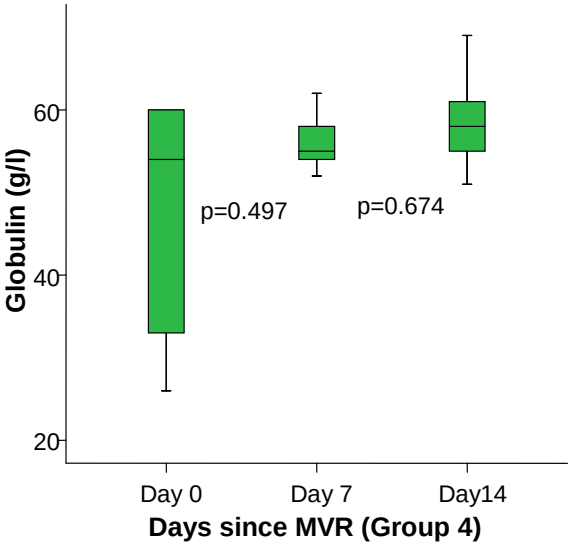


Figure 4.65: Globulin following MVR in Internal drainage group (n=6, Friedman Chi-Square=2.33, p=0.311)

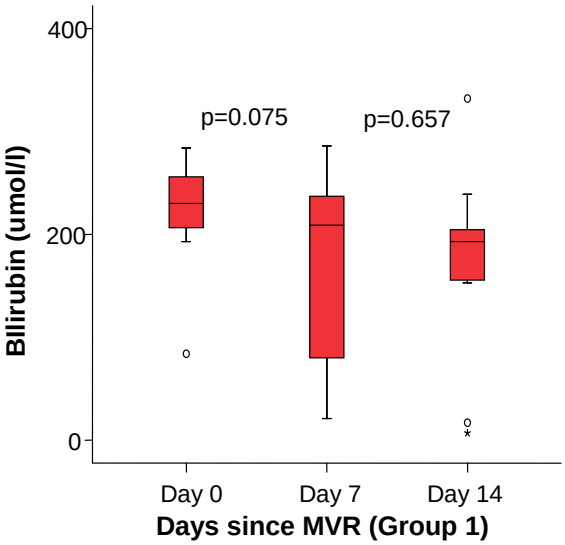


Figure 4.66: Bilirubin following MVR in undrained group (n=11, Friedman Chi-Square=5.628, df=2, p=0.060).

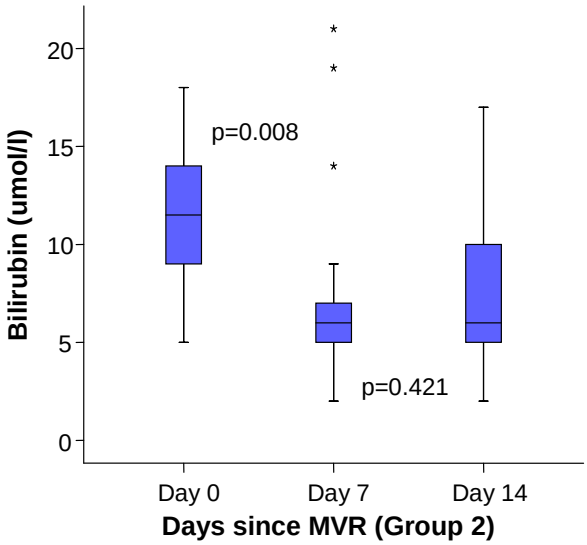


Figure 4.67: Changes in bilirubin following MVR in Sham group (n=18, Friedman Chi-Square=10.246, df=2, p=0.006).

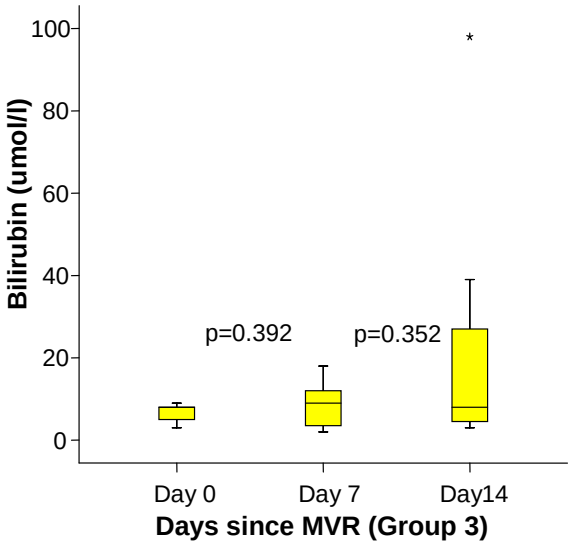


Figure 4.68: Bilirubin following MVR in External drainage group (n=7, Friedman Chi-Square=1.407, df=2, p=0.495).

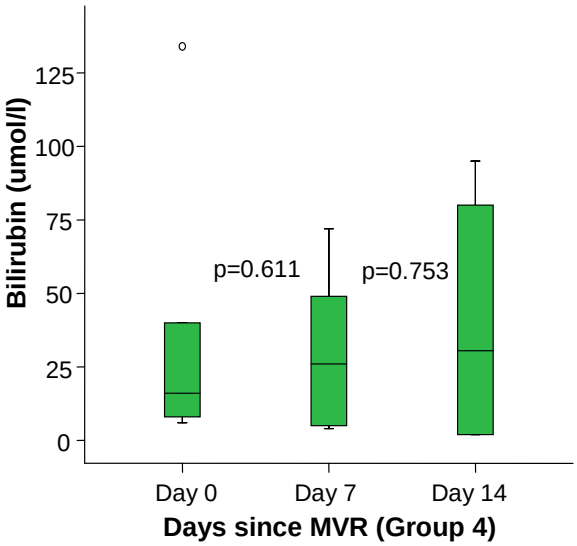


Figure 4.69: Bilirubin following MVR in Internal drainage group (n=6, Friedman Chi-Square=0.0, df=2, p=1.0).

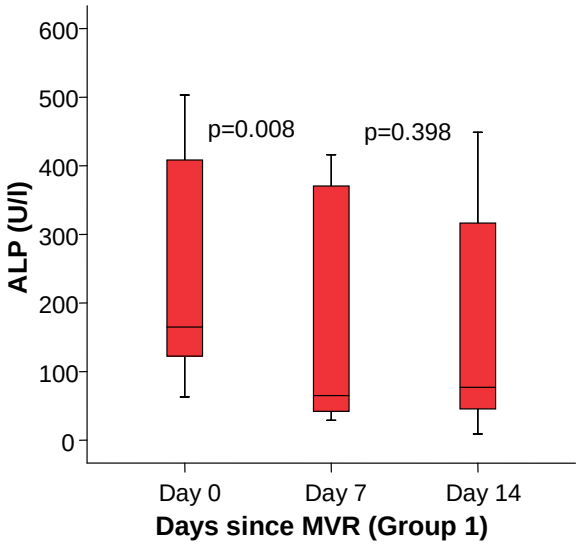


Figure 4.70: ALP following MVR in undrained group (n=11, Friedman Chi-Square=12.182, df=2, p=0.002).

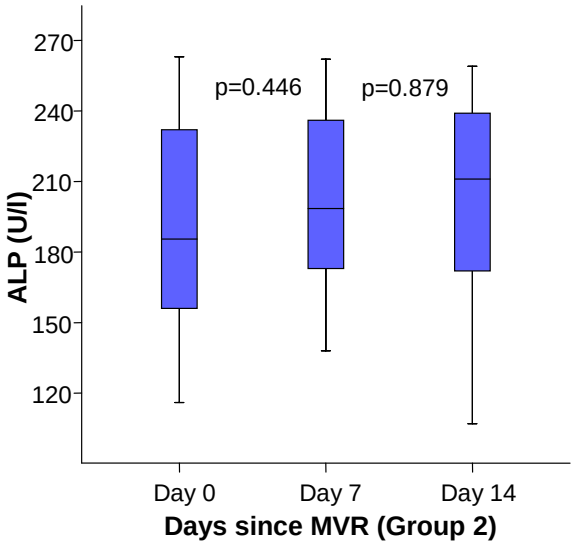


Figure 4.71: ALP following MVR in Sham group (n=18, Friedman Chi-Square=0.444, df=2, p=0.801).

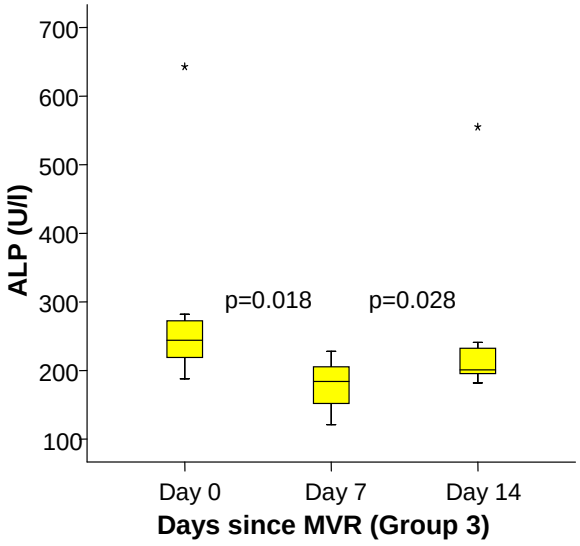


Figure 4.72: ALP following MVR in External drainage group (n=7, Friedman Chi-Square=8.85, df=2, p=0.012).

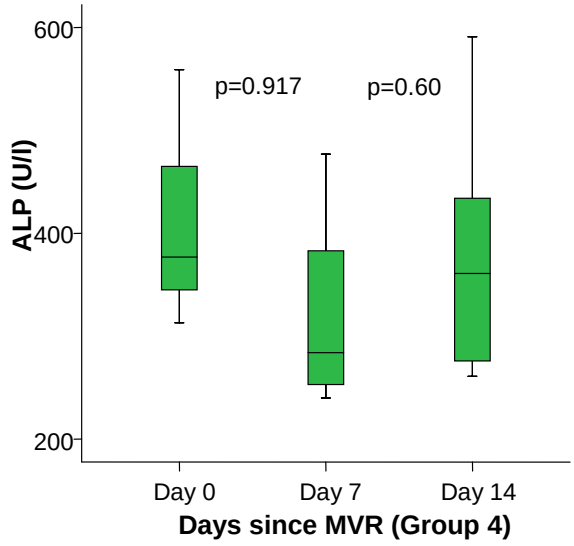


Figure 4.73: ALP following MVR in Internal drainage group (n=5, Friedman Chi-Square=1.20, df=2, p=0.549)

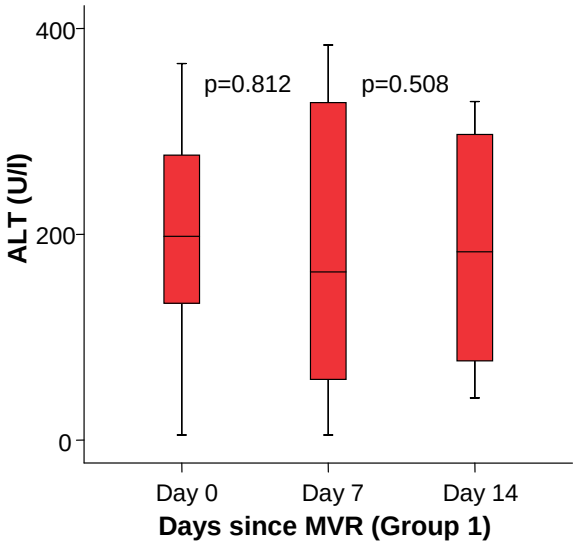


Figure 4.74: ALT following MVR in undrained group (n=10, Friedman Chi-Square=0.359, df=2, p=0.836)

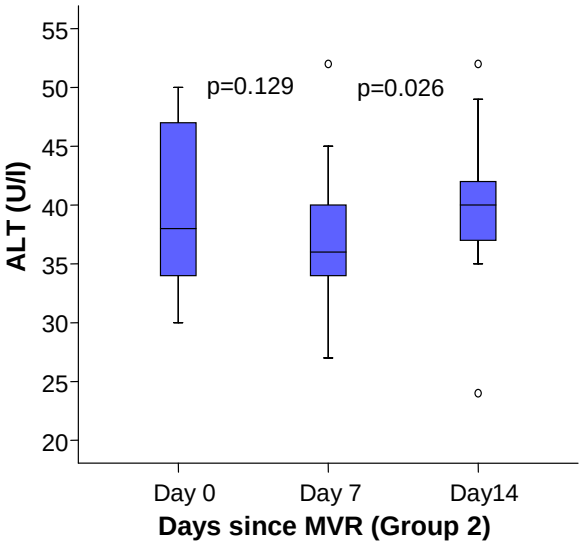


Figure 7.75: ALT following MVR in Sham group (n=17, Friedman Chi-Square=6.706, df=2, p=0.035).

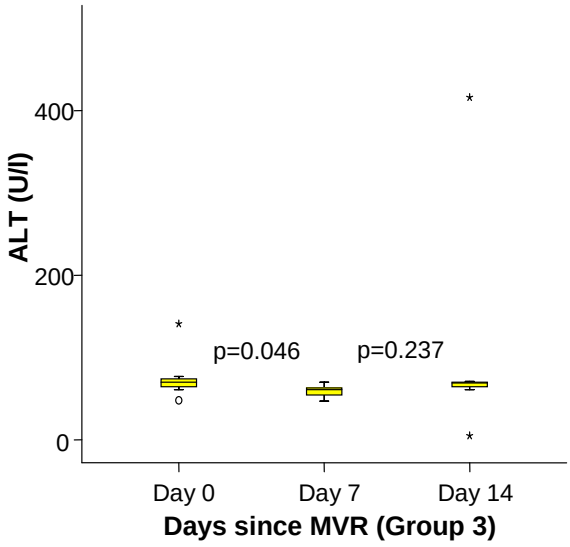


Figure 4.76: ALT following MVR in External drainage group (n=7, Friedman Chi-Square=2.846, df=2, p=0.241).

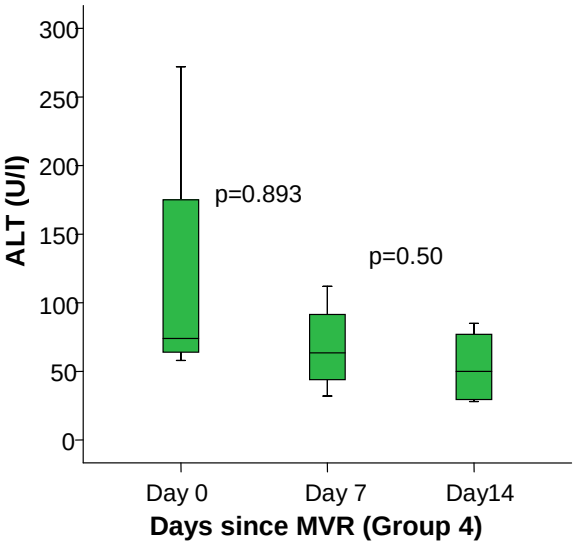


Figure 4.77: ALT following MVR in Internal drainage group (n=4, Friedman Chi-Square=2.0, df=2, p=0.368).

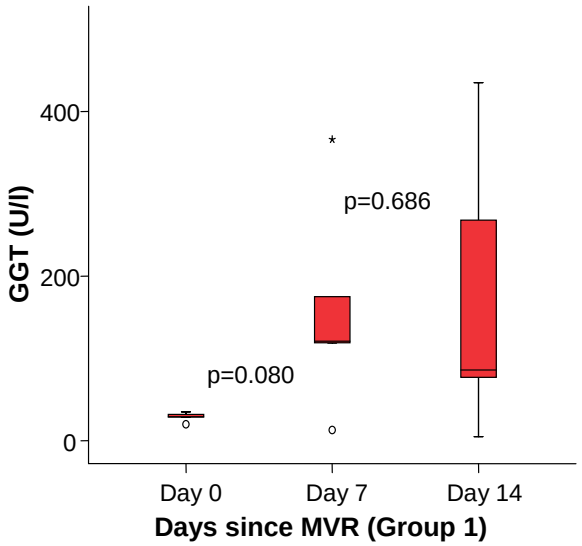


Figure 4.78: GGT following MVR in undrained group (n=5, Friedman Chi-Square=2.80, df=2, p=0.274)

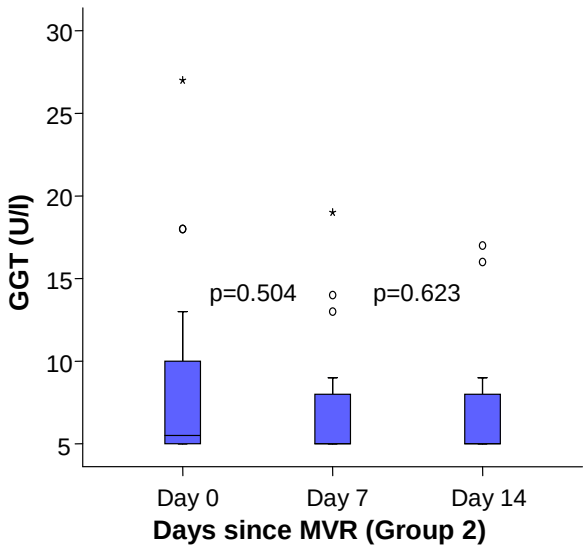


Figure 4.79: GGT following MVR in Sham group (n=18, Friedman Chi-Square=2.591, df=2, p=0.247).

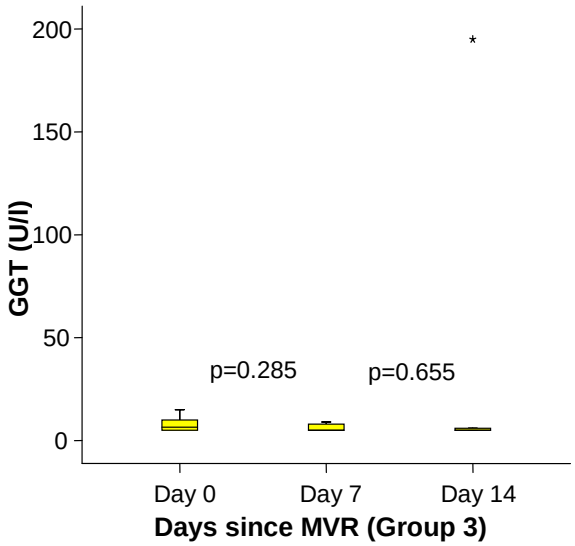


Figure 4.80: GGT following MVR in External drainage group (n=6, Friedman Chi-Square= 0.545, df=2, p=0.761)

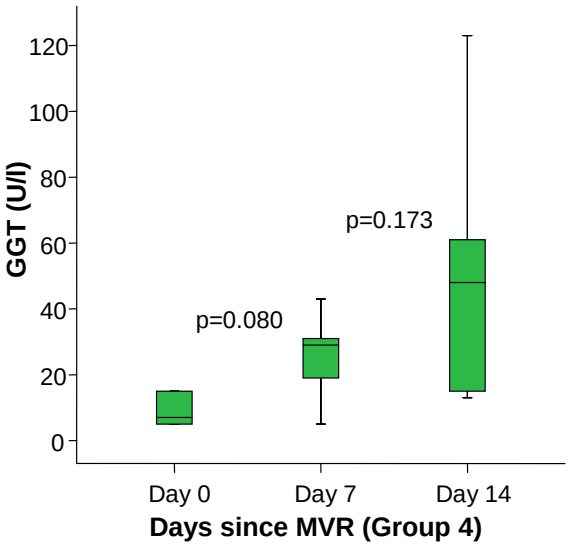


Figure 4.81: GGT following MVR in Internal drainage group (n=5, Friedman Chi-Square 5.20, df=2, p=0.074).

Variable	Group 1 (Undrained)			Group 2 (Sham)			Group 3 (External)			Group 4 (Internal)		
	Overall variability	0-7	7-14	Overall variability	0-7	7-14	Overall variability	0-7	7-14	Overall variability	0-7	7-14
Weight	Yes (p=0.005)	Decrease (p=0.007)	Increase (p=0.023)	Yes (p<0.001)	Decrease (p=0.008)	Increase (p<0.001)	Yes (p=0.039)	Decrease (p=0.028)	None (p=0.078)	No (p=0.135)	Decrease (p=0.017)	None (p=0.598)
Hb	Yes (p=0.036)	None (p=0.139)	Decrease (p=0.021)	Yes (p<0.001)	Decrease (p=0.004)	Decrease (p<0.001)	No (p=0.368)	None (p=0.352)	None (p=0.351)	No (p=0.20)	None (p=0.176)	None (p=0.080)
T Prot	No (p=0.386)	None (p=0.073)	None (p=0.513)	Yes (p=0.001)	Decrease (p=0.031)	Decrease (p=0.01)	Yes (p=0.048)	None (p=0.5)	None (p=0.225)	No (p=0.873)	None (p=0.672)	None (p=0.498)
Albumin	Yes (p=0.025)	Decrease (p=0.007)	None (p=0.491)	Yes (p<0.001)	Decrease (p<0.001)	Increase (p=0.02)	No (p=0.096)	None (p=0.171)	None (p=0.518)	No (p=0.186)	Decrease (p=0.027)	None p=0.892)
Globulin	No (p=0.294)	None (p=0.720)	None (p=0.609)	Yes (p=0.001)	None (p=0.702)	Decrease (p=0.002)	No (p=0.432)	None (p=0.932)	None (p=0.104)	No (p=0.311)	None (p=0.497)	None (p=0.674)
Bilirubin	No (p=0.060)	None (p=0.075)	None (p=0.657)	Yes (p=0.006)	Decrease (p=0.008)	None (p=0.421)	No (p=0.495)	None (p=0.397)	None (p=0.352)	No (p=1.0)	None (p=0.611)	None (p=0.753)
ALP	Yes (p=0.002)	Decrease (p=0.008)	None (p=0.398)	No (p=0.801)	None (p=0.446)	None (p=0.879)	Yes (p=0.012)	Decrease (p=0.018)	Increase (p=0.028)	No (p=0.549)	None (p=0.917)	None (p=0.60)
ALT	No (p=0.836)	None (p=0.812)	None (p=0.508)	Yes (p=0.035)	None (p=0.129)	Increase (p=0.023)	No (p=0.241)	Decrease (p=0.046)	None (p=0.237)	No (p=0.368)	None (p=0.893)	None (p=0.50)
GGT	No (p=0.247)	None (p=0.080)	None (p=0.686)	No (p=0.274)	None (p=0.504)	None (p=0.623)	No (p=0.761)	None (p=0.285)	None (p=0.655)	No (p=0.074)	None (p=0.080)	None (p=0.173)

Table 4.6: Summary of changes following major visceral resection.

Table 4.6 summarises all of the findings when each of the experimental groups are considered individually following major visceral resection.

In the sham group, it can be seen that there is overall variability seen in most of the measured parameters with the majority of the individual timepoints showing a decrease. Weight decreased at day 7 and then increased at day 14 following MVR while Hb and total protein both fell. For the first time the albumin levels in this group fell at seven days after the MVR but then recovered again significantly at day 14.

In the undrained group of rats (group 1) it can be seen that albumin fell again at day 7 post MVR but then remained unchanged after this. Apart from a fall then gain in weight and a drop in HB at day 14 the only other parameter to have significantly changed was a fall in ALP at day seven following MVR.

In the two biliary drainage groups there was no overall variability in albumin over the two week period. However the internal drainage rats did have a significant fall in albumin at day 7 post MVR and also had a fall in weight at that time. Despite these single timepoint changes however there was no significant variability in any measured parameter in the internally drained rats over the two week period following MVR.

4.5: Direct comparison of variability between groups (inter-group variability)

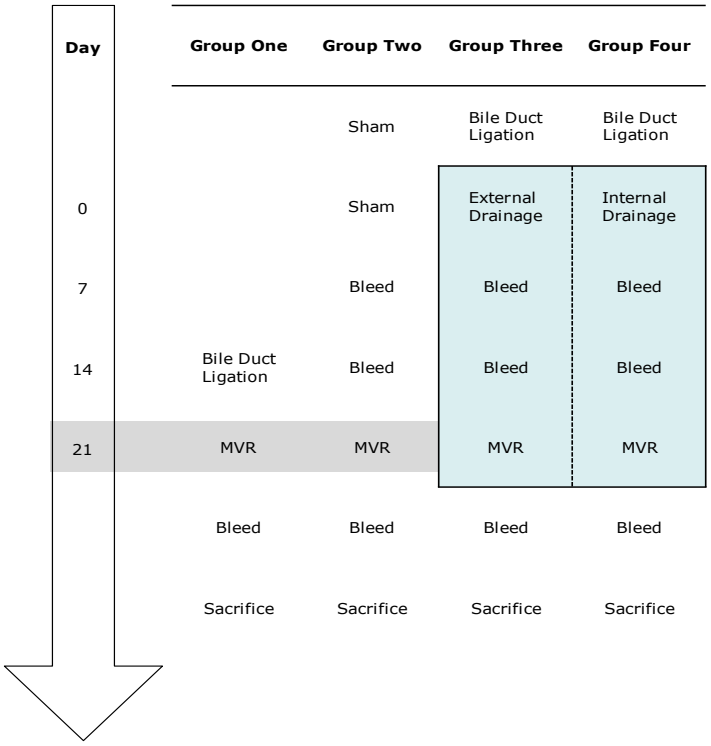
The next section compares the biochemical data and weight changes seen between different experimental groups following the two key timepoints in this experiment as discussed in the 'inter group variability' section 3.9 on statistical methods . Comparison of variability has been performed between;

- The external and internal drainage groups after the drainage procedure until the time of MVR
- All groups after major visceral resection. Each of the groups is directly compared to each of the other groups in turn.

4.5.i: Variability between external and internal biliary drainage prior to MVR

In this section, variation in weight and biochemical profiles between the internal and external biliary drainage groups prior to MVR have been compared. The groups were compared using multifactorial repeated measures ANOVA and the results shown in Table 4.7. This analysis compares the overall trend of change for each variable in each group *not* the actual differences in values at each time point or overall. All F statistics are for Greenhouse-Geisser estimates except for where Mauchlys test revealed that sphericity of data could be assumed (indicated with an asterisk in the data tables presented below). The data are sorted in descending order of F statistic value (with high F values indicating a greater degree of variation between groups). This format applies to all analyses in section 4.5 unless otherwise stated.

The following diagram demonstrates the groups and timepoints being assessed. The area of the chart shaded in light blue represents the groups that are presented below.



	F	Df (between group)	Df (within group)	P
Weight	11.299	1.877	31.914	<0.001
ALP	3.476	2.229	31.210	0.039
Haemoglobin*	2.387	3	51	0.080
Bilirubin	2.110	1.654	26.465	0.148
ALT	1.084	1.563	20.316	0.342
Total protein	0.794	1.742	27.870	0.446
Globulin	0.677	1.527	24.428	0.479
GGT	0.594	1.526	18.307	0.519
Albumin*	0.282	3	45	0.838

Table 4.7: Analysis of variation over time in weight and biochemical parameters between groups 3 and 4 following external or internal biliary drainage procedures.

Significant variation is only seen in weight and ALP. Specifically it can be seen that there are no differences in variation of albumin following 21 days of internal or external biliary drainage prior to MVR.

Those variables where significant variation over time has been identified are illustrated graphically below. Further analysis using Mann-Whitney U tests comparing the variable at each time point was performed. This demonstrates the differences between each variable specific to that timepoint. The point of variability between each group can be assessed visually on each graph by examining the trends over time. The results of these secondary analyses are shown below in Figures 4.82-4.83.

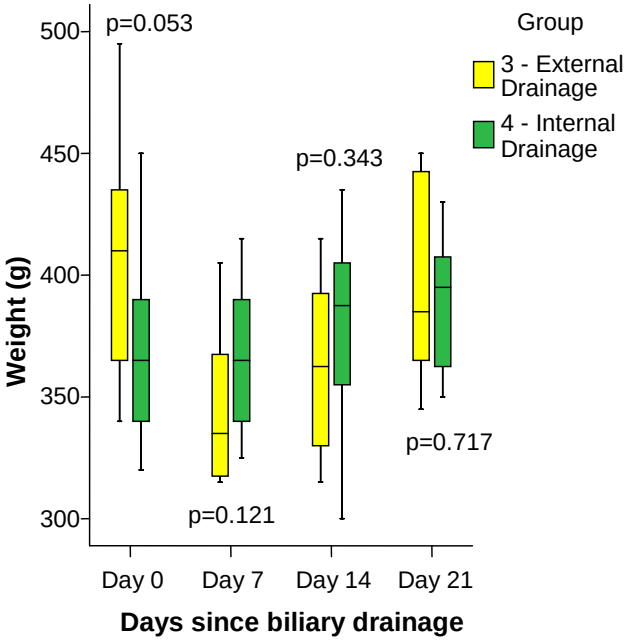


Figure 4.82: Differences in weight between groups 3 and 4 following biliary drainage. P values shown on the graph represent univariate analysis between the two groups at each timepoint. Overall value for variability p<0.001.

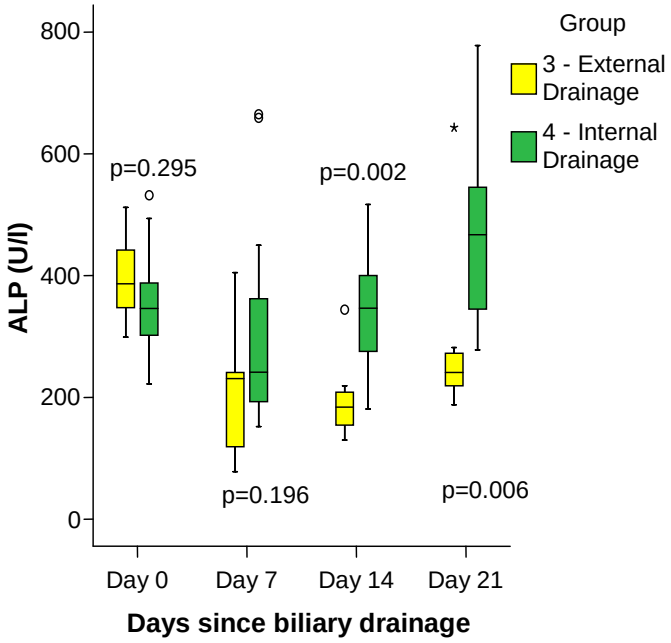


Figure 4.83: Differences in ALP between groups 3 and 4 following biliary drainage. P values shown on the graph represent univariate analysis between the two groups at each timepoint. Overall value for variability p=0.039.

These figures show that for weight (Figure 4.82), there is no difference between the two at any timepoint. However on examination of the graph it can be seen that the externally drained rats start at a higher point (heavier) which then falls at day 7 before recovering. The mean weight in the internally drained group does not fall at day 7 but instead remains steady before rising.

For ALP (Figure 4.83) there was no difference between the two groups on the day of drainage or at day 7 following drainage after which there is a significant difference between the two groups. Graphically it can be seen that ALP falls in both groups at day 7. Thereafter

the mean ALP falls again at day 14 before rising slowly. In the internally drained rats the ALP rises at both day 14 and 21 and to a greater degree than the externally drained rats. This may reflect the redevelopment of cholestasis in the internally drained group.

Summary

Following drainage, there were significant differences in the manner in which weight and ALP varied over time between the internal and externally drained groups. However there were no differences in the variation of albumin following either internal or external biliary drainage for three weeks.

4.5.ii: Variability between groups after MVR

In this section comparison between pairs of groups after MVR is presented. Each group is compared individually to all other groups, i.e. group 1 is compared to group 2, 3 and 4 in turn, followed by comparison of group 2 to groups 3 and 4 in turn until all groups have been compared to each other.

The diagram below demonstrates which groups and timepoints are being analysed. The area of the chart shaded in light blue represents the groups that are presented below.

4.5.ii.a: Group One (undrained) and Group Two (sham) following MVR

This section presents the variation in weight and biochemical parameters between the undrained group and sham operated animals following major visceral resection as shown below. The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.8.

Day	Group One	Group Two	Group Three	Group Four
		Sham	Bile Duct Ligation	Bile Duct Ligation
		Sham	External Drainage	Internal Drainage
		Bleed	Bleed	Bleed
	Bile Duct Ligation	Bleed	Bleed	Bleed
0	MVR	MVR	MVR	MVR
7	Bleed	Bleed	Bleed	Bleed
14	Sacrifice	Sacrifice	Sacrifice	Sacrifice

The figures are sorted in descending order of F values (with high F values meaning a greater degree of significant variation between groups).

	F	Df (between group)	Df (within group)	P
GGT	14.831	1.108	23.271	0.001
ALP	12.965	1.556	42.015	<0.001
Weight *	7.704	2	54	0.001
Bilirubin	3.602	1.545	41.722	0.047
Albumin	0.997	1.104	28.713	0.335
Globulin	0.283	1.265	32.882	0.653
Haemoglobin*	0.176	2	48	0.839
Total protein	0.104	1.268	34.223	0.808
ALT *	0.067	2	50	0.936

Table 4.8: Analysis of variation over time between undrained and sham group after MVR

From this it can be seen that there were significant differences between the sham and undrained group in the variation of bilirubin, ALP, GGT and weight after MVR. There was no significant variation over time in albumin, globulin, Hb, Total protein or ALT. For those variables where significant variation over time had been identified, further analysis using Mann-Whitney U tests at each time point was performed. The results of these analyses are shown graphically below in Figures 4.84-4.87.

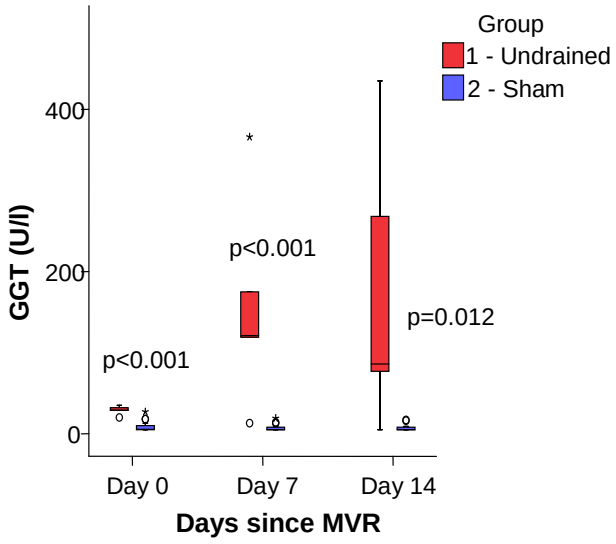


Figure 4.84: Overall variability in GGT post MVR (p=0.001). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

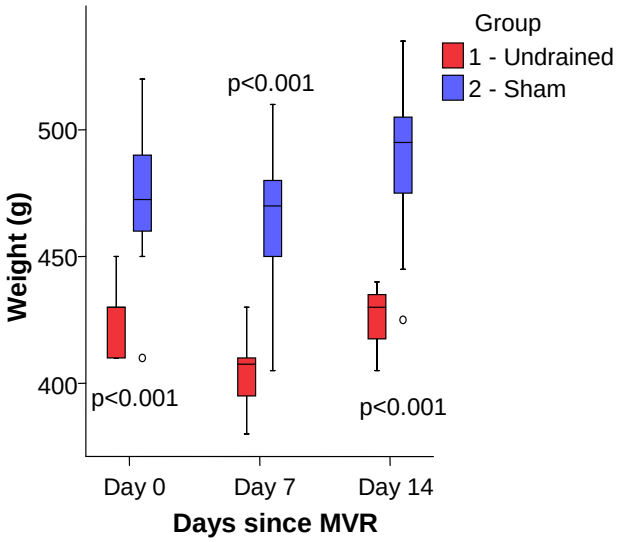


Figure 4.85: Overall variability in Weight post MVR (p=0.001). P values shown on the graphs represent univariate analysis between the two groups at each timepoint

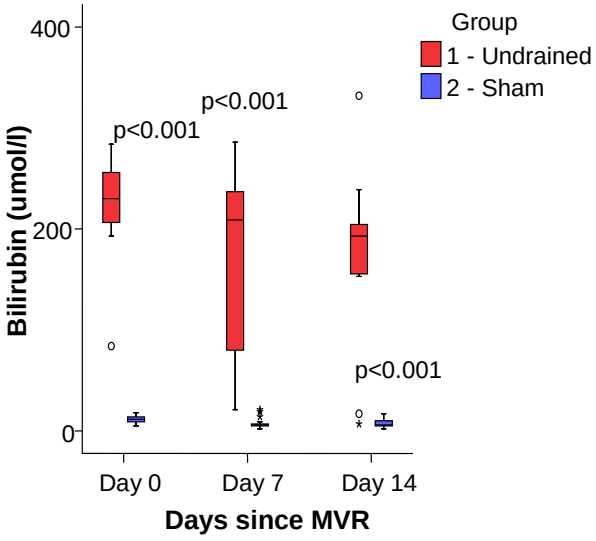


Figure 4.86: Changes in bilirubin post MVR (overall variability p=0.047). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

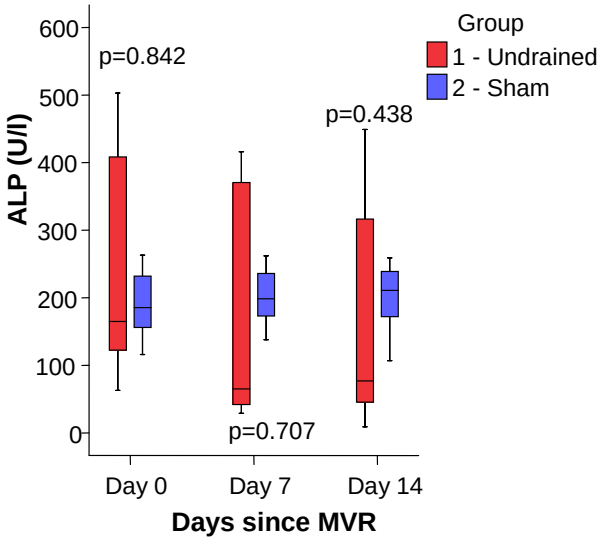


Figure 4.87: Changes in ALP post MVR (overall variability p<0.001). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

Figure 4.84 shows that, as would be expected, GGT continued to rise after MVR in the undrained group but remained static in the sham group, therefore accounting for both the significant differences at each timepoint and overall variability. Overall variability for bilirubin (figure 4.86) is significant with $p=0.047$, although the individual timepoints are significantly different $p<0.001$. This apparent disparity is due to the fact that bilirubin in the undrained groups is tending to trend downwards only slightly post MVR but remained static in the sham group.

While figure 4.85 is more difficult to interpret as the trend between the groups across the time period appears the same (i.e. stable weight from day 0 to seven followed by a rise from day 7 to 14), there is still significant variation over time between the groups. This may be due to the way the data points are distributed and in particular the wide range of weight seen in the sham group on day 7 post MVR.

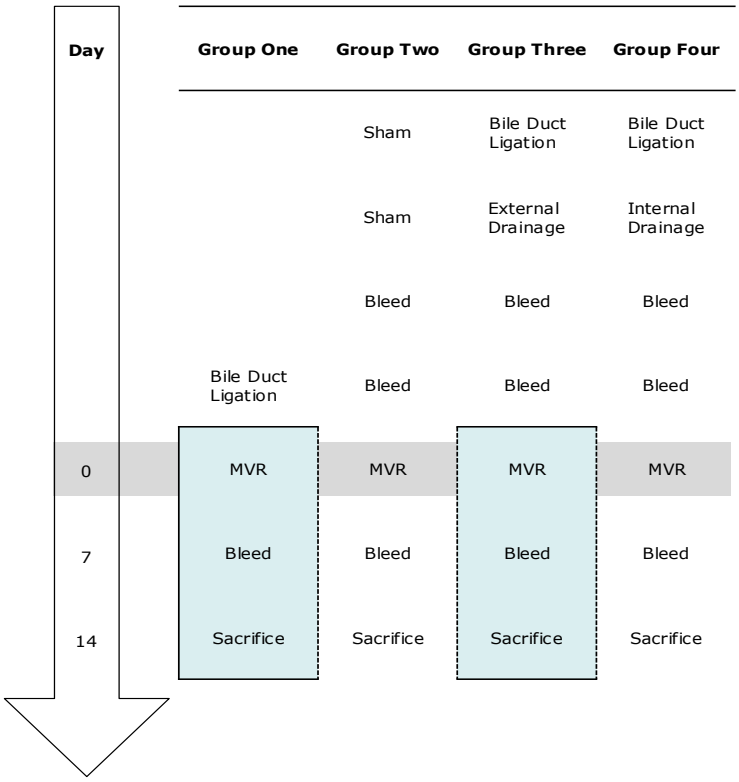
In figure 4.87 it can be seen that there is wide overlap in ALP ranges between the two groups and yet statistically there is variation. This may be accounted for by the fall in mean ALP in the undrained group from day 0 to 7 followed by a levelling off at day 7 to 14. In the sham laparotomy group ALP mean levels are fairly static over the experimental period.

Summary

Following MVR significant differences in the manner in which weight, GGT, ALT and ALP varied over time are seen between group one (undrained) and group two (sham). Specifically there is no variation in albumin between these groups following MVR.

4.5.ii.b: Group One (undrained) and Group Three (external drainage) following MVR

This section examines for variation between group 1 and group 3 following MVR in weight and biochemical parameters. The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.9.



The figures are sorted in descending order of F values (with high F values meaning a greater degree of significant variation between groups).

	F	Df (between group)	Df (within group)	P
Haemoglobin*	3.181	2	26	0.058
GGT	2.788	1.284	11.560	0.116
ALP *	2.456	2	32	0.110
Bilirubin *	2.136	2	32	0.135
ALT	1.378	1.041	15.611	0.260
Albumin	0.835	1.127	18.038	0.387
Total protein	0.605	1.157	18.513	0.470
Weight *	0.446	2	32	0.644
Globulin	0.023	1.192	19.075	0.914

Table 4.9: Pattern of variation over time between undrained and external biliary drainage groups.

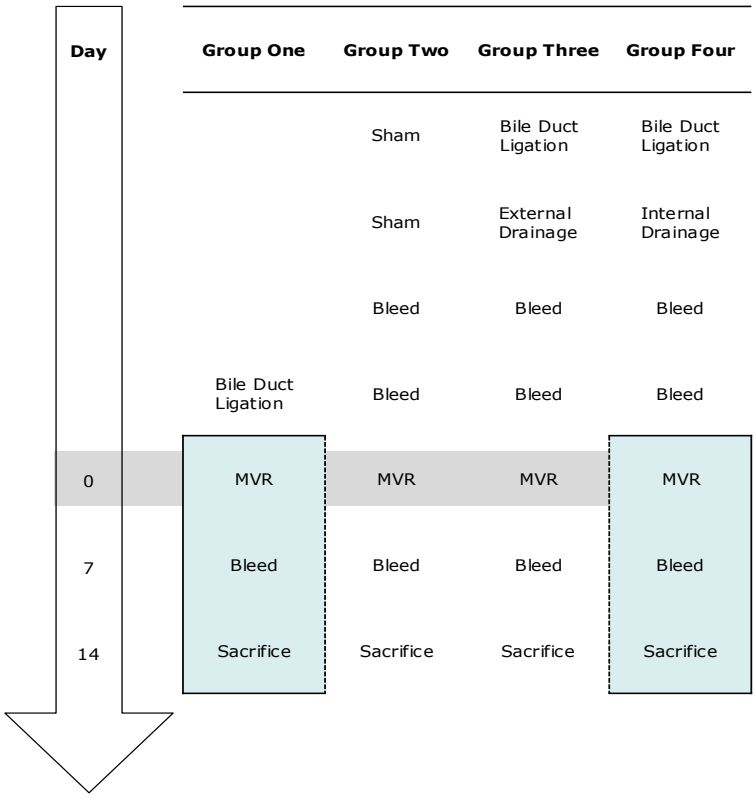
This table demonstrates that there was no significant variation between the undrained and external drainage groups in any of the measured parameters after MVR.

Summary

There is no significant variation in any measured parameters between the undrained group and externally drained group following major visceral resection. In particular there is no variation in albumin following MVR.

4.5.iii.c: Group One (undrained) and Group Four (internal drainage) following MVR

This section presents the final comparison for the undrained rats which is the comparison with the internally drained rats. The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.10.



The data are sorted in descending order of F statistic value (with high F values indicating a greater degree of variation between groups).

	F	Df (between group)	Df (within group)	P
Globulin *	4.301	2	30	0.023
Weight	3.221	1.286	19.292	0.080
GGT	1.990	1.245	9.958	0.190
Total protein	1.657	1.444	21.666	0.216
ALT	1.191	1.376	16.508	0.310
Bilirubin *	1.143	2	30	0.332
ALP *	0.824	2	28	0.449
Albumin	0.543	1.093	16.400	0.487
Haemoglobin*	0.357	2	24	0.703

Table 4.10: Pattern of variation over time between undrained and internal biliary drainage groups.

The only parameter showing significant variation over time was globulin and this is further analysed below in Figures 4.88.

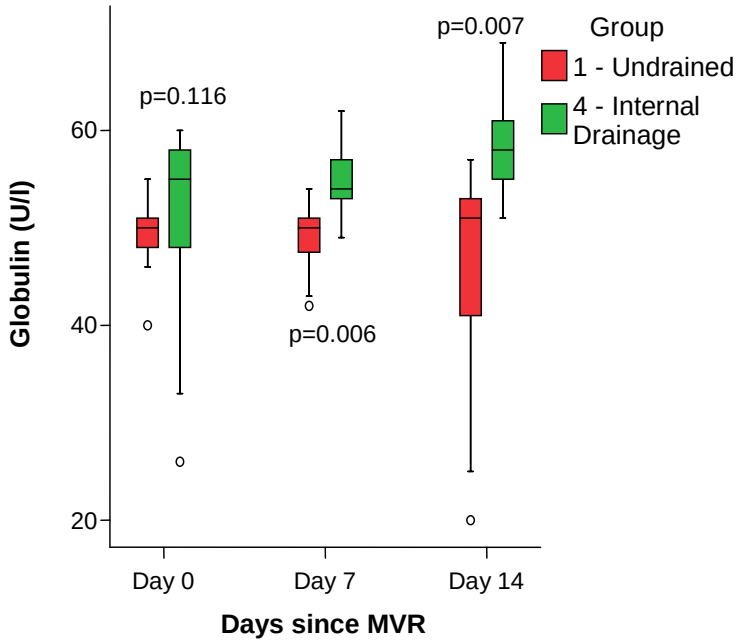


Figure 4.88: Changes in globulin following MVR between Group 1 and 4. (Overall variability p=0.023) P values shown on the graph represent univariate analysis between the two groups at each timepoint.

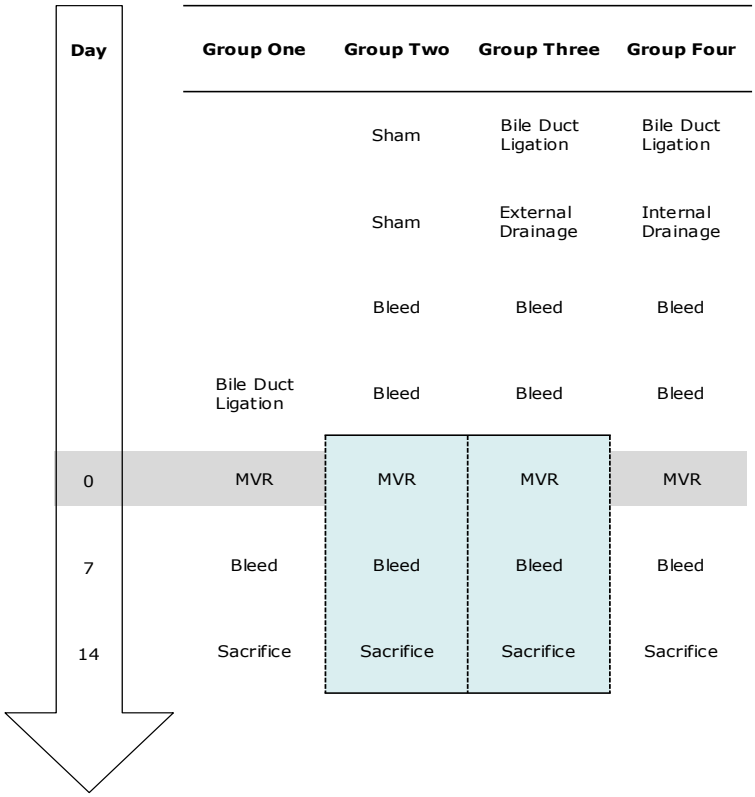
Figure 4.88 shows that globulin levels remained static in the undrained rats after MVR. Internally drained rats globulin was initially level from day 0 to 7 but rose at day 7 to 14 post MVR.

Summary

Following MVR, only globulin shows significant variation over time between and internally drained rats. There was no change in the variation of albumin between these groups.

4.5.iii.d: Group Two (sham) and Group Three (external) following MVR

In this section variability between the sham group and external biliary drainage group after MVR are presented. The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.11.



Day	Group One	Group Two	Group Three	Group Four
		Sham	Bile Duct Ligation	Bile Duct Ligation
		Sham	External Drainage	Internal Drainage
		Bleed	Bleed	Bleed
	Bile Duct Ligation	Bleed	Bleed	Bleed
0	MVR	MVR	MVR	MVR
7	Bleed	Bleed	Bleed	Bleed
14	Sacrifice	Sacrifice	Sacrifice	Sacrifice

The data are sorted in descending order of F statistic value (with high F values indicating a greater degree of variation between groups).

	F	Df (between group)	Df (within group)	Group 3 (External)
ALP	9.982	1.394	32.064	0.001
Haemoglobin*	5.575	2	46	0.007
Bilirubin	5.398	1.132	26.040	0.025
Weight *	5.391	2	46	0.008
GGT	3.067	1.058	26.266	0.091
ALT	2.413	1.001	22.021	0.135
Albumin *	1.973	2	44	0.151
Total protein	1.334	1.565	35.986	0.271
Globulin	0.837	1.482	32.604	0.410

Table 4.11: Comparison of pattern of variability between Group 2 (Sham) and Group 3 (External) following MVR.

From this it can be seen that significant variation between the groups occurs in ALP, haemoglobin, bilirubin and weight. There is no difference in variation in albumin. The parameters showing significant variation over time are further analysed below in Figures 4.89-4.92.

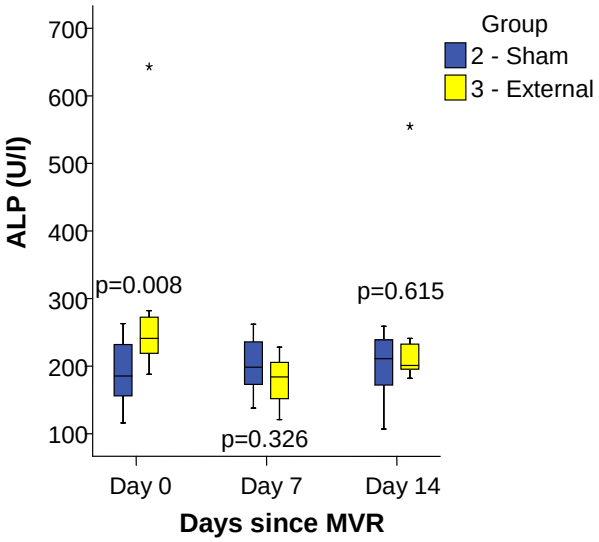


Figure 4.89: Changes in ALP following MVR (Overall variability p=0.001). P values shown on the graphs represent univariate analysis between the two groups at each timepoint

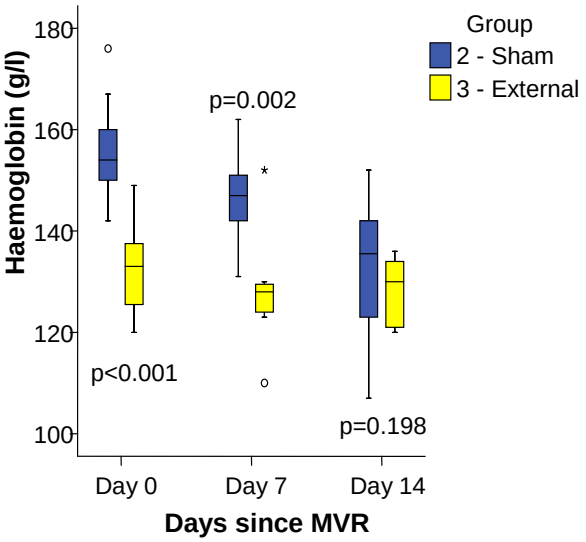


Figure 4.90: Changes in Hb following MVR (Overall variability p=0.007). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

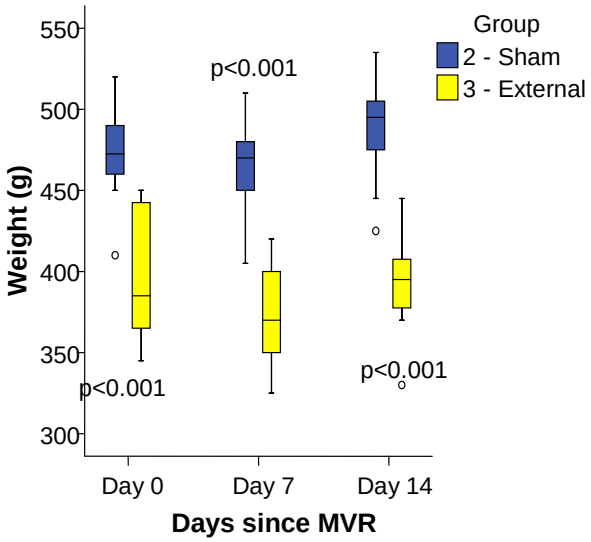


Figure 4.91: Changes in bilirubin following MVR (Overall variability p=0.025.) P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

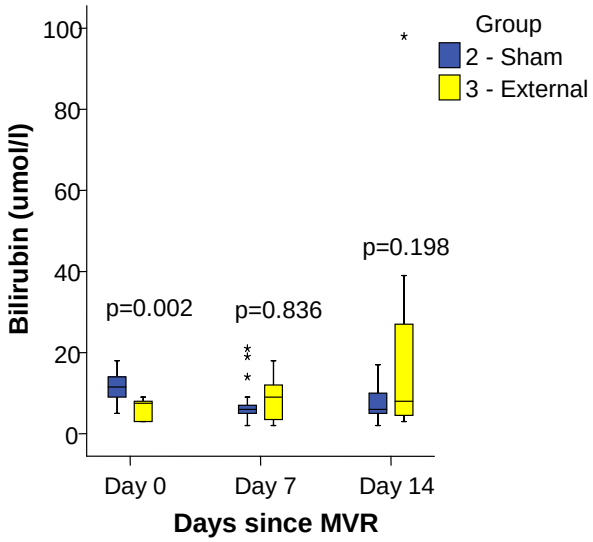


Figure 4.92: Changes in weight following MVR (Overall variability p=0.008). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

In figure 4.89 ALP is significantly different between the groups on the day of MVR, with the externally drained rats having a higher level compared to the sham group. Thereafter the ALP in the externally drained group fell again between day 0 and 7 whereby it remained stable until the end of the experiment. Haemoglobin (Figure 4.90) also showed variability between the groups. Haemoglobin in the sham rats fell at each timepoint after MVR, whereas in the externally drained rats it started at a lower point at the time of MVR but did not fall any further in the following two weeks. There was significant variation in bilirubin (Figure 4.91) between the external and sham rats. On looking at the graph the bilirubin was actually lower in the external drainage group at the time of MVR compared to sham rats, although both levels were very low and within the normal range. Between day 0 and 7 the bilirubin in the sham rats fell to a lower level than externally drained rats. The range of bilirubin at day 14 in the externally drained rats may also have contributed to the variability seen between the groups.

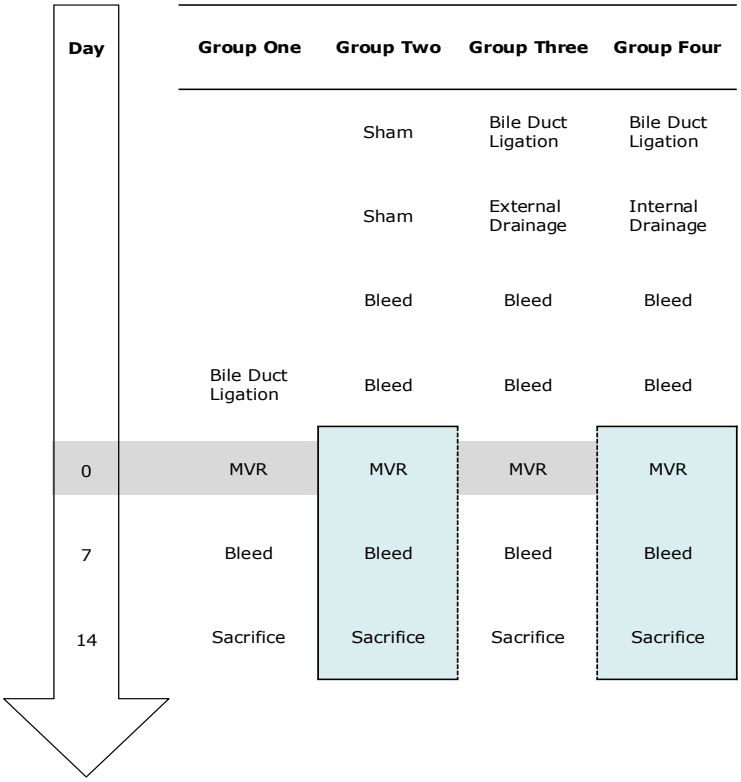
On inspection of weight in figure 4.92 the time of variability is again not clearly seen. However, although the externally drained rats are significantly lighter at each timepoint, between day 0 and 7 (as expected) the weight does fall before rising again, while the sham rats weights were static at day 0 to 7 before rising again.

Summary

Significantly variability is seen in ALP, Hb, bilirubin and weight between sham and externally drained rats following MVR. There is no variation in albumin between these two groups.

4.5.iii.d: Group Two (sham) and Group Four (internal) following MVR

This section presents variability between the sham group and internal biliary drainage group. The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.12.



Day	Group One	Group Two	Group Three	Group Four
		Sham	Bile Duct Ligation	Bile Duct Ligation
		Sham	External Drainage	Internal Drainage
		Bleed	Bleed	Bleed
	Bile Duct Ligation	Bleed	Bleed	Bleed
0	MVR	MVR	MVR	MVR
7	Bleed	Bleed	Bleed	Bleed
14	Sacrifice	Sacrifice	Sacrifice	Sacrifice

The data are sorted in descending order of F statistic value (with high F values indicating a greater degree of variation between groups).

	F	Df (between group)	Df (within group)	P
GGT	9.977	1.180	24.782	0.003
Total protein *	6.504	2	44	0.003
ALT	5.457	1.090	20.707	0.027
ALP *	2.623	2	42	0.090
Weight *	14.497	2	44	<0.001
Globulin *	11.449	2	42	<0.001
Bilirubin *	0.513	2	44	0.602
Albumin *	0.455	2	42	0.637
Haemoglobin *	0.338	2	44	0.715

Table 4.12: Multifactorial repeated measures ANOVA to evaluate pattern of overall variability between Sham and internal drainage rats following MVR.

From the above table it can be seen that significant variation occurs between the sham and internal drainage group in GGT, total protein, globulin, ALT and weight. The parameters showing significant variation over time are further analysed below in Figures 4.93-4.97. There was no variation in changes in albumin between the two groups.

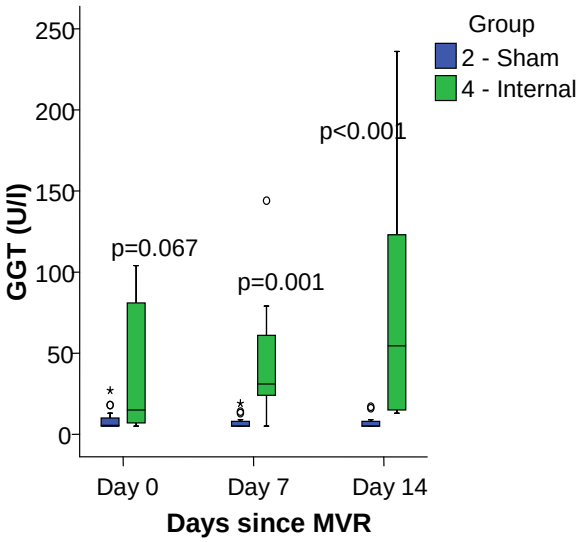


Figure 4.93: Changes in GGT following MVR (Overall variability p=0.003).

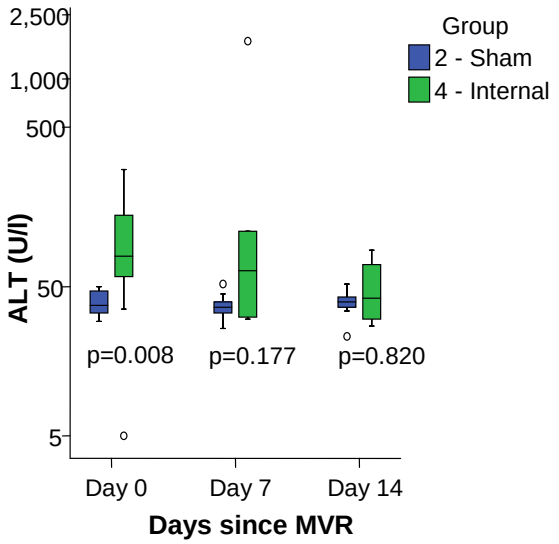


Figure 4.94: Changes in ALT following MVR (Overall variability p=0.027).

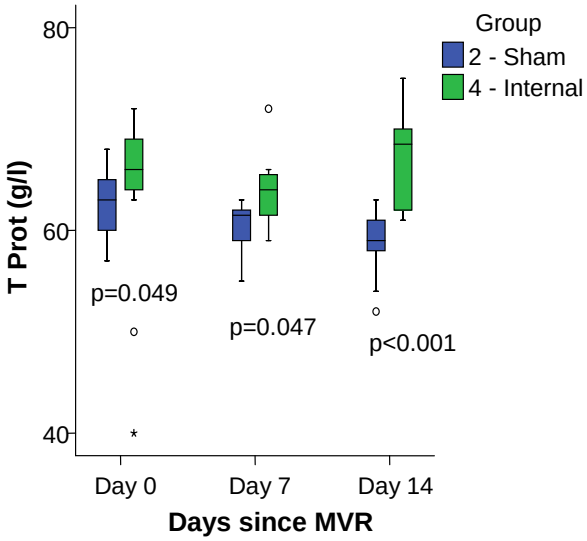


Figure 4.95: Changes in Total protein following MVR (Overall variability p=0.003).

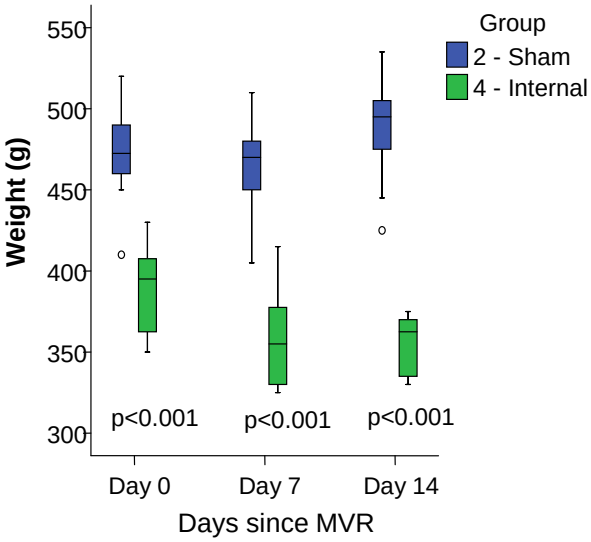


Figure 4.96: Changes in weight following MVR (Overall variability p<0.001).

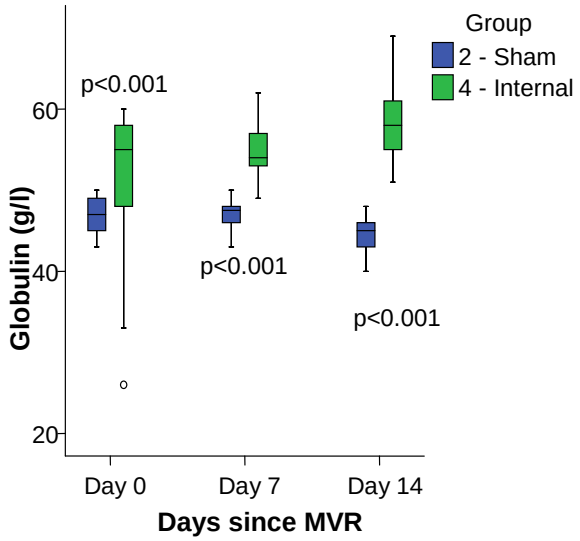


Figure 4.97: Changes in globulin following MVR
(Overall variability p<0.001)

In Figures 4.93 it can be seen that at day 0 GGT showed no differences between the two groups. Thereafter GGT rose in the internal drainage group but remained static in the sham group. In figure 4.94 it can be seen that ALT was significantly higher at the time of MVR but then fell in the internal drainage group whilst remaining static in the sham group. Figure 4.96 shows that weight was significantly higher at all points in the sham group following MVR. Weight in internally drained rats fell at day 7 post MVR but then remained static. Globulin levels between the groups (Figure 4.97) were different at all timepoints following MVR. At day 7 post MVR neither group had any change in trends but after 14 days globulin levels rose in internally drained rats but fell in the sham group.

Summary

Compared to sham rats, the internally drained rats showed increases in GGT, total protein, and globulin compared to the sham animals which showed stable levels of GGT and globulin but fall a in total protein. Internally drained rats lost more weight and demonstrated a fall in ALT following MVR compared to sham rats. There was no variability between the groups in albumin levels following MVR.

4.5.iii.e: Group Three (external) and Group Four (internal) following MVR

This final section presents the variation between the rats drained either internally or externally in weight and biochemical parameters following MVR. It is this section which will determine whether one drainage method has a greater effect on any of the measured variables following MVR.

The area of the chart shaded in light blue represents the groups that are presented below and the results are shown in Table 4.13.

Day	Group One	Group Two	Group Three	Group Four
		Sham	Bile Duct Ligation	Bile Duct Ligation
		Sham	External Drainage	Internal Drainage
		Bleed	Bleed	Bleed
	Bile Duct Ligation	Bleed	Bleed	Bleed
0	MVR	MVR	MVR	MVR
7	Bleed	Bleed	Bleed	Bleed
14	Sacrifice	Sacrifice	Sacrifice	Sacrifice

The data are sorted in descending order of F statistic value (with high F values indicating a greater degree of variation between groups).

	F	Df (between group)	Df (within group)	P
Globulin *	4.620	2	22	0.021
Total protein *	3.027	2	22	0.069
Haemoglobin *	1.966	2	22	0.164
Weight *	1.631	2	22	0.219
ALT	0.755	1.034	9.303	0.411
Bilirubin *	0.272	2	22	0.764
Albumin *	0.272	2	22	0.764
GGT	0.158	1.036	9.325	0.709
ALP *	0.077	2	20	0.920

Table 4.13: Multifactorial repeated measures ANOVA to evaluate pattern of overall variability between external and internally drained rats following MVR.

This table demonstrates that between the external and internal drainage groups the only parameter that demonstrated significant variability over time following MVR was globulin. This is further demonstrated below in Figure 4.98.

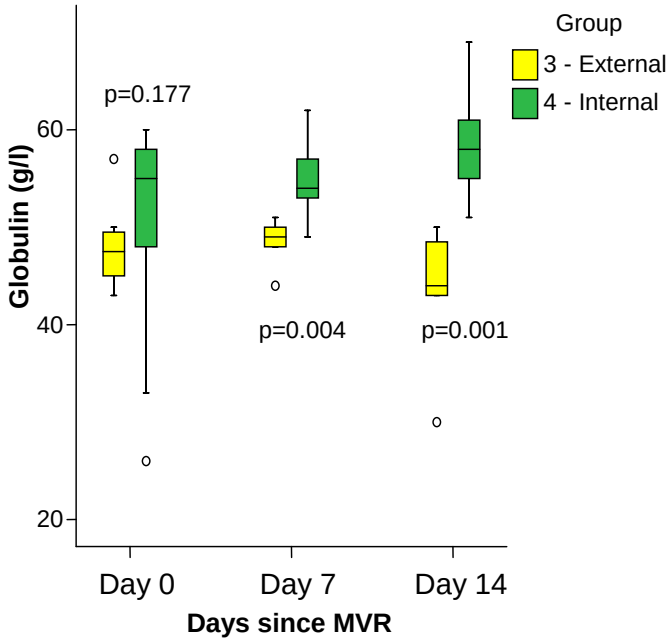


Figure 4.98: Changes in globulin following MVR. (Overall variability p=0.021). P values shown on the graphs represent univariate analysis between the two groups at each timepoint.

This shows that at the time of MVR there was no difference between the two groups but at day 7 and 14 the globulin had risen in the internal drainage group compared to the external drainage group where levels were seen to fall.

Summary

Following MVR only globulin showed a significant difference in the manner of variation over time, with an increase in globulin seen in the internally drained group. Importantly there is no difference in variation in albumin between externally or internally drained rats following major visceral resection.

- *4.6: Survival differences*

The following chart demonstrates the number of animals included at the start of each group. The inclusion and exclusion criteria are discussed earlier and the summaries of outcomes in each group are shown in more detail in Appendix A.

Day	Group One	Group Two	Group Three	Group Four
0		Sham n=18	Bile Duct Ligation n=12	Bile Duct Ligation n=14
7		Sham n=18	External Drainage n=12 2 died day 10 2 died day 11	Internal Drainage n=14 1 died day 8
14		Bleed n=18	Bleed n=8	Bleed n=13 1 died day 16
21	Bile Duct Ligation n=11	Bleed n=18	Bleed n=8	Bleed n=12 1 died day 24
28	MVR n=11	MVR n=18	MVR n=8 1 died at MVR	MVR n=11 1 died at MVR 1 died day 30 1 died day 33
35	Bleed n=11	Bleed n=18	Bleed n=7	Bleed n=9 1 died day 39
42	Sacrifice n=10 One animal met criteria for euthanasia according to distress score	Sacrifice n=18	Sacrifice n=7	Sacrifice n=7

As shown at the start of the chapter no animals in the sham group (group 2) died during the course of the experiment.

The single mortality in group one (undrained rats) is an important point of discussion as it has been classed as a death despite occurring on the day of the termination of the experiment (i.e. day 14 post MVR). It could be argued therefore that this animal technically survived to the end of the experiment as it was still alive 14 days after MVR which would make the post MVR mortality zero in this group. The reason that this case was chosen to represent a death was that the animal was obviously unwell on inspection and euthanased without delay. If this animal had been left for a few hours more it was likely that it would have died spontaneously anyway. It was the opinion of the author that this animal represented a death due to being jaundiced and having undergone the major visceral resection and should therefore be classed as such, despite this euthanasia occurring on the final day of the experiment. The remainder of the animals in the undrained group remained well until the experiment end.

In group three (external drainage), 4 animals died following the external biliary drainage procedure. Only one animal died following the major visceral resection and this animal died during recovery from anaesthesia.

In group four (internal drainage), 3 animals died following internal biliary drainage. Four animals died following major visceral resection, one during recovery from anaesthesia,

and the remaining animals died or were euthanased 2, 5 and 11 days after the major visceral resection.

The following figures (figures 4.99 – 4.103) demonstrate,

- Deaths following biliary drainage (Figure 4.99) – comparison of survival between the external and internally drained rats prior to the major visceral resection (i.e. deaths related to the drainage procedure itself).

- Deaths following major visceral resection comparing,
    - All intervention groups with sham group (Figures 4.100-4.102).
    - Drainage groups and undrained group (Figures 4.103-4.104).
    - External and internal drainage groups (Figure 4.105).
    - All groups (figure 4.106).

The following graphs show percentage survival over time, error bars show standard error.

4.6.i: Survival following biliary drainage

Survival differences between group 3 (external drainage) and group 4 (internal drainage) in the 3 weeks following the drainage procedure until the time of the major visceral resection are shown below in Figure 4.99.

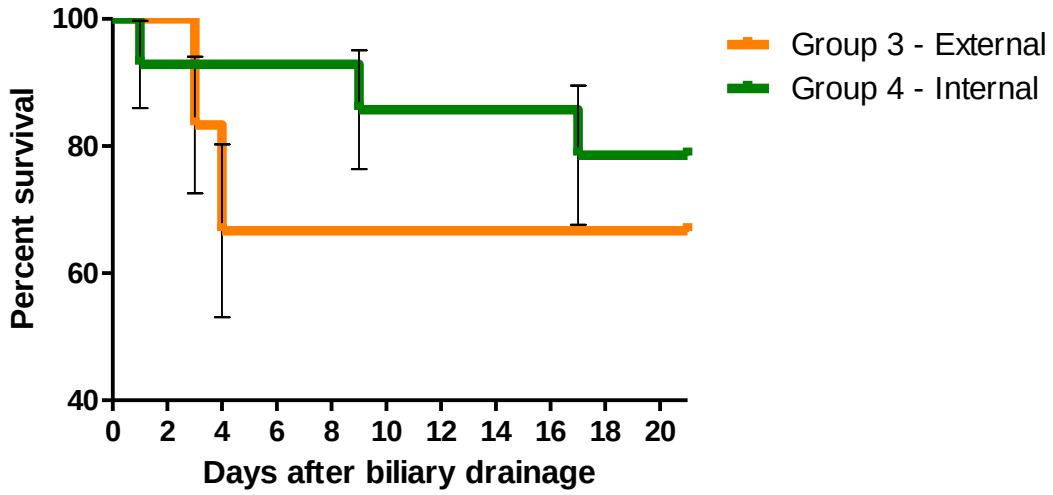


Figure 4.99: Comparison of mortality following external and internal biliary drainage, p=0.473 (Log-rank test, Chi-square=0.525, df=1).

Following biliary drainage there was no significant difference in overall survival between the internal and external biliary drainage groups (Figure 4.99). While there were similar numbers of deaths in both groups (4 external and 3 in the internal group) all deaths in the external group occurred within 4 days of the drainage procedure, while the deaths in the internal drainage group were spread out over the 21 days of drainage.

4.6.ii. Survival following Major Visceral Resection

4.6.ii.a: Comparison between all groups and sham laparotomy following MVR

Firstly each experimental group is compared with the survival following MVR in the sham laparotomy group and demonstrated below in figures (Figure 4.100–4.102).

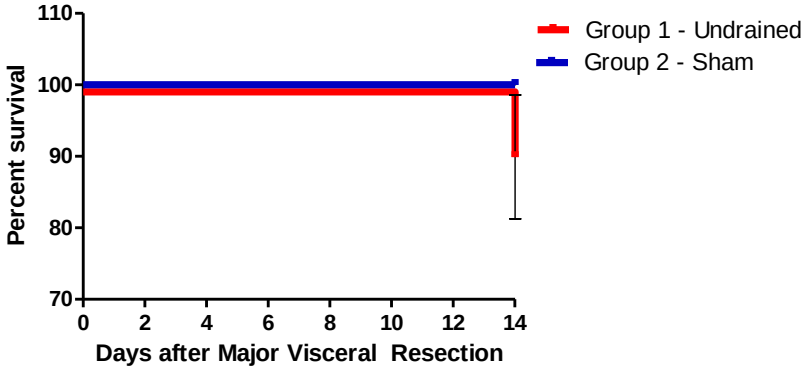


Figure 4.100: Survival difference between undrained and sham group following MVR (Log rank test. Chi-square=1.636, df=1, p=0.201).

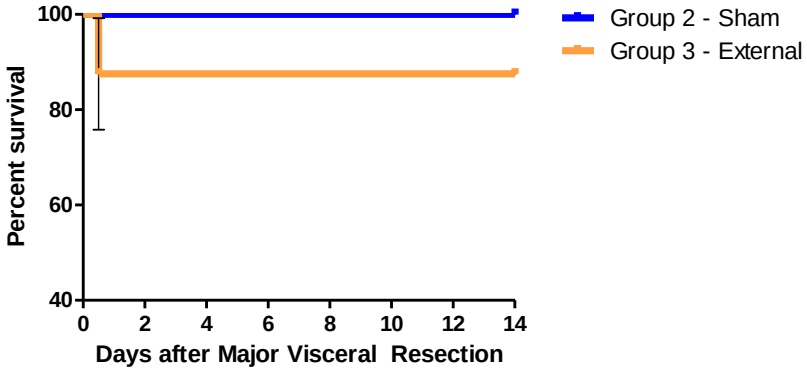


Figure 4.101: Survival difference between sham and external drainage group following MVR (Log rank test. Chi-square=2.250, df=1, p=0.134).

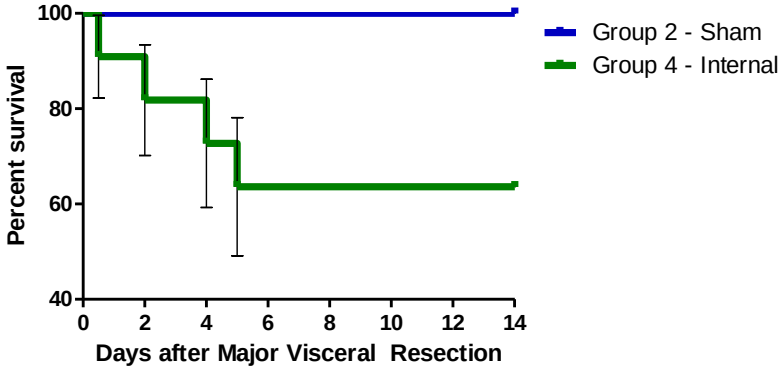


Figure 4.102: Survival difference between sham and internal drainage group following MVR (Log rank test. Chi-square=7.640, df=1, p=0.006).

There was no difference in survival between the sham group and either the undrained group (group 1) or external drainage rats (group 3). Only one animal died in both the undrained group and external biliary drainage group following MVR. There was a significant difference in survival between the sham animals and internally drained rats (Figure 4.102) as four animals died following MVR at day 0, 2, 5 and 11 days in the internal drainage group.

Summary

Only internally drained rats (group 4) had a significantly worse survival following MVR in comparison to the sham group.

4.6.ii.b: Comparison between the undrained group and both drainage groups following MVR

The following graphs (Figure 4.103-4.104) compare the survival of those rats having preoperative biliary drainage (external and internal) to those rats having no preoperative biliary drainage (undrained group) prior to major visceral resection.

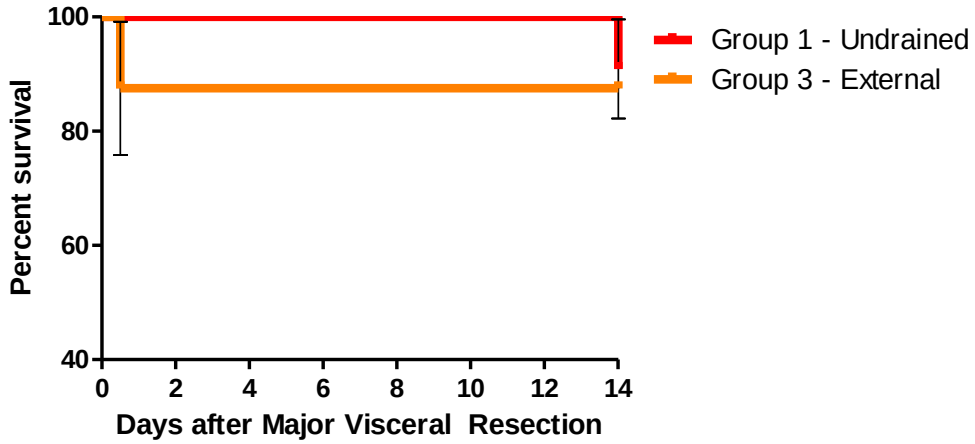


Figure 4.103: Survival difference between undrained and external drainage group following MVR (Log rank test. Chi-square=0.0969, df=1, p=0.755).

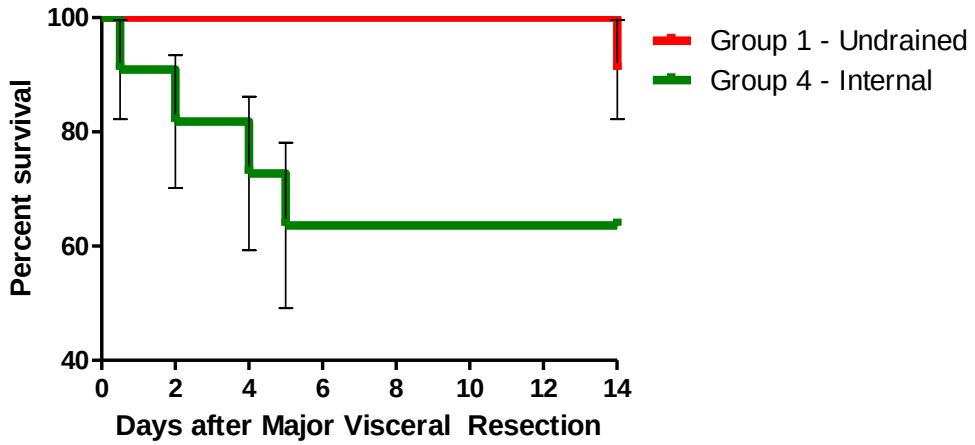


Figure 4.104: Survival difference between undrained and internal drainage group following MVR (Log rank test. Chi-square=2.233, df=1, p=0.111).

When comparing the survival between those rats that had no preoperative biliary drainage (group one) and those that had preoperative biliary drainage there was no difference in mortality between the externally drained and undrained rats (Figure 4.103). There is a trend towards an increased number of deaths in the internal biliary drainage group compared to the undrained group but this did not achieve statistical significance in this small group of animals.

Summary

Neither internal nor externally drained rats had a better outcome following major visceral resection in comparison to the undrained group, although a trend for higher mortality was seen in the internally drained rats.

4.6.ii.c: Comparison between external and internal drainage following MVR

The following graph (Figure 4.105) demonstrates survival differences on direct comparison between the internal and externally drained rats following major visceral resection.

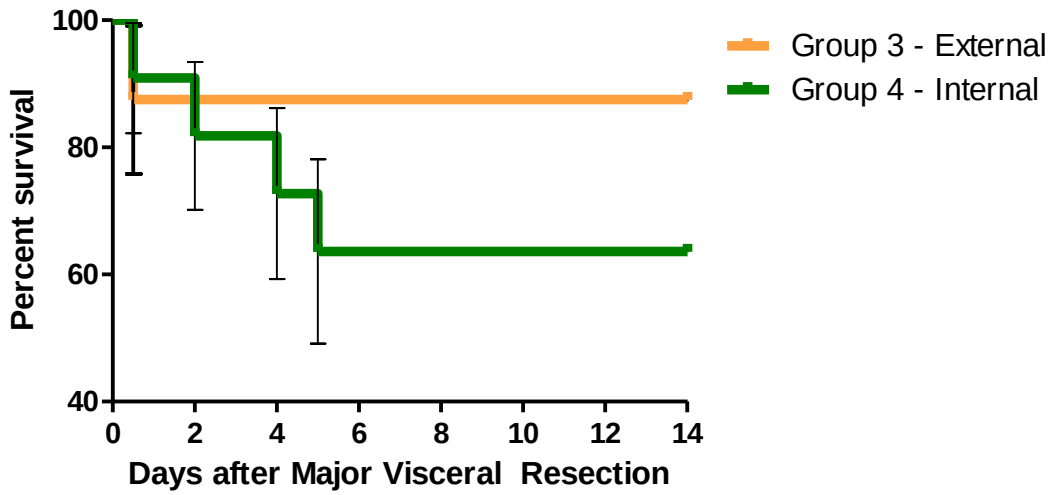


Figure 4.105: Survival difference between external and internal drainage group following MVR (Log rank test. Chi-square=0.912, df=1, p=0.339).

Summary

On direct comparison between the external and internal drainage groups there is no significant difference in mortality between internally drained rats and externally drained rats following major visceral resection. There was however a trend towards a higher mortality in the internal drainage group.

4.6.ii.d: Comparison of survival across all groups

A direct comparison in survival across all groups is shown below in Figure 4.106. This reflects the overall trend in mortality showing the survival following major visceral resection was worse in internally drained rats.

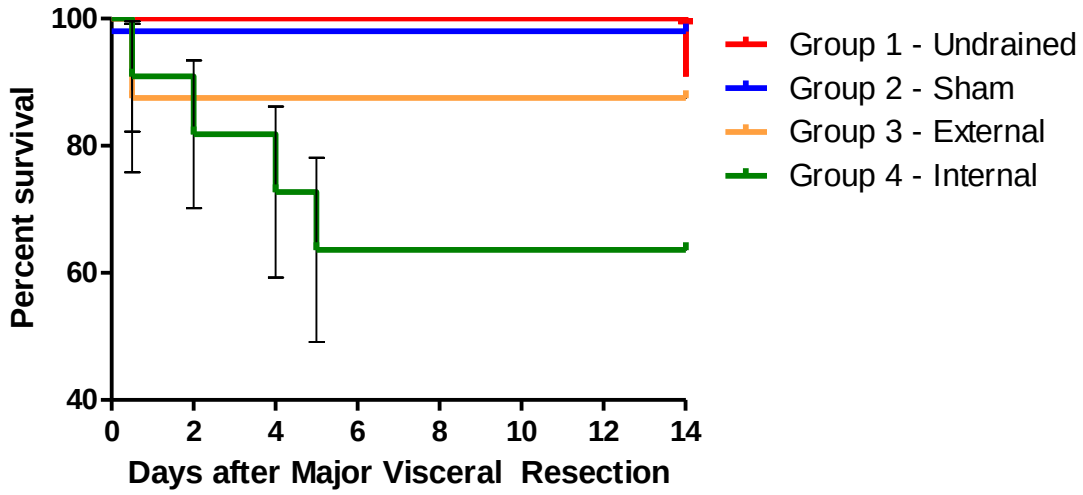


Figure 4.106: Survival difference between all groups following MVR (Log rank test. Chi-square=8.84, df=3, p=0.031).

Summary of survival differences

On examination of overall survival between all groups, as expected a significant difference is seen across all groups with Group 4 (internal drainage) having the lowest overall survival following MVR.

There is no statistically significant difference in mortality following MVR between no preoperative biliary drainage and those rats that underwent preoperative biliary drainage. Neither external nor internal drainage demonstrated a survival advantage over each other following major visceral resection.

5: Discussion

5.1: Summary of results

Following bile duct ligation, liver function tests followed the expected pattern, with elevation of bilirubin, ALP, ALT and GGT. Albumin and globulin levels fell significantly after bile duct ligation but total protein levels did not alter.

Similarly both external and internal drainage procedures resulted in significant falls in bilirubin. In the internally drained groups the bilirubin trended towards a rise again after 21 days of biliary drainage (although not significantly). ALT levels showed a significant increase after 14 days which then did not increase further. The external drainage group did not show any tendency for the LFTs to rise after the initial fall.

Neither drainage method resulted in a significant increase of albumin levels during the 21 days of biliary drainage prior to major visceral resection. On direct comparison between groups there was also no change in variability between the groups i.e. one group did not show an increase or decrease in albumin when compared to the other.

Sham operated animals showed a continuous gain in weight throughout the experiment up to the point of major visceral resection.

Following major visceral resection, there was no overall variability in albumin between the two drainage groups i.e. neither group showed a rise or fall in albumin

compared to the other. On individual group analysis, albumin levels in the internal drainage group fell 7 days following major visceral resection and then remained static for the remainder of the experiment. No change in albumin was seen in the externally drained rats after major visceral resection. The sham rats for the first time demonstrated a fall in albumin over the first seven days but which then increased at 14 days. The control rats also dropped their albumin levels in the first seven days after major visceral resection from an already lower mean than the other three groups.

Mortality rates in the three weeks following biliary drainage was no different in the external or internally drained rats. However deaths in the externally drained rats all occurred in the first week of the drainage period, whereas deaths in the internally drained animals were more evenly spread over the drainage period.

Direct comparison of mortality across all groups demonstrated a significant difference in mortality, with the lowest survival in the internally drained group. However on individual analysis only the sham operated vs internally drained rats had a statistically different survival rate. There was no statistically significant difference in mortality between no preoperative biliary drainage and those rats that underwent preoperative biliary drainage prior to major visceral resection. Neither external nor internal drainage demonstrated a survival advantage over each other following major visceral resection.

In summary these results support the null hypothesis as stated in the experimental aims that "different methods of preoperative biliary drainage will not affect either post operative outcome or albumin levels".

5.2: Relationship of this study to current literature

This study corresponds with both animal and human studies showing that the development of hypoalbuminaemia is associated with obstructive jaundice [3, 9, 256]. However there are few animal studies comparing drainage procedures prior to an experimental model of surgical stress such as the major visceral resection used in this study.

This study revealed no significant difference in mortality between animals undergoing MVR either with or without biliary drainage (irrespective of the type of drainage) which is in keeping with the metaanalysis by Sewnath *et al* [19] and other studies as discussed in the introduction chapter on biliary drainage.

Using renal ischaemia as a stressor Greve *et al* found internal biliary drainage increased survival [383]. Mok *et al* also found that obstructive jaundice in rats was associated with hypoalbuminaemia. They also found that internal drainage was superior in restoring albumin levels to normal, in animals where duration of jaundice prior to drainage was longer at least 3 weeks [9]. They found that shorter periods of drainage did not improve albumin recovery.

Li *et al* used a similar rodent model but found internal drainage superior to external drainage in correcting the abnormal liver function tests one week after drainage [384]. While we found that liver function tests also fell rapidly in both groups there was no improvement in one group compared with the other after this time. In fact the internal drainage group developed increased liver function tests over the course of the experiment (see below).

5.3: Justification of methods

The decision to use rats in this study was based upon the knowledge that it was a well established model for the investigation of obstructive jaundice. Once the experimental technique was established obstructive jaundice could be reliably reproduced allowing intra and intergroup comparison. As described in the introduction, total division of the bile duct has been shown to reduce recanalisation compared to simple ligation techniques. The initial experimental plan had been to drain bile externally via the bladder using a plastic cannula but as described below due to difficulties in establishing this model the simpler method of draining externally via the skin was used.

While the use of the major visceral resection used in this study as surgical model of surgical stress has not been previously described in the literature, it was used on the basis of unpublished work (by the group of Launois *et al*) which demonstrated a 70% mortality in this model for jaundiced rats following MVR. This model provides a suitably large operative stress but without provoking any element of ischaemia/reperfusion that is used in other stress models such as pulmonary compression

[385] or hepatic ischaemia [386] but does involve a higher surgical insult than simple models using laparotomy and bowel handling alone [387]. These examples however are in non jaundiced rats and there are very few references to surgical stress in jaundiced animal models. Greve *et al* used a model of bilateral renal ischaemia in jaundiced rats but all these animals died within a week following this procedure [383]. Bemelmans *et al* also used renal ischaemia in jaundiced mice as a surgical stress and showed a 36% mortality at one week following the surgical insult [388].

The control group (group one) was used to simulate the situation where a jaundiced patient immediately undergoes major surgery, such as a pancreaticoduodenectomy, without recourse to a prior drainage procedure. The two drainage groups were chosen to compare the effects of biliary drainage procedures prior to a major resection. The sham group was used to control for the effects of anaesthesia and laparotomy and the stress of the major visceral resection without jaundice. The one week interval between bile duct ligation and drainage procedure was chosen as a demonstrable drop in albumin is seen after this time. Following the drainage procedure the rats were allowed 3 weeks to recover as it has been shown albumin levels recovered towards normal after this time [383].

5.4: Strengths and weaknesses of this study

This study used similar methods to those previously described in the literature to produce obstructive jaundice and had good survivability once the model was established. The choice of major visceral resection as a surgical stress was also well tolerated and there were no technical complications with this part of the procedures. This was perhaps

somewhat surprising as the expected mortality for the jaundiced group was 70% but in this study only one of eleven rats in total died following the major visceral resection.

One of the major weaknesses of this study was the difficulties encountered in establishing the model i.e. initial problems in ligating the common bile duct resulting in frank bile leaks and death from biliary peritonitis. Animals that were found to have frank bile leaks and subsequently euthanased were excluded from the study as this complication was felt to be a technical error. Once the correct technique was identified very few bile leaks occurred but this did produce a problem due to the reduced total numbers of animals in each group (despite extension of the animal numbers by the ethics committee). This demonstrates the 'learning curve' necessary for establishing animal models in obstructive jaundice. Thompson *et al* [89] in their well described work in rat models of internal and external biliary drainage had a 25% mortality in internally drained rats (due to bile leaks from a choledochoduodenal anastomosis) which improved with further experience.

Initial technical problems were a particular problem in the original external drainage group where initial bile leaks were compounded by the death of animals having the choledochovesical cannula. The initial plan was to use external drainage by using an choledochovesical cannula as described by Diamond *et al* [389] although in their work the cannula was placed at the time of the initial bile duct ligation and then ligated intraperitoneally. Drainage was achieved by removal of this ligation allowing bile to drain into the bladder.

Initially using this method, the cannula was placed after one week of bile duct obstruction into the common bile duct and bladder. However 4 rats in this study died suddenly after insertion of the choledochovesical cannula with no apparent evidence of bile duct obstruction or leak on post mortem. Sepsis was thought to be a contributing factor despite the use of prophylactic antibiotics. Following a review of the literature and discussions with the ethics committee and veterinary officer an alternate method of external drainage using a cannula tunnelled through the skin was used as an alternative as it was felt that as it was a technically more straightforward it would be better tolerated.

The decision to use plastic tube drainage to simulate internal and external drainage was based on the use of similar techniques in other published work [308], [91] although in this model the tubes were placed after the bile duct had been obstructed for one week as opposed to placement of the cannulae at the time of bile duct ligation.

One of the main limitations in the external drainage group is the oral recirculation of bile due to grooming by the rats. This potential problem has not been widely discussed in published papers using techniques where bile is drained via the skin [315, 390] although one author did use bags to collect bile but did not state how these were drained and excluded any animals that damaged the bags. The use of long (30cm) external catheters has been described but sampling only occurred for 3 hours during which time the animals were kept in restraining cages [391] which is obviously neither practical nor ethical for the 5 weeks of external drainage required in this study. Other authors however have collected bile from rats for up to 3 weeks using long exteriorised

catheters but protected these catheters from interference by the rats using specialised rodent jackets [392]. Future work using external drainage should use this type of system to ensure no recirculation occurs.

Another limitation is that the external group rats were not internally drained at the time of MVR. This would simulate the conditions seen in the clinical scenario and another group should have been included to reflect this. This could have been easily performed under general anaesthesia at the time of the MVR by sectioning the external cannula and placing one end into the duodenum as per the internal drainage group. This would then allow restoration of the normal enterohepatic biliary circulation. Similarly the control group should have been drained internally at the time of the MVR. Both of the above however would have introduced considerably larger technical challenges and risk of subsequent complications.

One additional problem which became apparent during the course of the study was that despite double ligation and division of the bile duct, there was not 100% success in establishing definite total biliary obstruction. Several animals in the control group had evidence of falling bilirubin levels which either gradually trended down, or in two animals returned to within near normal range by the end of the experiment. The gradual tailing down could be due to changes in fluid balance with water and sodium retention but the dramatic decline of bilirubin seen in two animals was most probably the result of recanalisation of the common bile duct. However following euthanasia all of these animals had inspection of the abdominal cavity which showed evidence of a tense dilated common bile duct despite falling bilirubin levels with no evidence of bile

leaking in to the duodenum with gentle manual pressure on the bile duct. One animal did become jaundiced but not to the extent seen with the other animals and again the bilirubin levels did fall. On post mortem the bile duct was seen to be distended but on gentle pressure bile was seen to leak into the duodenum. It may be that in short span experiments (i.e. one week) there is not enough time for recanalisation and therefore ligation and division is adequate but as our rodents were jaundiced for 21 days this may have been sufficient time for this to occur. This could be avoided in future experimentation by actually transplanting and securing the distal end of the common bile duct behind the duodenum as described by Holmberg *et al* [363] who claim that use of this method results in a zero degree of recanalisation.

Using tubing to establish internal biliary drainage worked in the main part very well but in some cases the bilirubin was seen to fall following internal drainage but gradually rose over the following weeks. In some of these animals this did not affect their survival to the end of the experiment but were found to have dilated bile ducts at post mortem. In some cases where the animals were euthanased prior to completion of the experiment a dilated CBD was evident at post mortem. Over the course of the experiment the tube may become narrowed or blocked by food debris, leading to the less obvious elevations of bilirubin. This effect may also have led to the development of cholangitis which in turn may have influenced the chances of the animal dying. While only minor elevations of bilirubin were seen in the external drainage group over the course of the experiment one rat developed a retracted tube at the time of euthanasia with some bile leaking into the peritoneum at autopsy, with a concomitant increase in serum bilirubin. In general however problems with tube blockage were seen less

commonly in the external drainage group as one might expect. Other authors have advocated the use of a liquid diet to avoid the cannula becoming blocked by food particles [315].

Some animals did not develop a high rise in bilirubin after bile duct ligation seen in other animals but were found to have dilatation of the common bile duct at post mortem. It may be that in the rodent model a degree of biliary obstruction may be tolerated with relatively normal liver function tests.

5.5: Implication for future research

Results from these studies support the work from other groups and produce interesting data about the effect of a major surgical stress (MVR) in the context of significant obstructive jaundice. Biliary drainage not only does not improve survival after a surgical insult but is also associated with significant complications. In addition neither type of drainage procedure significantly improved albumin recovery prior to major visceral resection.

Future research should be directed toward prospective randomised trials in man. Questions that should be addressed include whether biliary drainage should be performed but also what type of drainage is the preferable, the optimal timing of drainage prior to surgery and whether or not the type of surgery makes a difference (hepatic resections, pancreatic surgery or palliative procedures). Levels of preoperative hepatic function in the jaundiced liver may also affect outcome and it remains unclear whether those with underlying preoperative cirrhosis or steatohepatitis would benefit

from drainage. The results of the DROP trial may help to answer some of these questions but the history of research into the effects of biliary obstruction suggest that it will pose new ones.

Appendix A

Establishing the experimental model and animal exclusion

Preliminary animal work

During the initial development of this project, 5 animals were authorized for use by the ethics committee in order to develop the techniques of bile duct ligation and division and major visceral resection. Following these procedures the rats were euthanased under general anaesthetic without regaining consciousness.

The common bile duct in the rat is easily visible throughout its course through the pancreas and for this reason this site was chosen to isolate the common bile duct and ligate and transect it to create bile duct obstruction as it was felt to be safer than dissection more proximally in or near the hilum.

Bile duct ligation

The first series of experiments commenced with group one (bile duct ligation and division followed by major visceral resection without biliary drainage) and the rats were designated 101 to 115. Three animals per day had bile duct ligation procedures so that the operative procedures for the whole group were completed between Monday to Friday. The initial 3 animals developed bile leaks and became unwell between day 3 and day 7 and were euthanased. The following two animals did not develop bile leaks despite using the same procedure as for the initial 3 animals. From the 15 animals that were operated on in that group 9 developed bile leaks after bile duct ligation and were

euthanased or died spontaneously. In some of these animals bile stained fluid was seen in the abdomen at the time of second laparotomy for MVR but with evidence of biliary dilatation. At this stage of the study it was thought the bile stained fluid may be bilious ascites due to obstructive jaundice and MVR was undertaken. However it soon became apparent soon after that the bilious fluid was due to bile leaks and when bile duct ligation occurred successfully, no fluid was found in the peritoneum and the common bile duct was very large and tense.

Internal biliary drainage

The second group to be operated on was experimental group four, in which the animals were scheduled for internal biliary drainage (rats 201 to 215). Of these animals, 10 developed early bile leaks with 5 developing obstructive jaundice without difficulty. One of these 5 rats one did however subsequently develop a bile leak nine days after internal drainage as at post mortem the tubing appeared to have damaged the friable common bile duct (which may or may not have occurred at the time of the drainage procedure). This animal was included in the study as the bile leak was felt to be a true complication of having a biliary drain, rather than a technical failure at the time of the procedure.

External biliary drainage

The group of rats initially allocated to external drainage (rats 401 to 415) was a disappointing group that was beset by a series of problems. One animal in this group (rat 413) was converted to experimental group one (no drainage) as at the time of second laparotomy the bile duct was poorly dilated and stuck behind densely adherent

adhesions. It was felt that attempts to dislodge these would result in possible bile duct damage which may have been irreparable.

The initial experimental plan was to perform external biliary drainage with the use of a plastic cannula connected between the dilated common bile duct and the bladder (choledocho-vesical cannula) as described by Burke *et al* [393] and Clements *et al* [292]. However Clements *et al* inserted a silastic catheter at the time of bile duct ligation and introduced a kink in the cannula that could be reversed to relieve biliary obstruction at a later date. Five animals in this group developed a bile leak after bile duct ligation (and prior to biliary drainage) and were euthanased. One animal did not recover from the anaesthetic following the drainage procedure and two developed bile leaks around the proximal end of the catheter, most probably due to technical complications at the time of surgery. Following these observations of proximal leakage the technique was altered to include a second purse string placed around the proximal end of the tubing.

One animal survived following placement of a choledochovesical cannula to the end of the experiment but four others became unwell and were euthanased prior to MVR. No obvious cause was apparent and there was no evidence of bile leak in any of these animals. One animal grew *morganella morganii* on blood cultures suggesting a septic cause of death (or at least a significant contribution).

Following the unexpected death of four animals a change in technique was clearly required and after discussion with the animal ethics committee it was decided that subsequent external drainage would be carried out via a skin tunnelled catheter

[315]. As described in the experimental methods it was felt that with one less anastomosis the operative procedure would be simpler and hopefully safer.

Investigation of bile leaks and change in technique

One of the major problems was determining why some animals developed bile leaks and others did not, despite an identical technique being used. Further advice was sought at this time from a previous researcher with experience of the technique. It was felt that the chosen site of ligation was too low and should be in the proximal bile duct prior to its course through the pancreas. From further examination of rat anatomy it was concluded that there must be smaller bile channels draining through the pancreas which join the main common bile duct again prior to its insertion into the duodenum. It is likely that in dissecting out the common bile duct pancreatic ducts were disrupted, causing erosion and leakage from the dilating and friable CBD. The rat pancreas does not have one main draining duct but rather several smaller ducts that drain directly into the common bile duct during its course through the pancreatic parenchyma. However, even these observations fail to fully explain why not all animals that were bile duct ligated in this way developed bile leaks.

Further animals were authorised by the ethics committee in order to repeat the external drainage group and to increase group numbers in the control and internal drainage groups in order to try to attain the 15 animals required in each group.

Following these changes only one of the next 15 animals undergoing bile duct ligation developed a bile leak prior to the second laparotomy for drainage. One animal

was euthanased intra operatively as the bile duct tore during insertion of the drainage tube and could not be repaired. Four developed bile leaks around the tubing following biliary drainage procedures and another was found to have developed a spontaneous perforation of the common bile duct in its lateral edge away from the position of the drainage tube.

After these changes incidences of bile leaks decreased dramatically, but not to zero. After further experience the incidence of bile leakage around the cannula also decreased.

Decisions regarding included and excluded rats

For the purposes of inclusion into the study several decisions were made about inclusion. Firstly any animal that had developed a bile leak due to technical failures (the development of biliary peritonitis prior to the second laparotomy or due to leakage around the drainage cannula) were excluded. Once satisfactory biliary obstruction was created any deaths after this were included (including death related to anaesthesia).

A full breakdown of the total numbers of animals used (105 in total) is given in Appendix B.

Appendix B

Summary of included and excluded rats

The following tables summarise the outcomes of each animal involved in the study. Those highlighted in green demonstrate the animals included for statistical analysis. The animals highlighted in red were excluded. Day zero is defined in these tables as the day of the first surgical procedure, either bile duct ligation or sham laparotomy. The headings of the groups are defined as per the numbering of the rats during progress of the practical work and not as the final numbering of group according to their drainage subtypes. The experimental groups are highlighted in the second column for clarity and summarised again below including the total numbers included and excluded.

Group	Experimental procedures	Included rats	Excluded rats
One	Bile duct ligation Major visceral resection	11	9
Two	Sham laparotomy Sham laparotomy Major visceral resection	18	0
Three	Bile duct ligation External biliary drainage Major visceral resection	12	24
Four	Bile duct ligation Internal biliary drainage Major visceral resection	14	17
Total		55	50

Group One (rats 101-115)

Rat	Procedure	Experimental Group	Outcome	Comment
101	Control	One	Euthanased day 8	Bile leak
102	Control	One	Euthanased day 8	Bile leak
103	Control	One	Euthanased day 4	Bile leak
104	Control	One	Survived to Experiment End	
105	Control	One	Survived to Experiment End	
106	Control	One	Euthanased day 5	Bile leak
107	Control	One	Survived to Experiment End	
108	Control	One	Survived to Experiment End	
109	Control	One	Survived to Experiment End	
110	Control	One	Euthanased day 9	Bile leak
111	Control	One	Euthanased day 3	Bile leak
112	Control	One	Survived to Experiment End	
113	Control	One	Died day 6	Bile leak
114	Control	One	Euthanased day 6	Bile leak
115	Control	One	Euthanased day 7	Bile leak

Group Two (rats 201-215)

Rat	Procedure	Outcome	Comment
201	Internal Drainage	Euthanased day 6	Bile leak
202	Internal Drainage	Euthanased day 5	Bile leak
203	Internal Drainage	Euthanased day 16 (post drain)	
204	Internal Drainage	Euthanased day 9	Hole in CBD ? from tube
205	Internal Drainage	Euthanased day 6	Bile leak
206	Internal Drainage	Survived to Experiment End	
207	Internal Drainage	Survived to Experiment End	
208	Internal Drainage	Euthanased day 3	Bile leak
209	Internal Drainage	Survived to Experiment End	
210	Internal Drainage	Euthanased day 3	Bloody fluid in abdomen
211	Internal Drainage	Euthanased day 7	Bile leak
212	Internal Drainage	Euthanased day 8	Bile leak
213	Internal Drainage	Euthanased day 5	Bile leak
214	Internal Drainage	Survived to Experiment End	
215	Internal Drainage	Euthanased day 11	Bile leak

Group Three and Group Seven

This group (rats 301-315 and 701-703) were sham, sham, MVR rats. All rats survived the experiment and were all included.

Group Four (rats 401-415)

Rat	Procedure	Outcome	Comment
401	External to bladder	Euthanased day 8	Bile leak around cannula
402	External to bladder	Euthanased day 8	Bile leak around cannula
403	External to bladder	Euthanased day 3	? pancreatitis
404	External to bladder	Euthanased day 5	Bile leak
405	External to bladder	Euthanased day 5	Bile leak
406	External to bladder	Euthanased day 2	Bile leak
407	External to bladder	Euthanased day 7	Bile leak
408	External to bladder	Euthanased day 7	Bile leak
409	External to bladder	Euthanased day 10	No cause found
410	External to bladder	Died day 7	During recovery from GA
411	External to bladder	Euthanased day 10	No cause found
412	External to bladder	Survived to Experiment End	
413	Control	Survived to Experiment End	
414	External to bladder	Died day 10	No cause found
415	External to bladder	Euthanased day 10	? sepsis Morganella morganii cultured

Group Five (rats 501-515)

Rat	Procedure	Outcome	Comment
501	Internal Drainage	Euthanased day 8 (post drain)	? gastric outlet obstruction
502	Internal Drainage	Survived to Experiment End	
503	Internal Drainage	Died day 33 (post MVR)	Coliforms, Proteus and Enterococcus in bile culture
504	Internal Drainage	Died day 39 (post MVR)	
505	Internal Drainage	Euthanased day 6	Bile leak
506	Internal Drainage	Died day 24 (post MVR)	
507	Internal Drainage	Survived to Experiment End	
508	Internal Drainage	Euthanased day 9 (post drain)	Bile leak
509	Internal Drainage	Euthanased day 30 (post MVR)	Coliforms and streptococcus in bile culture
510	Internal Drainage	Euthanased day 10 (post drain)	Bile leak
511	Control	Euthanased day 21 (14 days post MVR)	
512	Internal Drainage	Euthanased day 7	Bile leak
513	Internal Drainage	Euthanased day 7	Damaged CBD
514	Internal Drainage	Survived to Experiment End	
515	Internal Drainage	Euthanased day 8	Bile leak

Group Six (rats 601-615)

Rat	Procedure	Outcome	Comment
601	External to skin	Euthanased day 11 (post drainage)	
602	External to skin	Survived to Experiment End	
603	External to skin	Survived to Experiment End	
604	External to skin	Euthanased day 7	Bile leak
605	Internal Drainage	Died day 28 (at MVR)	Died under GA
606	External drainage	Euthanased day 7	Bile leak
607	External drainage	Euthanased day 7	Irreparable CBD damage
608	External drainage	Euthanased day 7	Bile leak
609	External drainage	Euthanased day 5	Bile leak
610	External drainage	Euthanased day 6	Bile leak
611	External drainage	Survived to Experiment End	
612	External drainage	Died day 10 (post drainage)	
613	External drainage	Euthanased day 7	Died under GA
614	External to skin	Euthanased day 7	Irreparable CBD damage
615	External to skin	Survived to Experiment End	

Group Eight (rats 801-812)

Rat	Procedure	Outcome	Comment
801	Control	Survived to Experiment End	
802	Internal Drainage	Died day 6	Bile leak
803	External to skin	Died day 12	Bile leak around cannula
804	Control	Survived to Experiment End	
805	External to skin	Survived to Experiment End	
806	Control	Survived to Experiment End	
807	External to skin	Died day 7	Bile leak
808	External to skin	Survived to Experiment End	
809	External to skin	Died day 28 (at MVR)	Died under GA
810	External to skin	Survived to Experiment End	
811	External to skin	Euthanased day 11	Urinary retention
812	External to skin	Died day 10	

Appendix C

Clinical Record Sheet

Investigator –
Rat I.D. no - Date of Arrival -
Starting weight (grams) -
Planned Procedures –

Date	Weight (g)	% Loss	Distress Score	Wound	Comments / procedures / analgesia given	Initials	Hb

Weight loss greater than 15 % euthanase regardless of score, convulsions, coma or score of 3 or 4 is reached in any category

DAILY DISTRESS MEASUREMENTS

VARIABLE		SCORE
Body weight changes	Normal	0
	< 10 % Weight loss	1
	10 - 15 % Weight loss	2
	>15 % Weight loss (euthanasia limit)	-
Physical appearance	Normal	0
	Lack of grooming	1
	Rough coat, nasal/ocular discharge	2
	Very rough coat, abnormal posture, enlarged pupils	3/4
Measurable clinical signs	Normal	0
	Small changes of potential significance	1
	Temperature changes of 1-20C, cardiac and respiratory rates increased up to 30%	2
	Temperature changes of > 2 ^{o}C, cardiac and respiratory rates increased up to 50%, or markedly reduced	3/4
Unprovoked behaviour	Normal	0
	Minor changes	1
	Abnormal; reduced mobility, decreased alertness, inactive	2
	Unsolicited vocalisations, self mutilation, restlessness, immobility	3/4
Behavioural responses to external stimuli	Normal	0
	Minor depression/exaggeration of responses	1
	Moderately abnormal responses	2
	Violent reactions, comatose	3/4

Adapted from: Moreton DB and Griffiths PMH (1985) "Guidelines on the recognition of pain, distress. Source : Adapted from University of Newcastle, Australia. Research and Research Training Website

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