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Title:
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Date:
2019-04-01

Citation:
Bishop, B., Hannah, N., Doyle, A., Amico, F., Hockey, B., Moore, D., Sood, S., Gorelik, A., Liew, D., Njoku, D. & Nicoll, A. (2019). A prospective study of the incidence of drug-induced liver injury by the modern volatile anaesthetics sevoflurane and desflurane. Alimentary Pharmacology and Therapeutics, 49 (7), pp.940-951. https://doi.org/10.1111/apt.15168.

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Article type : Original Scientific Paper

A prospective study of the incidence of drug-induced liver injury by the modern volatile anaesthetics sevoflurane and desflurane

A prospective study of the incidence of modern VA-DILI

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Author contributions

AN, BH, DM, and DL: study design. BH, DM, SS, and AN: recruitment supervision and data collection. BB, NH, AD, and FA: patient recruitment and data collection. DN: laboratory work. AG: statistical analysis. BB, AN, and DN: manuscript drafting. All: data analysis and final manuscript preparation.

Conflicts of interest

Prof Nicoll would like to declare grants from MSD, Bayer and BMS, outside this work. All other authors have no conflicts of interest to disclose.

Key words

Hepatitis, liver function tests, hepatotoxicity, sevoflurane, desflurane

Footnotes

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List of abbreviations

- VA-DILI: volatile anaesthetic drug induced liver injury
- ALT: alanine transaminase
- GGT: gamma-glutamyltranspeptidase
- ALP: alkaline phosphatase
- TFA: trifluoroacetylated lipid and protein adducts
- CYP 2E1: cytochrome P450 2E1
- BMI: body mass index
- MAC: minimum alveolar concentration
- MAC hour: MAC per hour, a calculation of anaesthetic dosage over time
- RUCAM: Roussel Uclaf Causality Assessment Method

Financial support

This study was funded by the Departments of Gastroenterology and Hepatology, and Anaesthesia and Pain Management, Special Purposes Funds, Royal Melbourne Hospital. Specialist Laboratory work was funded by the Departments of Anesthesiology and Critical Care Medicine, Pediatrics, and Pathology, John Hopkins University, Baltimore, U.S.A.

Word count

4798 words, excluding title page, foot notes, summary, references, tables and figures (summary: 299 words)

Summary

Background: Volatile anaesthetics are known to cause drug-induced liver injury, a hepatotoxic reaction characterised by antibodies to trifluoroacetylated lipid and protein adducts and cytochrome p4502E1. The incidence of volatile anaesthetic drug induced liver injury from older agents has been described, but modern agents have not been prospectively studied.

Aims: Our aim was to prospectively determine the incidence of volatile anaesthetic drug induced liver injury from sevoflurane and desflurane.

Methods: Adult surgical patients with a predicted post-operative stay of at least four days were recruited. If volatile anaesthetic was administered, liver biochemistry was performed regularly. Medications, observations, and other investigations were documented. Patients with abnormal liver biochemistry were classified as likely volatile anaesthetic drug induced liver injury or not based on clinical assessment, Roussel Uclaf Causality Assessment Method score, and the absence of other likely pathology. Some patients were also tested for antibodies to both trifluoroacetylated lipid and protein adducts, and cytochrome p4502E1.

Results: 209 patients were recruited, of which 121 were included for analysis. Post-operative liver biochemistry was abnormal in 62 patients (51.2%); further classified as not volatile anaesthetic drug induced liver injury in 47 cases (38.8%), and likely volatile anaesthetic drug induced liver injury in 15 cases (12.4%). Of the likely volatile anaesthetic drug induced liver injury patients, only one had severe disease with alanine transaminase greater than five times the upper limit of normal, while four cases had moderate disease with alanine transaminase greater than three times the upper limit of normal. Thus, the incidence of clinically significant volatile anaesthetic drug induced liver injury was 4.1%. No risk factors were identified.

Conclusions: Volatile anaesthetic drug induced liver injury from modern agents seems to be as common (4.1%) as previously reported with older agents (3%), and may identify patients at risk of severe acute liver injury with subsequent re-exposure.

Introduction

Drug induced liver injury, or drug-related hepatotoxicity, is injury to the liver due to exposure to a drug, as demonstrated by impaired liver function tests (1). Volatile anaesthetics are commonly used inhaled halogenated agents that are a recognised cause of drug induced liver injury (2). Volatile anaesthetic drug-induced liver injury (VA-DILI) typically causes a rise in the alanine transaminase (ALT) two to 14 days post-operatively (3), and can manifest as a spectrum of disease, from asymptomatic derangement in liver biochemistry (4), to acute severe hepatitis (4-16), and rarely, fatal hepatic necrosis (17, 18).

The most well-known type of VA-DILI is “halothane hepatitis” (19). Halothane was widely used until it was found to cause liver injury in up to 24.4% of patients (20, 21), and has since been replaced by newer volatile anaesthetics. Volatile anaesthetics are primarily metabolised by the cytochrome p450 enzyme, CYP2E1, which generates intermediates that can covalently bind to native cellular components, creating trifluoroacetylated lipid and protein adducts (TFA) (22). Immune-mediated drug induced liver injury has been demonstrated in patients following modern volatile anaesthetics, but is less common than with halothane or enflurane (2, 22).

Modern volatile anaesthetics that are widely used include isoflurane (introduced in 1981), desflurane (1992) and sevoflurane (1995). These newer agents are thought to be much safer, with a lower risk of causing VA-DILI, since they have a lower rate of hepatic metabolism and thus generate less TFA (20, 23). In fact, sevoflurane is thought not to undergo hepatic oxidative metabolism at a clinically relevant level (24). However, there are case reports (4-18), as well as retrospective data (2), that suggest that VA-DILI with modern volatile anaesthetics can still occur.

The diagnosis of VA-DILI is difficult and requires exclusion of other causes of deranged liver biochemistry, such as infection, ischaemia, and other drug-induced or autoimmune phenomena. VA-DILI is characterised by a rise in ALT in the two to 14 days post-exposure, and is often accompanied by fever and eosinophilia in severe cases (25). Liver injury from volatile anaesthetics is due to a combination of toxicity and autoimmunity, with the former occurring early in the post exposure period, and the latter manifesting later (26). Most cases resolve, but evolution into fulminant and sometimes fatal hepatic failure, as well as chronic liver disease, has been described (4).

The aim of this study was to prospectively estimate the incidence of VA-DILI from the newer generation volatile anaesthetics, sevoflurane and desflurane, using accepted methods to diagnose drug induced liver injury, including careful case review, exclusion of other possible aetiologies, and calculation of the Roussel Uclaf Causality Assessment Method (RUCAM) score (27). Appreciation of VA-DILI with modern agents may aid hepatologists in recognising possible VA-DILI, and help anaesthetists identify those at risk and minimise the chance of severe VA-DILI occurring with rechallenge. This study was a pilot, proof of concept study, to determine if a prospective study of drug induced liver injury was possible, and to guide future work in this field.

Methods

Patient recruitment

A pilot prospective cohort study of patients recruited between June 2012 and June 2015 was performed. Patients were recruited from emergency trauma admissions in 2012 from June to November inclusive. Elective surgery admissions at the Royal Melbourne Hospital, including general, orthopaedic, ear, nose and throat, urology, plastics, oncology, breast and endocrine surgical lists were recruited from March to June inclusive in 2013, 2014 and 2015. Patients were recruited from Pre-Admission Clinic or the Day of Surgery admissions ward, and were required to give written informed consent prior to participating in the study. Data on other patients was not admissible without written consent, limiting recruitment to a small sample size of all patients receiving volatile anaesthetic during this period.

Inclusion criteria were age greater than 18 years, predicted post-operative length of stay of at least four days to allow for sufficient testing, and normal pre-operative liver function tests at baseline. Patients with pre-existing liver disease, abnormal pre-operative liver function tests, and hepatobiliary trauma or surgery were excluded due to their potential for confounding abnormalities in liver biochemistry. Patients on immunosuppressant medication were similarly excluded due to the possible effect on any subsequent immune-mediated drug induced liver injury. The final exclusion criterion was the incapacity to provide informed consent due to cognitive impairment or inadequate English language comprehension.

This study is registered with the Australian and New Zealand Clinical Trials Registry, Australian Clinical Trial Reference Number 12612000286842. Ethics approval was granted by the Melbourne Health Human Research Ethics Committee (EC00243), ethics approval number 2012.009.

Data collection

Baseline data collected at the point of patient recruitment included age, sex, body mass index (BMI), history of past volatile anaesthetic exposure, current medications, medication allergies, alcohol intake, date and type of surgery planned, and relevant past medical history, including prior and current liver disease, diabetes and dyslipidaemia.

Alcohol intake was calculated in grams per week based on the number of standard drinks, defined as ten grams of alcohol, consumed weekly as estimated by the patient. Based on the Australian National Health and Medical Research Council guidelines, greater than 120g per week of alcohol was considered “heavy” alcohol consumption (28).

The anaesthetic report was reviewed post-operatively to determine if volatile anaesthetic was used, record intra-operative medications, and identify any intra-operative complications such as hypotension or excessive blood loss. If volatile anaesthetic was used, the minimum alveolar concentration (MAC) and duration of anaesthesia was noted, from which the average MAC hour was calculated. MAC is defined as the alveolar concentration of volatile anaesthetic at which 50% of patients will not show a motor response to a standardised surgical incision, and is influenced by the solubility and equilibration of the volatile gas amongst the tissues, along with metabolism, excretion, cardiac output, and minute ventilation (29). The MAC hour is a measure of anaesthetic dosage over time, calculated by multiplying MAC by duration of volatile anaesthetic exposure in hours, and allows the comparison and summation of dosages of different volatile anaesthetic in different patients (2, 20).

The presence of fever (defined as temperature >37.5°C), eosinophilia (>0.5x10^9 cells/L), sepsis, hepatic trauma and hepatic ischaemia were documented, as was the requirement for blood transfusion. The

medications were carefully documented, paying particular attention to other potential causes of drug-induced liver injury such as antibiotics and paracetamol (30). Liver biochemistry was measured on post-operative days one and four, and then every four days until discharge.

Classification of liver function tests

Patients with abnormal post-operative liver biochemistry were subject to further assessment to determine the likelihood of VA-DILI. Differential diagnoses of VA-DILI were considered, including ischaemic hepatitis and rhabdomyolysis, particularly in trauma patients, as well as other drug induced liver injury, such as due to antibiotics or paracetamol.

The admission notes, operation reports, and the pattern of liver biochemistry over time were reviewed. Particular attention was paid to the degree and rapidity of ALT rise in relation to exposure to volatile anaesthetic and other medications, as per the RUCAM score (27).

A RUCAM score was calculated to aid in objectively identifying VA-DILI (27, 31). This score takes into account chronological criteria, the course of the reaction, risk factors, concomitant therapy, exclusion of other causes, previous reactions, and rechallenge reactions. The total RUCAM score generates a number which relates to likelihood of the drug, in this case volatile anaesthetic, as the cause of the deranged liver function tests. Where a patient had received multiple potentially hepatotoxic medications, a RUCAM score was calculated for each to help determine the more likely culprit.

The RUCAM score is interpreted as follows: 0 or less means that the drug is “excluded” as a cause, 1 to 2 is “unlikely,” 3 to 5 is “possible,” 6 to 8 is “probable,” and greater than 8 is “highly probable” (2, 4, 27). Patients with a RUCAM score greater than or equal to 3 were considered to be at least possible VA-DILI, with the clinical picture and pattern of liver biochemistry over time aiding the final classification following separate independent review by consultant hepatologists, authors AN and SS, with the plan to resolve any disagreements with a third independent hepatologist. However, there were no discrepancies in assessment by the two hepatologists and so adjudication was not required.

Based on this, patients with abnormal liver biochemistry were categorised into two groups: not VA-DILI, and likely VA-DILI. Any rise in post-operative ALT was considered, not only those cases fulfilling the criteria for drug induced liver injury (32), as capturing mild cases allowed for the assessment of the possible scope of this phenomenon.

Investigations for other causes of abnormal liver biochemistry were performed where appropriate, including but not limited to hepatitis A, B and C virus serology (and hepatitis D and E where appropriate),

other viral serology (e.g. Epstein-Barr virus, cytomegalovirus, herpes simplex virus), autoimmune serology (anti-nuclear antibody, anti-smooth muscle antibody, anti-liver-kidney microsomal antibodies, and anti-mitochondrial antibodies), markers of other causes of liver disease (caeruloplasmin, alpha-1-antitrypsin, iron studies), hepatobiliary ultrasound, and liver biopsy. We defined cumulative paracetamol excess that might cause hepatotoxicity as four grams of paracetamol per day for greater than or equal to four consecutive days.

The severity of VA-DILI was classified based on the maximum ALT level relative to the upper limit of normal (2, 32). The three categories were defined as follows: mild, ALT 55-119 IU/L (1.8 to 3 times the upper limit of normal); moderate ALT 120-199 IU/L (3 to 5 times the upper limit of normal); and severe, ALT ≥200 IU/L (greater than 5 times the upper limit of normal). Any rise in bilirubin in conjunction with raised ALT, not explained by blood transfusion or haemolysis, was also considered severe. Our criteria for severe VA-DILI are consistent with those outlined by the Drug-Induced Liver Injury Network (33). Only patients with moderate or severe VA-DILI were included to calculate the incidence of clinically relevant VA-DILI.

If the patient was discharged after developing abnormal liver biochemistry, ongoing monitoring occurred as an outpatient where possible.

Serological testing

A subset of patients with abnormal post-operative liver biochemistry also had serum tested for anti-TFA and anti-CYP2E1 IgG4 autoantibodies in the laboratory of author, DN, which is accredited to carry out this technique for research purposes. All participants did not have this testing as many were discharged after four days. Therefore, this group was enriched to include some patients who likely had VA-DILI on initial assessment. IgG4 autoantibodies to TFA and CYP2E1 were detected by enzyme linked immunosorbent assays, and positive values were two standard deviations above median control values, as previously described (34). While awaiting serology results, these patients were further assessed along with the remainder of the cohort and classified based on clinical assessment and RUCAM criteria as likely VA-DILI and not VA-DILI (7, 22, 34, 35).

Statistical analyses

Sample size calculation was performed using data from our retrospective study (2) and other studies (19, 36), and used to calculate standard deviations for the incidence of VA-DILI of 1.1-6.4%. This gave a recruitment target of 200 participants for sufficient power. The incidence was calculated as total number of patients with VA-DILI divided by the total sample. The primary outcome of interest was incidence of clinically significant, i.e. moderate and severe, VA-DILI from sevoflurane and desflurane, while

secondary outcomes included the difference in clinical characteristics between groups in order to identify putative risk factors, and the presence of autoantibodies and their correlation with disease.

The Student t-test and ANOVA was used to analyse normally distributed continuous variables, while the Mann-Whitney U and Kruskal-Wallis tests were used for non-normally distributed continuous variables. The Fisher's exact test was used to compare categorical variables. The data stored in FileMaker Pro 13.0v5 (Filemaker; Filemaker, Inc.Santa Clara CA, U.S.A.), exported to Microsoft Excel 2007 12.0.4518.1014 (Microsoft Corportation, Redmond WA, U.S.A.), and was analysed using Stata 12.1 for Windows (StataCorp LP, College Station TX, U.S.A.). P-values less than 0.05 were considered to be significant.

Results

Patients

Over four periods from 2012 to 2015, 209 patients were recruited. Due to the short stay of patients in the trauma unit, and the subsequent difficulty in obtaining day four and beyond liver biochemistry results, a decision was made to switch to recruiting elective patients from 2013 onwards. The pool of patients this sample of 209 patients was derived from comprised 750 trauma patients, 42 of which were recruited, and 5,884 elective surgery patients, 167 of which recruited.

A total of 88 patients (42.1%) excluded from analysis – 13 trauma patients in 2012, and 75 elective patients from 2013 to 2015 inclusive. Of these excluded patients, 13 patients recruited in pre-admission clinic did not have their operation within the recruitment time frame, 15 patients did not receive volatile anaesthetic, and 43 patients had insufficient liver biochemistry as they were either discharged prior to four days, or lacked normal pre-operative liver biochemistry within the previous three months. Furthermore, 17 patients were excluded due to screen failure on recruitment: 15 had significant pre-existing liver disease, and two were on long term immunosuppressant medication. No patients withdrew from the study. Therefore, a total number of 121 patients were included in the analysis, as demonstrated in Figure 1.

The demographic and clinical characteristics of the patient cohort are presented in Table 1. Of the 121 patients, 66 were male (54.5%) and 55 were female (45.5%), with a median age of 59 years. Nearly two thirds of patients underwent either trauma, orthopaedic, or colorectal surgery, comprising 24.0%, 24.0% and 15.7% of cases respectively. The vast majority of patients (91.7%) received sevoflurane alone as their volatile anaesthetic, while two cases (1.7%) received a combination of sevoflurane and desflurane, and the remaining eight cases (6.6%) received desflurane alone. A similarly large majority of patients (88.4%) had previously been exposed to volatile anaesthetic.

Post-operative liver biochemistry was normal in 59 patients (48.8%), and abnormal in 62 patients (51.2%). Of the 62 patients with abnormal post-operative liver biochemistry, 47 patients (79.7% of abnormal liver biochemistry patients) were regarded as not due to volatile anaesthetic exposure, as there were other more probable causes explaining their deranged liver biochemistry. The presumptive diagnoses of these cases were considered to be drug induced liver injury from antibiotics in 26 cases (55.3%), drug induced liver injury from cumulative paracetamol excess in 14 cases (29.8%), hepatic ischaemia in three cases (6.4%), and other causes such as bone disease and isolated hyperbilirubinaemia in the remaining four cases (8.5%). The most commonly implicated class of antibiotics were the cephalosporins, while other medications considered to possibly cause drug induced liver injury were phenytoin, non-steroidal anti-inflammatory medications, isoniazid, HMG-CoA reductase inhibitors, angiotensin II receptor antagonists, and excess alcohol intake. The clinical characteristics of these subgroups are shown in Table 2.

As shown in Table 3, a total of 15 patients (24.2% of abnormal liver biochemistry patients, 12.4% of total cohort) were regarded as likely VA-DILI, given the characteristic rise in ALT, the absence of more probable differential diagnoses, and a RUCAM score greater than three. However, only five of these cases had at least moderate VA-DILI with ALT greater than three times the upper limit of normal. This subgroup, whose ALT patterns over time are demonstrated in Figure 3, includes only one patient with severe VA-DILI, fulfilling the Drug-Induced Liver Injury Network criteria of ALT greater than five times the upper limit of normal. Thus, the incidence of clinically significant, i.e. moderate and severe, VA-DILI in our cohort was 4.1%, while mild hepatitis following volatile anaesthetic exposure occurred in ten patients, or 8.3% of the cohort, as shown in Figure 2. Of note, some cases of VA-DILI were potentially missed as it was not possible to get liver biochemistry from some patients beyond four days despite requesting them after discharge.

Only one of the 15 patients with likely VA-DILI was rechallenged with a second volatile anaesthetic exposure during their admission. This second exposure occurred three days after the first operation, while the peak ALT, which falls into the mild elevation of ALT category, occurred four days later, i.e., seven days after the first operation.

The clinical features of the three patient groups, i.e., normal liver biochemistry, abnormal liver biochemistry but not due to VA-DILI, and abnormal liver biochemistry likely due to VA-DILI, were compared as shown in Table 2. There were no significant differences between the three groups with respect to age ($p = 0.967$), sex ($p = 0.904$), BMI ($p = 0.319$), dyslipidaemia ($p = 0.201$), diabetes ($p = 0.493$), allergies ($p = 0.891$), or heavy alcohol consumption ($p = 0.197$). While there was also no significant difference in terms of prior volatile anaesthetic exposure and MAC hour, there was a trend, suggesting that these may be clinically important but could not be demonstrated here due to small numbers. In addition, concurrent

cephalosporin use was significantly greater in the abnormal liver biochemistry but not VA-DILI group (p = 0.018), reflecting that the use of a cephalosporin cast doubt on volatile anaesthetic being the cause of the deranged liver function tests, as per the RUCAM score.

The large number of cases lost to follow up (43 patients, 21%) reflects the difficulty in engaging patients once their immediate health problem has resolved. This was particularly a challenge with the young trauma patients. The ethics approval for this study allowed us to request that patients have follow up biochemistry post discharge, but did not permit us contacting people who did not follow through with this. However, there were no differences at admission between cases included and those lost to follow up, so it is unlikely that this would change the results.

Serological analysis

A subset of 13 patients from the cohort had anti-TFA IgG4 and anti-CYP2E1 IgG4 autoantibody testing because their early results suggested that they may have VA-DILI. Of the seven patients who on final analysis had likely VA-DILI, only four had positive autoantibodies, with two being positive for both anti-TFA and anti-CYP2E1, and two being positive for anti-CYP2E1 alone. All five of the patients classified as not VA-DILI had positive anti-TFA and anti-CYP2E1 serology. One patient with normal post-operative LFTs was tested and had significantly raised levels of both anti-CYP2E1 and anti-TFA. These results are summarised in Table 5.

Discussion

This is the first prospective study to our knowledge to attempt to examine the incidence and risk factors of VA-DILI with modern volatile anaesthetics. The incidence of VA-DILI in patients receiving sevoflurane or desflurane was found to be 4.1%, with another 8.3% with volatile anaesthetic hepatitis that did not fulfil the minimum criteria for drug induced liver injury of ALT greater than three times the upper limit of normal. These figures are similar to the incidence of 3% estimated in our previous retrospective study (2), and the ALT elevation was similarly mild in the majority of cases, and none resulted in jaundice.

VA-DILI causes a hepatitic pattern of liver biochemistry derangement, and normally manifests as a rise in ALT in the two to 14 days post-operatively. This is in contrast to hypotension and hepatic ischaemia, which causes a rise in ALT within 24 hours and tends to improve before 48 hours; antibiotic drug induced liver injury, which more commonly creates a cholestatic pattern and occurs seven to 28 days post exposure (37); and paracetamol drug induced liver injury, which usually requires at least four days of cumulative regular dosing (32). Often a diagnostic challenge, drug-induced liver injury is the most common cause of acute liver failure (38), however it is an evolving area of research and much is still not understood about its

pathophysiology and clinical consequences (39). Patients with abnormal post-operative liver function tests, whether due to VA-DILI or other liver injury, are usually asymptomatic. Therefore, relying on clinical signs and symptoms alone to diagnose drug induced liver injury means that only very severe cases are detected. Our study monitored post-operative liver biochemistry, and although this results in identifying a number of patients with clinically mild liver injury not fulfilling the criteria of drug induced liver injury, it does provide a more accurate estimation of the overall extent of this effect. There is also concern that patients identified with mild elevations of ALT after volatile anaesthetic exposure may develop severe VA-DILI with rechallenge (4, 25, 40, 41), so identifying milder cases may assist anaesthetists in future to exercise caution with re-exposure. Importantly, severe drug induced liver injury from volatile anaesthetics with jaundice remains rare, although our study was not powered to demonstrate this. It should also be noted that other causes of severe hepatitis, with or without jaundice, should always be considered in post-operative patients, such as that due to antibiotics, excessive paracetamol, and ischaemia.

The Drug-Induced Liver Injury Network Causality Assessment Criteria (33) was selected for classification of liver injury in our patient population as it is more inclusive than other scoring systems, such as that outlined by Aithal et al. (32), and has the validity and reproducibility for hepatotoxicity causality that other scores, like the Naranjo Adverse Drug Reactions Probability Scale, lack (42). The severity of liver injury in this guideline is categorised based on degree of hyperbilirubinaemia, while DILI itself is defined by much higher rises in ALT and ALP. We acknowledge that in applying these criteria, only one patient would satisfy the definition of drug induced liver injury, and that the severity would be classified as mild. However, the use of a more inclusive scoring system facilitates the capture of mild cases of VA-DILI in order to assess the scope of the problem. Furthermore, this classification is consistent with previous research conducted by our group (2), allowing for a more accurate comparison between retrospective and prospective data.

One of the surprising findings in this study is the high level of over prescription of paracetamol post-operatively. Many surgeons choose to avoid opiate or non-steroidal analgesia by using regular paracetamol, four grams per day orally or intravenously, even in patients who are fasting or have sepsis. The safety profile of paracetamol is regularly overestimated, and accidental or iatrogenic paracetamol toxicity is a significant reason for referral for liver transplantation assessment, equal to intentional paracetamol overdose (43, 44). It is clearly important to raise awareness of safe paracetamol dosing in surgical units.

Abnormal liver biochemistry post-operatively is often attributed to the peri-operative antibiotics, sepsis, or liver ischaemia. In this study we were careful to exclude these by checking for signs of sepsis or markers of possible organ hypoperfusion, e.g. intra-operative hypotension and transfusion requirements, and looking

at the characteristics of the liver biochemistry pattern. The most commonly used peri-operative antibiotic was a cephalosporin. However, drug induced liver injury from cephalosporins is uncommon and characteristically manifests as a cholestatic pattern of deranged liver biochemistry that occurs at least one week after the drug, not during the first few days post-operatively (45-47). It should be noted that ceftriaxone differs from other cephalosporins, and has been associated with biliary sludge, cholecystitis and cholestatic jaundice (48-50).

From retrospective studies (2, 36, 51) and case report data (4-18), proposed risk factors for VA-DILI comprise (21, 40, 41) non-modifiable characteristics such as genetic predisposition via an association with human leukocyte antigens, HLA-DR3 and DR4; previous exposure to volatile anaesthetic, including occupational exposure; older age, with a peak incidence at 50-60 years; female sex; and a history of atopy, allergy or autoimmune disease. Modifiable risk factors include obesity and altered hepatic metabolism, including cytochrome P-450 enzyme induction by certain drugs, such as ethanol (52). There are some factors that are thought to be unrelated to the risk of VA-DILI, including type or duration of surgery, and presence of pre-existing liver disease. This study was not powered to determine risk factors for VA DILI; however, age, sex, BMI, MAC hour, past history of volatile anaesthetic exposure, dyslipidaemia, diabetes, allergies, and heavy alcohol consumption were analysed. No significant risk factors for developing VA-DILI were identified in this study, although trends such as increased volatile anaesthetic exposure, i.e. MAC hour, past exposure, hyperlipidaemia and excess alcohol intake will need to be explored in larger cohorts.

Most patients received sevoflurane as their volatile anaesthetic. Sevoflurane is generally believed to be an uncommon cause of VA-DILI due to its minimal hepatic metabolism (20). However, it is metabolised to a limited extent by CYP2E1, and thus may produce the TFA neoantigens implicated in the pathogenesis of VA-DILI (24). There are a number of case reports of VA-DILI from sevoflurane supporting this (4, 12-14, 17, 18), and it is thought to be more common in the very young or in the elderly.

Anti-TFA and anti-CYPE2E1 IgG4 subclass autoantibodies are the only clinically validated serological test for VA-DILI, and are seen in 40% and 45-70% of cases respectively, whereas IgG1 subclass autoantibodies are associated with volatile anaesthetic exposure but not disease (34, 35, 53, 54). While VA-DILI can occur through either a toxic or immunological mechanism (26), it is accepted that patients commonly develop anti-TFA and anti-CYP2E1 antibodies of the IgG4 subclass (35). Furthermore, while IgG4 autoantibodies are the rarer of the immunoglobulin subclasses, they are strongly associated with IgE signalling in autoimmune diseases and allergy, such as thyroiditis (55) and asthma (56), suggesting that autoimmunity contributes to the pathophysiology of VA-DILI. Proteomic analyses in cases of drug-induced liver injury have also

identified proteins involved in immune system activation and inflammation (57). However, the exact role of the humoral system in VA-DILI has not been completely characterised.

Based on the proposed pathophysiology above, anti-TFA and anti-CYP2E1 IgG4 serological testing was performed on a small number of patients in this cohort. Testing was performed in an accredited laboratory employing a technique in which autoantibody levels are measured and compared to negative controls. These results show that specific antibodies consistent with VA-DILI were produced in some patients exposed to modern volatile anaesthetic agents. Consistent with the literature (34, 35, 53, 54), only half of the patients with clinical VA-DILI had positive anti-CYP2E1 antibodies. Furthermore, given serum was collected for serological testing early in the patient's admission, it may be the case that there was not enough time for the autoantibodies to be produced at detectable levels. On the other hand, all five of the patients with abnormal LFTs classified as not VA-DILI had positive anti-TFA and anti-CYP2E1 serology. All of these cases may have been VA-DILI, but as they received antibiotics or excessive paracetamol, and the rise in ALT was later than expected, it was deemed clinically more likely that other drugs were responsible for the hepatitis. Recognising that testing a small subset of patients is less robust, a future study planned by our group will examine pre- and post-operative serology of the entire patient cohort. In addition, testing patients' serology pre-operatively will identify those with pre-existing antibodies.

While prospective in nature, this study is limited by the small sample size. This is partially attributable to the logistical challenges of obtaining written informed consent as part of the recruitment process. The target of 200 patients for analysis was not reached as 88 patients of the 209 recruited had to be excluded. However, our initial assumption of an incidence of 3% (1.1-6.4%) was accurate, with 4.1% of participants having LFT patterns (ALT greater than three times the upper limit of normal) and RUCAM scores (>3) consistent with VA-DILI. In this case, 121 participants should be accurate for an incidence of 0.9-8%. This study was underpowered to detect the risk factors for VA-DILI but did show some interesting trends. Furthermore, patients were recruited at a single site, and so the sample cohort may not be representative of the general population, thus creating a potential selection bias.

Other logistical and financial constraints of this pilot study meant that it was not possible to test all patients for anti-TFA and anti-CYP2E1 serology, and we were unable to assemble an adjudication committee for assessment of VA-DILI as used in the Drug Induced Liver injury Network (33). In addition, while it would have been ideal, we were ethically unable to subject patients to daily venepuncture to test LFTs if it was otherwise clinically not indicated. Nevertheless, performing this preliminary prospective study has paved the way for further research into this field by our group.

Conclusion

This preliminary prospective study suggests that moderate to severe VA-DILI from modern volatile anaesthetics is relatively frequent with an incidence of 4.1% in our patient population, similar to our previous retrospective study (2). Earlier reports had suggested that sevoflurane and desflurane VA-DILI were extremely rare (20), although it is possible only severe cases have been previously identified, as a further 8.3% of patients in our cohort had mild VA-DILI. As such, most VA-DILI is mild and clinically insignificant, and other differential diagnoses of moderate to severe post-operative liver impairment must be excluded before attributing it to VA-DILI. Nevertheless, awareness of VA-DILI may improve recognition of milder cases, and prevent re-exposure and severe liver injury for some patients. Ongoing work in this area by our group will aim to characterise this drug induced liver injury further.

Acknowledgements

The authors would like to acknowledge the support and assistance of staff at the Royal Melbourne Hospital, including Ms. Rochelle Cotter, Ms. Marcelle Stewart and Ms. Nicolette Zingerle, Department of Anaesthesia and Pain Management; Mr. Thomas Baker, Department of Health Information Services; Pre-Admission Clinic and Day of Surgery Admissions staff; Mrs. Virginia Bird, Mrs. Zafirah Khan, and Ms. Brooke Flanders, Department of Gastroenterology and Hepatology, and the collaborating surgeons. We would also like to thank the patients that consented for their clinical information to be included in this study.

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Illustrations, figures and tables

Table 1: Clinical characteristics of the patient cohort

	Number (%) Total n = 121
Sex (%)	
Male	66 (54.5%)
Female	55 (45.5%)
Age (IQR)	
Median (years)	59 (42 – 70)
Body Mass Index (kg/m^2) (%)	
Normal, <25	43 (35.5%)
Overweight, 25 - 30	38 (31.4%)
Obese, >30	36 (29.8%)
Unknown	4 (3.3%)
Concomitant hepatotoxic drugs (%)	
Antibiotics[1]	98 (81.0%)
Acetaminophen (>4g/day, >4 days)	47 (38.8%)
Non-steroidal anti-inflammatory drugs	38 (31.4%)
HMG-CoA reductase inhibitors (statins)	28 (23.1%)
Other[2]	11 (9.1%)
Pre-operative LFTs (IQR)	
ALT (IU/L), median (normal range <34IU/L)	23 (16 – 32)
ALP (IU/L), median (normal range 30-120IU/L)	71 (57 – 87)
Bilirubin (IU/L), median (normal range <19IU/L)	8 (6 – 12)
Post-operative markers of autoimmunity (%)	
Fever (T >37.5°C)	28 (23.1%)
Eosinophilia (>0.5x10^9 cells/L)	9 (7.4%)
Procedure (%)	
Trauma surgery	29 (24.0%)
Orthopaedics	29 (24.0%)
Colorectal	19 (15.7%
General surgery[3]	9 (7.4%)
Ear, nose and throat	8 (6.6%)
Renal	8 (6.6%)
Urology	6 (5.0%)
Plastics	5 (4.1%)

Vascular	5 (4.1%)
Breast, oncology and endocrine	2 (1.7%)
Neurosurgery	1 (0.8%)
Volatile anaesthetic (primary procedure) (%)	
Sevoflurane	111 (91.7%)
Desflurane	8 (6.6%)
Both	2 (1.7%)
Previous volatile anaesthetic exposure	107 (88.4%)
>1 VA exposure during admission (%)	18 (14.9%)

Table 1: LFTs: liver function tests, ALT: alanine aminotransferase, ALP: alkaline phosphatase, IQR: interquartile range, T: temperature

1. Antibiotics included: cephalosporins, nitrofurantoin, isoniazid, and minocycline.

2. Other hepatotoxic drugs included: irbesartan, anti-retrovirals, and the oral contraceptive pill.

3. General surgery included: gastroesophageal surgery, abdominal wall hernia repair, and excision of simple skin lesions, such as lipoma.

Table 2: Comparison of clinical characteristics and analysis of risk factors associated with VA-DILI, compared to normal LFTs, and to other causes of abnormal LFTs

	Normal (n = 59)	Abnormal LFTs, likely VA-DILI (n = 15)	Abnormal LFTs, not VA-DILI					p value[2]
			Total not VA-DILI (n = 47)	Antibiotics (n = 26)	Paracetamol (n = 14)	Hepatic ischaemia (n = 3)	Other causes[1] (n = 4)	
Age[median (IQR)]	60 (42 – 72)	57 (48 – 66)	57 (42 – 68)	59 (44 – 68)	49 (34 – 66)	32 (29 – 54)	68 (58 – 75)	0.967
Sex [M:F]	31:28	8:7	27:20	14:12	9:5	2:1	2:2	0.904
BMI [mean ± σ]	27.9 ± 6.3	29.9 ± 6.4	27.1 ± 6.0	27.8 ± 6.0	24.5 ± 5.1	32.4 ± 8.4	27.8 ± 6.1	0.319
MAC hour[median (IQR)]	3.3 (1.8 – 4.8)	4.0 (3.2 – 6.6)	4.2 (2.4 – 7.1)	7.1 (4.2 – 9.5)	4.5 (2.4 – 5.5)	2.5 (1.8 – 3.1)	6.7 (5.2 – 7.2)	0.068
Prior VA exposure [n(%)]	49 (83.1%)	13 (86.7%)	39 (83.0%)	22 (84.6%)	12 (85.7%)	2 (66.7%)	3 (75%)	0.071
Dyslipidaemia [n (%)]	14 (23.7%)	6 (40.0%)	13 (27.7%)	8 (30.8%)	2 (14.3%)	1 (33.3%)	2 (50%)	0.201
Diabetes [n(%)]	11 (18.6%)	1 (6.7%)	5 (11.4%)	3 (11.5%)	1 (7.1%)	0 (0%)	1 (25%)	0.493
Allergies [n(%)]	19 (32.2%)	4 (26.7%)	11 (23.4%)	6 (23.1%)	3 (21.4%)	1 (33.3%)	1 (25%)	0.891
Heavy alcohol consumption [n(%)]	4 (6.8%)	3 (20.0%)	8 (17.0%)	3 (11.5%)	3 (21.4%)	1 (33.3%)	1 (25%)	0.197
Concurrent cephalosporin use [n(%)]	44 (74.6%)	12 (80%)	44 (93.6%)	25 (96.2%)	11 (78.6%)	3 (100%)	3 (75%)	0.018
Peak post-operative ALT [median (IQR)]	18 (13 – 27)	111 (71.5 – 142)	47 (33.5 – 76)	45.5 (31.3 – 78.3)	48.5 (37.8 – 62.5)	200 (156 – 237)	27 (21.3 – 35.8)	< 0.00001

Peak post-operative ALP [median (IQR)]	71 (57.5 – 89)	100 (90.5 – 129.5)	142 (73.5 – 201)	177.5 (142.5 – 239.3)	73 (59.3 – 100.5)	145 (108 – 227)	62 (57.7 – 105)	0.001

IQR: interquartile range, M: male, F: female, BMI: body mass index, σ: standard deviation, MAC: minimum alveolar concentration, VA: volatile anaesthetic.LFTs: liver function tests, ALT: alanine aminotransferase, ALP: alkaline phosphatase, IQR: interquartile range.

1. Other causes included: bone disease and isolated hyperbilirubinaemia.

2. p value is for comparison of the following groups: normal, total abnormal LFTs but not VA-DILI, and likely VA-DILI

Table 3: RUCAM scores and clinical characteristics of patients with likely VA-DILI

Pt	RUCAM score[1]	Age	Sex	Operation	Previous VA exposure	Number of VA exposures during admission	Pre-op ALT	ALT peak	Day of ALT peak	Anti-TFA	Anti-CYP 2E1	Fever	Eosinophilia	Severity of VA-DILI
1	12	74	F	Elective	Y	1	37	204	2	-	-	No	No	Severe (ALT>5xULN)
2	12	59	F	Elective	Y	1	18	164	2	+	+	No	No	Moderate(ALT>3xULN)
3	10	57	F	Elective	Y	1	41	128	3	N/A	N/A	No	No	Moderate (ALT>3xULN)
4	7	73	F	Elective	Y	1	24	119	1	-	-	Yes	Yes	Mild (ALT>1.8xALN)
5	7	65	M	Elective	Y	1	32	73	6	+	+	Yes	No	Mild (ALT>1.8xALN)
6	7	53	M	Elective	Y	1	39	66	1	N/A	N/A	No	No	Mild (ALT>1.8xALN)
7	7	66	F	Elective	Y	1	18	111	2	N/A	N/A	No	No	Mild (ALT>1.8xALN)
8	6	38	M	Trauma	Y	1	22	156	8	N/A	N/A	No	No	Moderate (ALT>3xULN)
9	5	70	M	Elective	Y	1	34	119	3	-	+	Yes	No	Mild (ALT>1.8xALN)
10	5	48	M	Elective	Y	1	19	57	8	N/A	N/A	No	No	Mild (ALT>1.8xALN)
11	5	39	F	Elective	N	1	14	79	6	N/A	N/A	No	No	Mild (ALT>1.8xALN)
12	5	48	M	Trauma	Y	1	28	70	8	N/A	N/A	No	No	Mild (ALT>1.8xALN)
13	4	65	M	Elective	Y	1	30	157	1	-	-	Yes	No	Moderate (ALT>3xULN)

14	4	48	F	Elective	Y	1	25	74	10	-	+	Yes	No	Mild (ALT>1.8xALN)
15	3	31	M	Trauma	N	2	49	61	7	N/A	N/A	No	Yes	Mild (ALT>1.8xALN)

Table 3: Pt: patient. VA-DILI: volatile anaesthetic drug-induced liver injury. RUCAM: Roussel Uclaf Causality Assessment Method. LFTs: liver function tests, ALT: alanine aminotransferase. M: male, F: female. Y: yes, N: no. Anti-TFA: antibodies to trifluoroacetylated lipid and protein adducts, anti-CYP2E1: antibodies to cytochrome p4502E1. +: positive, -: negative, N/A: not applicable, i.e., serology not performed. ULN: upper limit of normal

1. RUCAM score was not calculated on re-exposure

Table 4: Serological analysis of anti-TFA and anti-CYP2E1 autoantibodies

Pt	Age	Sex	Operation	Previous VA exposure	IgG4 serology		Post-operative LFTs				RUCAM score
					Anti-CYP2E1	Anti-TFA	Normal	Not VA-DILI	VA-DILI	Severity of LFTs if VA-DILI	
1	32	M	Trauma	N	+	+	✓				N/A
2	65	M	Elective	Y	-	-			✓	Moderate (ALT>3xULN)	4
3	48	F	Elective	Y	+	-			✓	Mild (ALT>1.8xULN)	4
4	74	F	Elective	Y	-	-			✓	Severe (ALT>5xULN)	12
5	59	F	Elective	Y	+	+			✓	Moderate (ALT>3xULN)	12
6	73	F	Elective	Y	-	-			✓	Mild (ALT>1.8xULN)	7
7	70	M	Elective	Y	+	-			✓	Mild (ALT>1.8xULN)	6
8	65	M	Elective	Y	+	+			✓	Mild (ALT>1.8xULN)	7
9	32	F	Elective	Y	+	+		✓			1
10	26	M	Trauma	N	+	-		✓			4
11	61	M	Trauma	Y	+	-		✓			-1
12	35	M	Trauma	Y	+	-		✓			1
13	27	M	Trauma	Y	+	+		✓			3

Table 4: Pt: patient, VA: volatile anaesthetic, IgG4: immunoglobulin G4, anti-CYP2E1: antibodies to cytochrome p450 2E1, anti-TFA: antibodies to trifluoroacetylated lipid and protein adducts, LFTs: liver function tests, ALT: alanine aminotransferase, VA-DILI: volatile anaesthetic drug-induced liver injury, RUCAM: Roussel Uclaf Causality Assessment Method, Y: yes, N: no, +: positive, -: negative, ULN: upper limit of normal.

Figure 1: Flow chart of patient recruitment and exclusion criteria

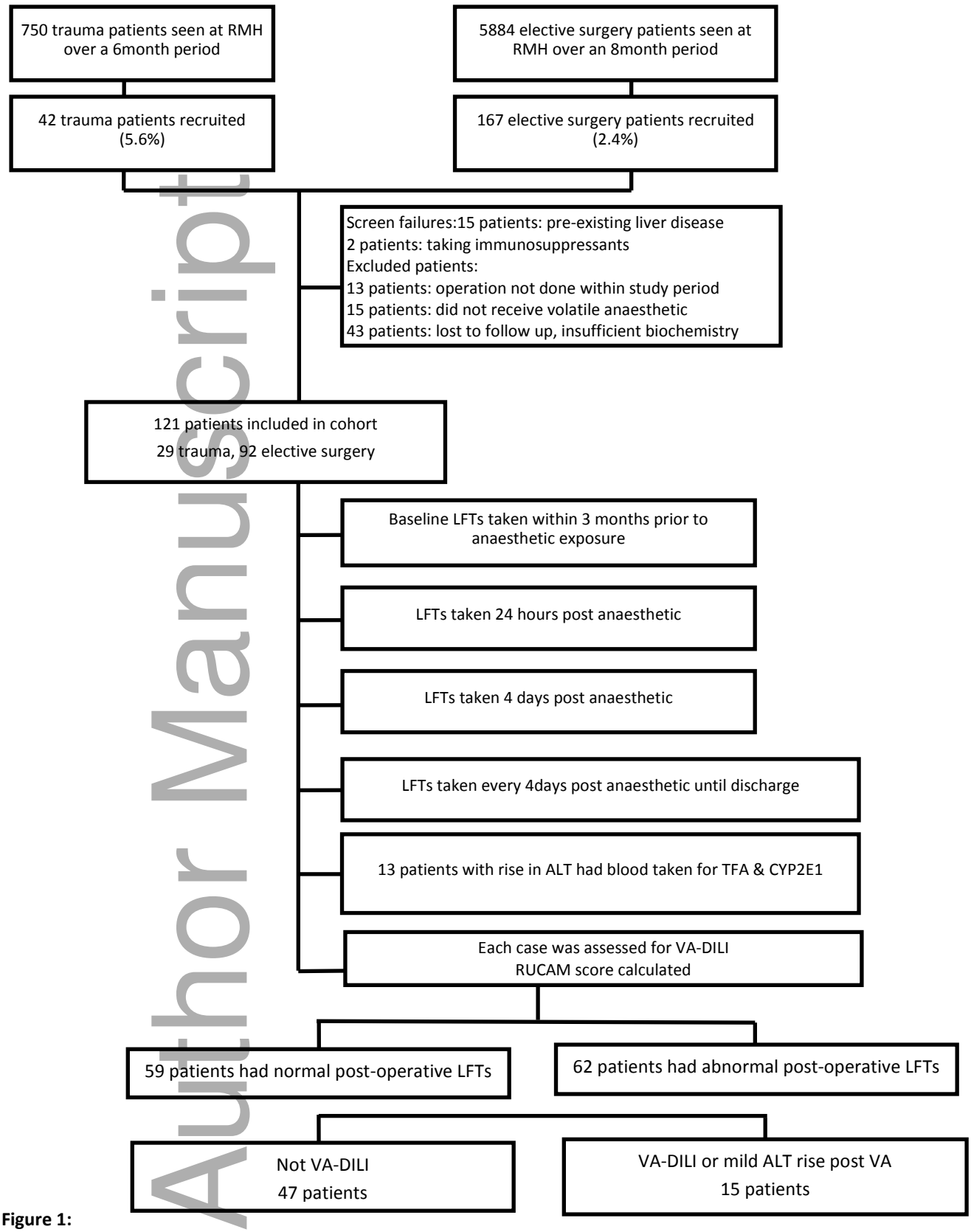

Figure 1:

RMH: Royal Melbourne Hospital. VA-DILI: volatile anaesthetic drug-induced liver injury. RUCAM: Roussel Uclaf Causality Assessment Method. Normal liver function tests (LFTs) are defined by alanine aminotransferase (ALT) <55 IU/L. Anti-TFA: antibodies to trifluoroacetylated protein and lipid adducts. CYP2E1: antibodies to cytochrome p4502E1.

Figure 2: Number of patients classified as normal liver biochemistry, abnormal liver biochemistry but not VA-DILI, and likely VA-DILI, based on post-operative LFTs and RUCAM; sub-classified into aetiology of abnormal LFTs that are not VA-DILI, and severity of LFTs that are likely VA-DILI

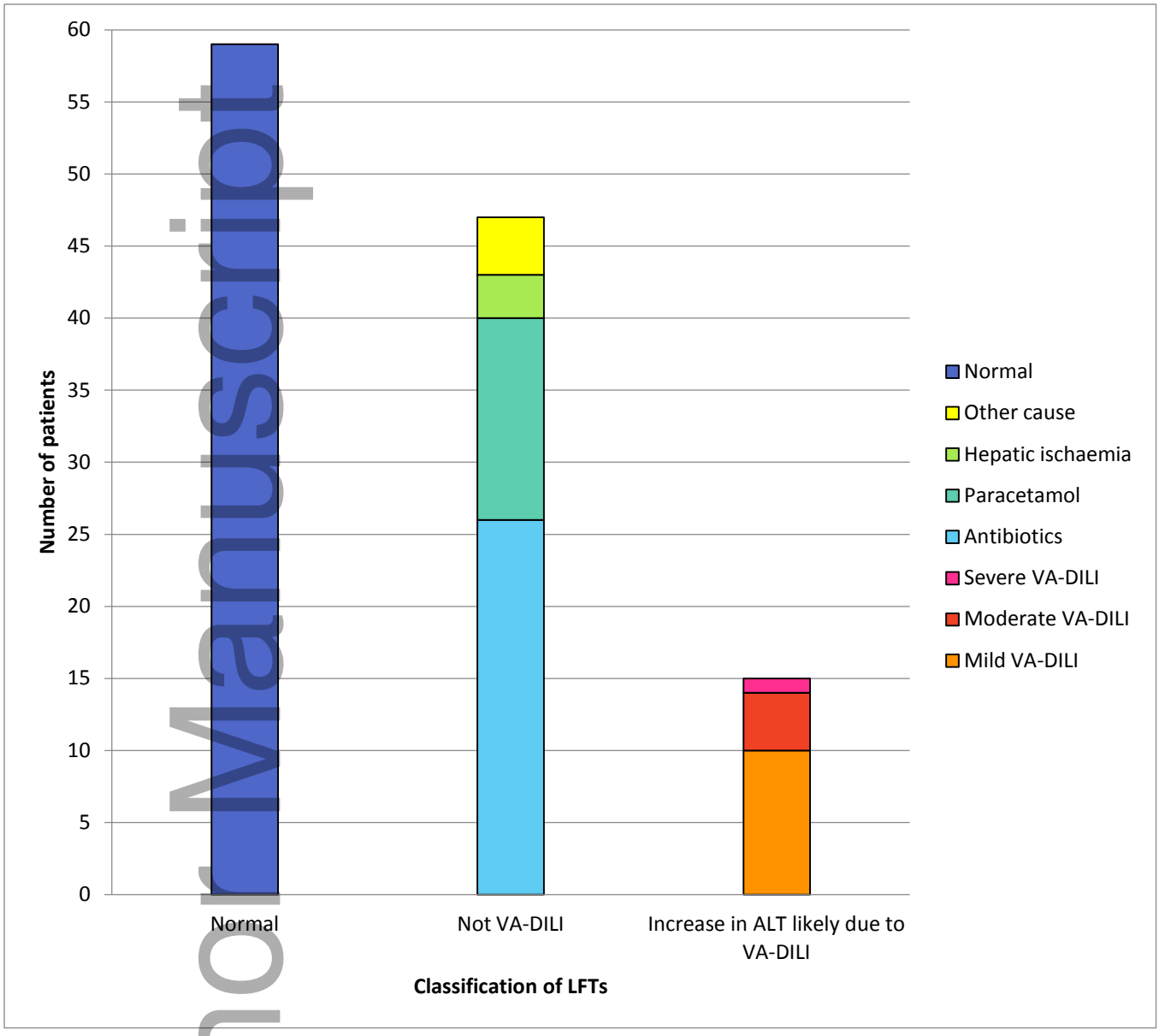

Figure 1:
VA-DILI: volatile anaesthetic drug-induced liver injury. RUCAM: Roussel Uclaf Causality Assessment Method. Normal liver function tests (LFTs) are defined by alanine aminotransferase (ALT)<55. The ALT rise presumed to be caused by the volatile anaesthetic was classified based on the maximum ALT level relative to the upper limit of normal (ULN). The three categories were defined as follows: ALT 55-119 IU/L (<3 xULN) were mild, ALT 120-199 IU/L (3-5x ULN) were moderate, and ALT ≥200 IU/L (>5 xULN) were consistent with VA-DILI.

Figure 3: Pattern of ALT rise over time in patients with moderate and severe VA-DILI

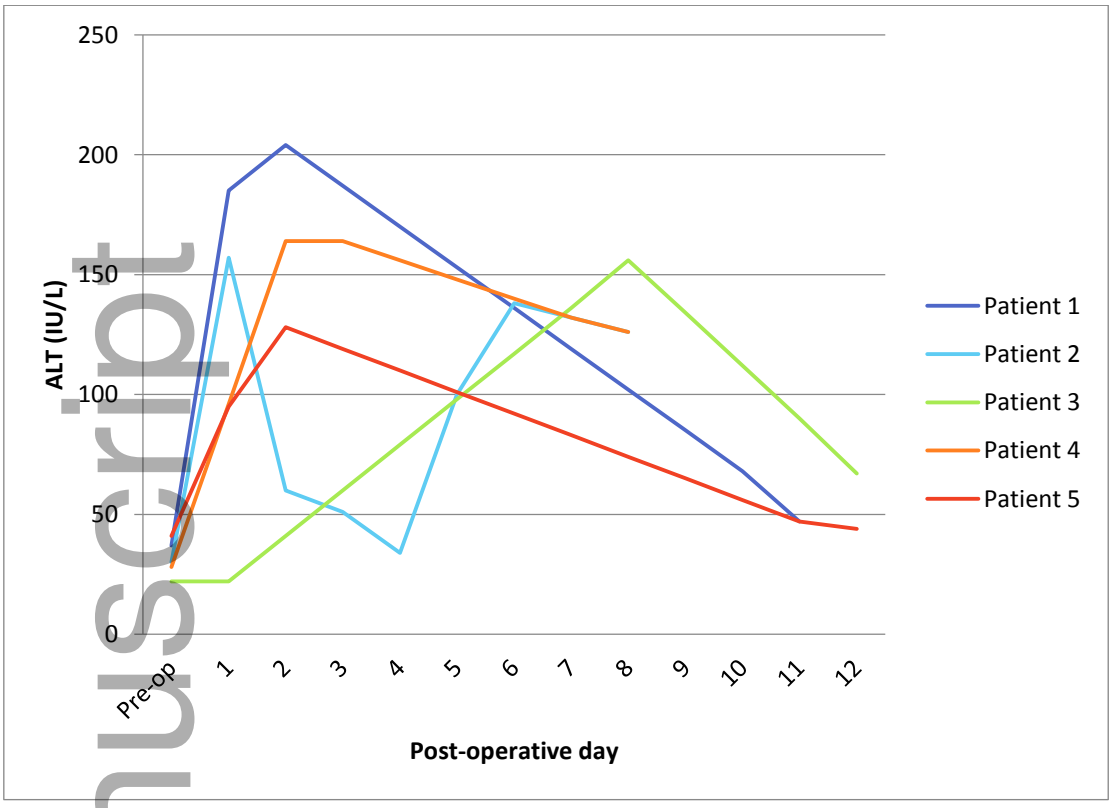

Figure 3:

VA-DILI: volatile anaesthetic drug-induced liver injury. ALT: alanine aminotransferase. Patients with likely VA-DILI and ALT 120-199 IU/L (three to five times the upper limit of normal) were moderate, while an ALT ≥200 IU/L (greater than five times the upper limit of normal) were classified as severe. Patient 1 had severe VA-DILI, while patients 2 to 5 inclusive had moderate VA-DILI.