Review of liver biopsies at Red Cross War Memorial Children's Hospital over a six-year period

By

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DECLARATION

I, TSHEPANG MOKOTO, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or other university.

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ABSTRACT

Background: Liver biopsy is a fundamental diagnostic tool in clinical hepatology, also playing a crucial role in the prognostication and management of liver diseases. Previous

studies at various centres have examined liver biopsies in the context of liver disease workups, including indications, histological findings and procedural complications.

Objectives: To ascertain the role of liver biopsy in the evaluation of patients with liver diseases at Red Cross War Memorial Children's Hospital (RCWMCH) over a six-year period.

Method: This retrospective descriptive study includes all paediatric patients who underwent liver biopsies at RCWMCH between 01/01/2018 and 30/06/2023.

Results: Seventy-five patients were screened for eligibility; six were excluded due to missing data and files, subsequently the study comprised of sixty-nine participants. Most liver biopsies were performed percutaneously under ultrasound guidance (n=45, 65%). There were three (4.3%) major complications, and no minor complication. The tissue yield was 95.7% (n = 66), with histopathological findings guiding clinical management in 50 patients (72%). Most frequent diagnoses were biliary atresia and autoimmune hepatitis. The commonest indications for liver biopsy were hyperbilirubinemia and suspected graft rejection.

Conclusion: Although liver biopsy is an invasive procedure, if guidelines are adhered to and performed by experienced staff it can be justified when using standard indications as it has a low complication rate in our setting and directly influence management in the majority of cases

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To my family, who have supported me through every phase of this journey and motivated me to persevere, I express my heartfelt thanks.

Most importantly, I am profoundly grateful to God for granting me the opportunity to learn and grow through this experience.

Tshepang

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ABBREVIATIONS

ACR: Acute Cellular Rejection

AIH: Auto Immune Hepatitis

ESPGHAN: European Society for Paediatric Gastroenterology Hepatology and Nutrition

INR: International Normalised Ratio

JPGN: Journal of Paediatric Gastroenterology and Nutrition

MASLD: Metabolic dysfunction Associated Steatotic Liver Disease

NHLS: National Health Laboratory Service

POPI: Protection Of Personal Information act

TB: Tuberculosis

UCT: University of Cape Town

CHAPTER 1: INTRODUCTION

1.1 Context

Despite advancements in radiological and genetic diagnostic modalities, liver biopsy remains integral to the management of liver disease, facilitating diagnosis, therapeutic decisions, and prognostic assessment [1]. Previous studies have consistently demonstrated the high diagnostic yield of liver biopsy; however, its clinical impact on patient management varies considerably, with reported utility ranging between 36.2% to 70% [2,3,4, 5, 6 &7]

The advent of genetic testing has significantly reshaped the role of liver biopsy in diagnosing neonatal cholestasis. Next-generation sequencing (NGS) technologies enable rapid and

comprehensive genome analysis, facilitating the identification of novel genetic causes of neonatal cholestasis [8].

Targeted gene panels have enhanced the diagnostic precision for various genetic disorders, including: Alagille syndrome, associated with *JAGGED1* and *NOTCH2* mutations, Citrin deficiency, caused by *SLC25A13* mutations, and Progressive familial intrahepatic cholestasis type 3, linked to *ABCB4* mutations [8].

Previous work has demonstrated the clinical utility of next-generation sequencing in diagnosing intrahepatic cholestasis. For instance, Wang et al. identified potential genetic diagnoses in 22% of patients by sequencing 61 cholestasis-related genes in 141 individuals. Similarly, a study in Japan reported a 26% genetic diagnosis rate in 109 patients with neonatal cholestasis [9,10]. However, the role of genetic testing in diagnosing neonatal cholestasis within the South African context remains to be fully elucidated.

According to the 2015 guidelines published by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), indications for liver biopsies in children are categorized into two primary groups: biopsies of the graft liver and biopsies of the native liver. The latter category is further subdivided into diagnostic biopsies and biopsies in patients with known liver disease. Table: Supplemental Digital Content 1 below delineates the specific indications within each category [1].

In a study conducted in Mexico, suspected autoimmune hepatitis (AIH) and transaminitis of unknown origin were the most frequent indications for liver biopsy in the native liver [11]. Similarly, a single-centre study at the British Columbia Children's Hospital identified elevated liver enzymes and cholestasis as the primary indications for liver biopsy in the native liver [12].

A study in Sudan reported cholestatic jaundice and hepatomegaly as frequent indications for liver biopsy [13]. However, comparable data specific to the South African context remains scarce, with limited published studies addressing the indications for liver biopsy in this setting.

The main indication for liver biopsy on a graft liver is suspected graft rejection, other indications include work up for suspected recurrence of the primary disease or per protocol liver biopsy.

Histological findings in the native liver, as reported in one study, included chronic hepatitis, metabolic liver disease, cirrhosis, and neonatal hepatitis [14]. In 2000, the Ga-Rankuwa

Hospital Histopathology Laboratory in Pretoria identified neonatal hepatitis and biliary atresia as common histological findings, while metabolic liver disease was noted to be rare. [15]

Common histopathological findings in graft liver include fibrosis, chronic hepatitis, and steatosis, all of which are associated with late rejection or poor compliance with immunosuppressive therapy [16,17].

Complications associated with liver biopsy are broadly categorized as major and minor. Major complications encompass significant bleeding requiring blood transfusion, pneumothorax, and mortality, whereas minor complications include post-procedural pain, sub-capsular bleeding that does not necessitate transfusion, and minor bile leakage [1].

Minor complications occur more frequently than major complications, with post-procedural pain being the most reported minor complications, while symptomatic bleeding is the most prevalent major complication [12,18]

Findings from a retrospective cohort study in Beijing highlighted thrombocytopenia, age under 18 years, and multiple needles passes as significant independent risk factors for bleeding [19]

A Japanese study reported a major complication prevalence of 0.5%, with a higher occurrence in native liver patients compared to graft liver patients (1% vs. 0.2%). Among native liver patients, identified risk factors for major complications included younger age, liver malignancies, and coagulopathy, whereas younger age alone was a significant risk factor in graft liver recipients [20]. The heightened risk associated with liver biopsy in younger patients underscores the necessity of exploring alternative investigative modalities for neonatal cholestasis.

The incidence of pneumothorax has been linked to the intercostal approach in liver biopsy. However, its prevalence remains low and was reported to be 0.2% in 1 study [21]. Likewise, the risk of mortality following liver biopsy is minimal, further reinforcing the procedure's favourable safety profile [12,22]

1.2 Aim:

Our aim is to describe the experience of liver biopsies at Red Cross War Memorial Children's Hospital (RCWMCH), one of the two centres in the Western Cape province who perform liver biopsies and the only centre performing paediatric liver transplants in the province.

We aim to document our indications, histological findings-including liver cirrhosis- methods, approach, the team performing the liver biopsy, number of passes, baseline coagulation status and if in line with internationally acceptable guidelines. We will compare the native and transplant group

1.3 Objectives:

The objectives of this study encompass the identification of major and minor complications, alongside an evaluation of the risk factors contributing to these adverse events. Additionally, the study seeks to determine whether our complication rates align with internationally established rates. Furthermore, it aims to assess the clinical impact of liver biopsy findings on subsequent patient management strategies.

Major complications are defined as either bleeding that require blood transfusion, development of a pneumothorax or death within 3 days of the liver biopsy. Minor complications are defined as pain or bleeding that does not require blood transfusion.

Bleeding will be assessed based on documented ultrasound findings in cases of percutaneous ultrasound-guided liver biopsy, intraoperative observations during laparoscopic liver biopsy or laparotomy, and instances where bleeding was identified during relook laparotomy following the liver biopsy.

Pain will be evaluated based on recorded complaints of discomfort or the requirement for additional analgesia beyond the prescribed regimen.

Clinical utility of the liver biopsy results will be assessed on how the biopsy results impacted on further management of the patients. This encompasses initiating new therapeutic interventions, continuing existing treatment, opting for conservative management, and disease prognostication informed by the stage of progression as indicated by the presence of liver cirrhosis.

Liver biopsy samples deemed inadequate for histological evaluation, and those failing to directly influence patient management, will be considered clinically non-contributory

1.4 Chosen journal for publication

We have selected the Journal of Paediatric Gastroenterology and Nutrition (JPGN) as the prospective publication venue for our study. JPGN is a paediatric medical journal dedicated to research in nutrition, hepatology, and gastroenterology. Liver biopsies have been extensively studied at various centres globally; our research will contribute to the existing body of knowledge within the South African context, enabling comparative analyses with findings from other centres.

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CHAPTER TWO: PUBLICATION – READY MANUSCRIPT

TITLE: Review of liver biopsies at Red Cross War Memorial Children's Hospital over a six-year period

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ABSTRACT

Background: Liver biopsy is the cornerstone of diagnosis in clinical hepatology. It also plays a cardinal role in prognostication of liver disease and guiding management.

Objectives: To determine the role of liver biopsy in the work up of patients with liver diseases at Red Cross War Memorial Children's Hospital (RCWMCH) over a six-year period.

Method: This is a retrospective descriptive study of children who had liver biopsies performed at RCWMCH between the 01/01/2018 and 30/06/2023.

Results: Seventy-five patients were screened for eligibility; six were excluded due to missing data and files, subsequently the study comprised of sixty-nine participants. Most liver biopsies were performed percutaneously under ultrasound guidance (n=45, 65%). There were three (4.3%) major complications, and no minor complication. The tissue yield was 95.7% (n = 66), with histopathological findings guiding clinical management in 50 patients (72%). Most frequent diagnoses were biliary atresia and autoimmune hepatitis. The commonest indications for liver biopsy were hyperbilirubinemia and suspected graft rejection.

Conclusion: Although liver biopsy is an invasive procedure, if guidelines are adhered to and performed by experienced staff it can be justified when using standard indications as it has a

low complication rate in our setting and directly influence management in the majority of cases

Key Words: Indications; histological results; tissue yield; complications, impact on patient management.

What is known	• The impact of liver biopsy findings on patient management varies, with reported influence ranging from 36% to 70% • Minor complications are more prevalent than major complications following liver biopsy • Metabolic dysfunction Associated Steatotic Liver Disease (MASLD) is on the rise in the paediatric population
What is new	• Liver biopsy has clinical impact on most patients in our setting • With appropriate methods and use of guidelines liver biopsy is safe with no minor complications and few major complications • Hepatic steatosis is uncommon in our setting.

INTRODUCTION

1.1 Context

Liver biopsy plays a vital role in management of liver disease including diagnosis, treatment and prognostic decision making despite advances in radiological and genetic diagnostic methods [1]. Several studies have reported a high diagnostic yield from liver biopsies, however, its clinical impact on patient management varies considerably, with reported utility ranging between 36.2% to 70% [2,3,4,5,6&7].

The advent of advanced genetic testing technologies, including next-generation sequencing, has significantly reshaped the role of liver biopsy in the diagnosis of liver disease. Also facilitating the identification of novel genetic aetiologies underlying neonatal cholestasis. Previous studies have demonstrated a prevalence of genetic causes in neonatal intrahepatic cholestasis, estimated at 22-26%. However, in the South African context, the clinical utility and diagnostic implications of genetic testing in neonatal cholestasis remain incompletely characterized [8,9&10]

The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has set out several indications for liver biopsies to mitigate unnecessary invasive procedures. (Table: Supplemental Digital Content 1).

Previous studies conducted both internationally and across the African continent have reported similar indications for liver biopsy in the native liver, including suspected autoimmune hepatitis (AIH), evaluation of cholestasis, and investigation of transaminitis of unknown aetiology [1,11,12,13]. However, no published data currently exist on this subject within the South African context.

The main indication for liver biopsy on a graft liver is suspected graft rejection, other indications include work up for suspected recurrence of the primary disease or per protocol liver biopsy.

Prior studies report chronic hepatitis, neonatal hepatitis and biliary atresia as the most frequent histological findings on native liver biopsy [14,15]. In the graft liver common histology findings include fibrosis, chronic hepatitis, and steatosis [16,17].

Complications associated with liver biopsy are broadly categorized as major and minor. Major complications encompass significant bleeding requiring blood transfusion, pneumothorax, and mortality, whereas minor complications include post-procedural pain, sub-capsular bleeding that does not necessitate transfusion, and minor bile leakage [1].

Minor complications occur more frequently than major complications, with post-procedural pain being the most reported minor complications, while symptomatic bleeding is the most prevalent major complication [12,18]

Findings from a retrospective cohort study in Beijing highlighted thrombocytopenia, age under 18 years, and multiple needles passes as significant independent risk factors for bleeding [19]

A Japanese study reported a major complication prevalence of 0.5%, with a higher occurrence in native liver patients compared to graft liver patients (1% vs. 0.2%). Among native liver patients, identified risk factors for major complications included younger age, liver malignancies, and coagulopathy, whereas younger age alone was a significant risk factor in graft liver recipients [20]. The heightened risk associated with liver biopsy in younger patients underscores the necessity of exploring alternative investigative modalities for neonatal cholestasis.

The incidence of pneumothorax has been linked to the intercostal approach in liver biopsy. However, its prevalence remains low and was reported to be 0.2% in 1 study [21]. Likewise,

the risk of mortality following liver biopsy is minimal, further reinforcing the procedure's favourable safety profile [12,22]

1.2 Aim:

Our aim is to describe the experience of liver biopsies at Red Cross War Memorial Children's Hospital (RCWMCH), one of the two centres in the Western Cape province who perform liver biopsies and the only centre performing paediatric liver transplants in the province.

We aim to document our indications, histological findings-including liver cirrhosis- methods, approach, the team performing the liver biopsy, number of passes, baseline coagulation status and if in line with internationally acceptable guidelines. We will compare the native and transplant group

1.3 Objectives:

The objectives of this study encompass the identification of major and minor complications, alongside an evaluation of the risk factors contributing to these adverse events. Additionally, the study seeks to determine whether our complication rates align with internationally established rates. Furthermore, it aims to assess the clinical impact of liver biopsy findings on subsequent patient management strategies.

Major complications are defined as either bleeding that require blood transfusion, development of a pneumothorax or death within 3 days of the liver biopsy. Minor complications are defined as pain or bleeding that does not require blood transfusion.

Bleeding will be assessed based on documented ultrasound findings in cases of percutaneous ultrasound-guided liver biopsy, intraoperative observations during laparoscopic liver biopsy or laparotomy, and instances where bleeding was identified during relook laparotomy following the liver biopsy.

Pain will be evaluated based on recorded complaints of discomfort or the requirement for additional analgesia beyond the prescribed regimen.

Clinical utility of the liver biopsy results will be assessed on how the biopsy results impacted on further management of the patients. This encompasses initiating new therapeutic interventions, continuing existing treatment, opting for conservative management, and disease prognostication informed by the stage of progression as indicated by the presence of liver cirrhosis or fibrosis.

Liver biopsy samples deemed inadequate for histological evaluation, and those failing to directly influence patient management, will be considered clinically non-contributory.

METHODS

Study setting:

This study was conducted in the Paediatric Gastroenterology Department at Red Cross War Memorial Children's Hospital (RCWMCH), a tertiary paediatric facility in Cape Town, South Africa. RCWMCH is affiliated with the University of Cape Town (UCT) and provides specialized care in paediatric surgery, hepatology, and liver transplantation.

Liver biopsies were performed by both the paediatric gastroenterology and paediatric surgical teams, including registrars, fellows, and consultants. All procedures were conducted under the supervision of a consultant within the respective units.

Percutaneous ultrasound-guided and blind liver biopsies were performed under conscious sedation by the gastroenterology team. Laparoscopic and open laparotomy biopsies were conducted under general anaesthesia by the surgical team.

During the procedures, patients received both local and systemic analgesia, followed by systemic analgesia post-procedure. They were subsequently admitted to the high-care unit for close monitoring of cardiopulmonary status, for pain management and assessment for potential bleeding.

An immediate ultrasound evaluation was performed post-procedure for all patients who underwent percutaneous ultrasound-guided liver biopsies, assessing for potential bleeding, including subcapsular haemorrhages.

Repeat ultrasound evaluations were performed for patients who underwent liver biopsies via laparotomy, laparoscopy or blind only when clinically indicated, such as in cases of haemodynamic instability or declining haemoglobin levels.

Routine post-procedural chest radiography was performed for patients who underwent liver biopsies via the intercostal approach to assess for pneumothorax.

Study design:

This was a retrospective descriptive study in children aged 0-15 years who underwent liver biopsies at RCWMCH between the 01/01/2018 and 31/06/2023.

Study population:

This study included all patients aged 0–15 years who underwent liver biopsies performed by the paediatric gastroenterology or surgical team at RCWMCH during the review period.

A total of 75 liver biopsies were conducted; however, six patients were excluded due to missing data or files, resulting in a final study cohort of 69 participants

Inclusion criteria:

All children aged 0-15 years who had liver biopsies performed at RCWMCH during the period under review.

Exclusion criteria:

Participants with missing data and files, and those who had liver biopsies performed at other institutions.

Data collection:

Entries were retrieved from the unit liver biopsy registry and the liver biopsy checklists and exported into Microsoft Excel spreadsheet. Biopsies performed by both the paediatric surgical and gastroenterology team were captured on the liver biopsy registry. Missing entries were acquired through records review and verified from the National Health Laboratory Service (NHLS) database. All liver biopsies conducted during the study period were cross-verified with histopathological laboratory records to ensure complete and accurate data capture for the study.

Relevant general and clinical characteristics were retrieved (refer to appendix). General characteristics included age, gender, weight, Human Immuno-deficiency Virus (HIV) status and the team responsible for performing the liver biopsy.

Clinical characteristics include indications for liver biopsy, method, approach, baseline coagulation status, number of passes and cores attained. Additionally, histological findings and procedural complications we documented.

The primary outcome included the evaluation of both major and minor complications, while the secondary outcome focused on assessing the clinical impact of histological findings on patient management.

We also compared the clinical characteristics of patients in the transplant group to those of patients in the native liver group.

Data Analysis:

Data were collected and entered Microsoft Excel spreadsheet. Statistical analyses were performed using Stata version 13.1. Associations between the native and graft liver groups were assessed using Pearson's Chi-square test for categorical variables, with Fisher's exact test applied when appropriate. Continuous variables were analysed using Mann-Whitney U tests. Statistical significance was defined as a p-value <0.05.

Ethical consideration:

Consent was obtained from the hospital research review committee at RCWMCH (RCC 379/WC_202306_044), the National Health Research Database (WC 202306 044) and the Human Research Ethics Committee of the University of Cape Town (HREC REF:385/2023) to conduct research at RCWMCH. No further consent was required from the participants and caregivers due to the retrospective nature of the study.

To ensure confidentiality, anonymized identifiers were utilized, and the study was conducted in accordance with the Helsinki Declaration. Both the liver biopsy registry and the Microsoft Excel spreadsheet containing exported data were secured with password protection.

Potential risks:

The study involves retrospective review of medical records without any direct contact with subjects and hence no human risks or harms were incurred.

Potential benefits:

We anticipate that the histopathological findings from liver biopsies in this study will inform and refine patient management strategies for liver diseases. Additionally, this study aims to expand our understanding of prevalent liver biopsy findings and complications specific to our clinical setting.

The results of the study will be presented at the annual Department of Paediatrics and Child Health Research Day at the University of Cape Town (UCT) in October 2024. The manuscript will also be submitted to the Journal of Paediatric Gastroenterology and Nutrition (JPGN) for publication.

RESULTS

A total of 75 patients were screened for eligibility, with six excluded due to incomplete data or missing records, resulting in a final study cohort of 69 participants. Most liver biopsies were performed by the paediatric gastroenterology team (p=0.01), with percutaneous ultrasound-guided biopsy being the predominant method (Figure 1).

Among the cohort, three patients underwent multiple liver biopsies during the study period; however, none required a repeat biopsy due to inadequate tissue sampling during the initial procedure.

The general characteristics of the participants are summarized in Table 2 below. The study demonstrated a predominance of female participants, comprising 55% of the population, compared to 45% males. The median age at liver biopsy was 36 months (interquartile range

[IQR]: 5–84 months), with an overall age range of 1–168 months. Patients in the native liver group were younger than those in the graft liver group (p=0.03). HIV testing was conducted for 64 patients, of whom 4.7% (n=3) yielded positive results.

Major complications were observed in two patients, both belonging to the native liver group, with no minor complications reported (Table 3). One patient developed a pneumothorax, while another demised due to severe major bleeding.

A pneumothorax was noted in a patient following liver biopsy via the intercostal approach. Mortality was reported in a high-risk patient with pre-existing coagulopathy and a suspected liver malignancy, where the biopsy was appropriately performed laparoscopically. Notably, all complications occurred in older patients.

Platelet counts and International Normalised Ratio (INR) were normal in most patients, only 3 had coagulopathy (INR>1.5) hence liver biopsies in these patients were performed using a laparoscopic approach. In most patients 2 passes were required to obtain adequate liver tissue samples.

The tissue yield in our study was 95.7 % (n=66), with 4.3% (n=3) biopsies deemed suboptimal for histological evaluation. Histological findings influenced clinical decision-making in 72% (n=50) of patients who had an adequate tissue sample, facilitating changes in treatment plans, initiation of new therapeutic approaches, and evaluation of prognostic outcomes.

A total of 28% (n=19) of biopsies were deemed clinically non-contributory. This subset comprised suboptimal biopsies (n=3, 4,3%), cases of non-specific hepatitis (n=13, 19%), and neonatal hepatitis (n=3, 4%).

In the native liver group biliary atresia and autoimmune hepatitis were the most frequent diagnoses. Hepatic glycogenosis was observed in three out of the four patients presenting with hepatomegaly, with no instances of steatohepatitis (Figure 2). Among the hepatic glycogenosis cases 2 patients had glycogen storage disease while 1 was diagnosed with Mauriac syndrome.

Graft rejection was observed in 64% (n=9) of patients within the graft liver group, comprising eight cases of acute cellular rejection and one case of chronic ductopenic rejection.

In the native liver group, hyperbilirubinemia represented the commonest indication for liver biopsy, whereas in the graft liver group, suspected graft rejection was the most frequently

observed indication. Notably, all biopsies performed on transplanted livers were conducted to assess for graft rejection, with none undertaken as part of a routine surveillance protocol (see Table 4).

DISCUSSION

Liver biopsy at our institution is safe, has a good yield and directly influence management in most patients, whether diagnostically in liver disease work-up, or when assessing post liver transplant patients for complications.

The high tissue yield, and clinical impact observed in our study align closely with findings from previous research, reinforcing its relevance within the existing body of evidence [2.3.4.5 & 7].

No minor complications were identified in our study, contrasting with findings reported in previous literature [7,13]. The incidence of major complications remained low, aligning with rates reported in the existing literature [13, 21].

All major complications occurred in the native liver cohort, likely attributable to the intrinsic clotting dysfunction in these patients. In contrast, transplanted livers synthesize normal clotting factors, and biopsies in this group were performed for graft rejection rather than chronic liver disease, which inherently carries greater procedural risk.

In this cohort, identified risk factors—baseline coagulopathy, malignancy and the intercostal approach were consistent with established findings, reinforcing previously documented associations [20,21].

Despite the recommendation for 1 pass and more passes being associated with more complications, finding that more patients had 2 passes, there were no minor complications and 3 major complications [1,19]. Although young age has been associated with an increased risk of complications, no complications were observed in younger patients within this cohort [20]

Our findings demonstrate a comparable tissue yield to that reported in previous studies, thereby validating the reliability of our liver biopsy methodology [2,3,4&5]. Consistent with findings by Ahmed A et al., the high clinical utility of our biopsy results supports its role as a reliable tool for assessment of liver disease [6].

Biliary atresia and autoimmune hepatitis were the most predominant histological findings in the native liver group, consistent with previous studies conducted both locally and internationally. The only South African study referenced was conducted 25 years ago in a non-transplant center, reporting a biliary atresia incidence of 20.8%, which aligns with our findings [6,7 & 12**].**

The rising prevalence of paediatric obesity has corresponded with an increasing incidence of metabolic dysfunction-associated steatotic liver disease (MASLD). In the United States, childhood obesity was reported at 19.3% between 2017 and 2018, with the highest prevalence observed among the Hispanic Black population. Previous studies have estimated the incidence of MASLD at 9.6% with highest rates among obese children, underscoring the growing burden of obesity-related liver disease in paediatric cohorts [23, 24].

In South Africa, childhood obesity prevalence was reported at 7.2% in 2016. However, no published national data exist on the prevalence of paediatric fatty liver disease based on liver histology. Notably, no cases of hepatic steatosis were identified in our study, an unexpected finding. Assessing the body mass index of our cohort may provide further insight into this absence.

The incidence of both acute and chronic graft rejection in our study aligns with internationally reported rates at other transplant centres, reaffirming the appropriateness of our immunosuppressive therapy protocol and its concordance with international guideline**s [**25,26, 27&28]

One of the main indications for liver biopsy is work up for cholestasis, it is therefore understandable that patients in the native liver group are of a younger age. The mean age at liver transplant at our institution is 4 years 6 months hence the older age at liver biopsy in the transplant group.

The indications for liver biopsy in our study were consistent with those observed in other institutions and aligned with the ESPGHAN guidelines for paediatric liver biopsies. Specifically, biopsies were conducted for the assessment of hyperbilirubinemia, suspected graft

rejection, and malignancy in cases where imaging failed to establish a definitive diagnosis [1,6,7 & 8].

The management of post-liver transplant patients is inherently complex, with immunosuppressive therapy playing a critical role in preventing graft rejection. However, this therapy is associated with risks, including malignancy, infectious complications, and metabolic, renal, and cardiovascular adverse effects.

Some centres implement protocol biopsies to enable early detection of rejection, facilitate timely intervention, personalize immunosuppressive regimens, and promote immune tolerance, thereby mitigating complications associated with immunosuppression. At our institution, post-transplant liver biopsies are performed exclusively for the evaluation of suspected graft rejection. Protocol liver biopsies are not conducted due to resource limitations [19, 20].

Our high diagnostic yield is reassuring that we are obtaining adequate samples with limited complications, and although liver biopsy is an invasive procedure it can be justified if using standard indications as it directly influences management in most patients.

Study limitations:

Given the study's retrospective nature and its execution across two different departments, variability in interpretation may have influenced the findings. The absence of routine post-procedural ultrasound assessments following laparotomy or laparoscopy liver biopsies may have led to an underestimation of minor haemorrhages. Assessing pain in paediatric patients is inherently challenging, and the reported rates in this study may not fully reflect actual patient experiences.

CONCLUSION

Although liver biopsy is an invasive procedure, if guidelines are adhered to and performed by experienced staff it can be justified when using standard indications as it has a low complication rate in our setting and directly influence management in the majority of cases

Recommendation:

Our study revealed that liver biopsies can be safe, with high tissue yield and clinical impact. On this basis use of expert societal guidelines including ESPGHAN and local guidelines to select the appropriate indication and method for liver biopsy is recommended.

Future research should focus on pain as a complication for liver biopsy using appropriate paediatric pain scores

Conflicts of interest: The authors declare that there was no conflict of interest in the manuscript, including financial, institutional and other relationships that may result in bias.

Source of funding: The study was self-funded

Authors' Contributions:

TM: Literature search, proposal writing, data collection, data analysis, manuscript drafts, critical editing of manuscript for important intellectual content.

RJDL: Study conceptualization, critical editing of the proposal, and manuscript for important intellectual content including review of data and results.

LR: Critical editing of the proposal and manuscript for important intellectual content.

RAB: Critical editing of the proposal and manuscript for important intellectual content.

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Table 1: Supplemental digital content 1: Indications for liver biopsies (adapted from ESPGHAN 2015 guidelines for liver biopsies

Native liver	**Graft liver**
Diagnosis: • MASLD • AIH • Cryptogenic transaminitis	• Deranged liver function test of unknown aetiology • Suspected ACR • Suspected chronic ductopenic rejection • Suspected infection when serological tests yield negative results • Protocol biopsies to assess graft function • Suspected recurrence of the primary disease • Suspected de novo hepatitis
Assessment of a known liver disease: • To stage fibrosis and guide screening for portal hypertension • Histological diagnosis of malignancy if diagnosis is not made on imaging • To guide the need for treatment (eg necroinflammation in chronic active hepatitis B infection)	
Notes: Abbreviations **MASLD** (Metabolic dysfunction Associated Steatotic Liver Disease), **AIH** (Auto Immune Hepatitis), **ACR** (Acute Cellular Rejection)	

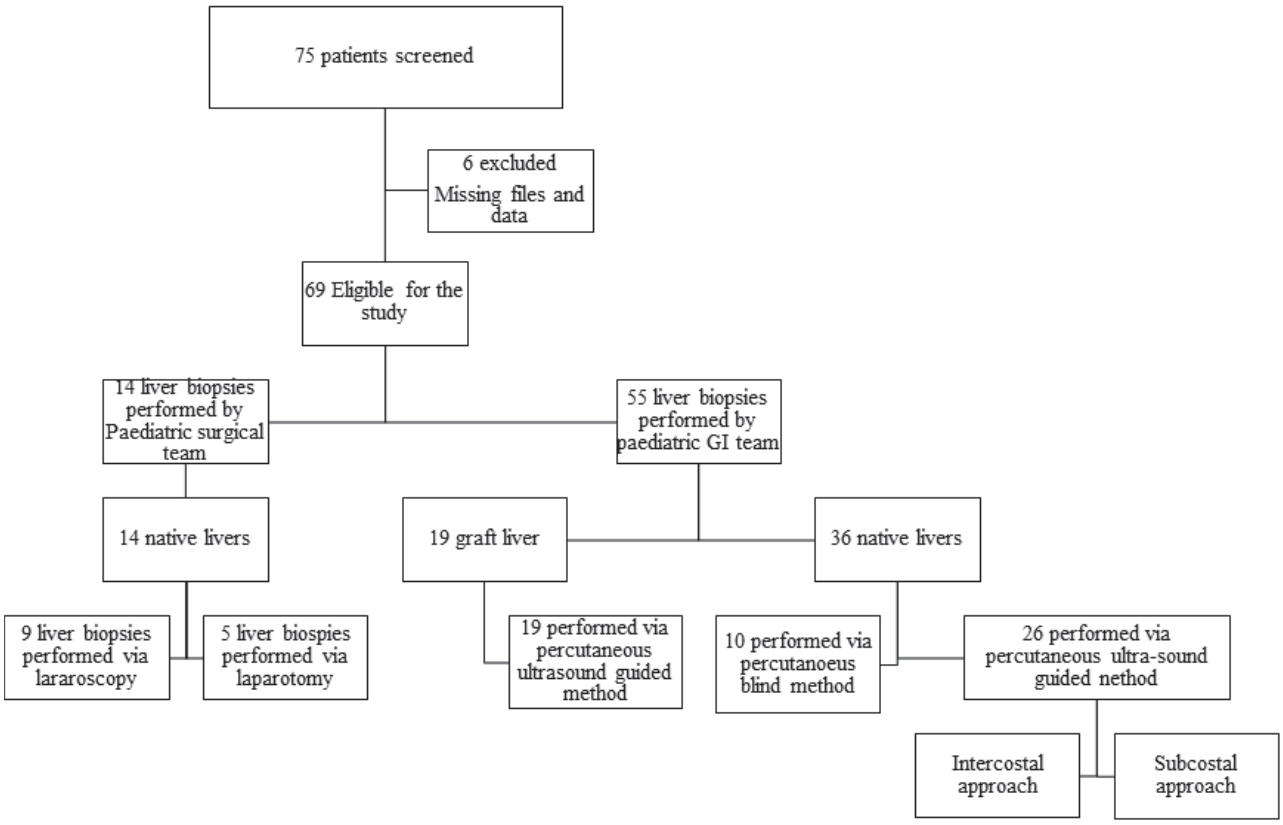

Figure 1: Randomisation

Table 2: General characteristics

Variable	General population	Native liver	Graft liver	P-value
Male-no/total (%)	31/69 (45)	24/50 (48)	7/19 (37)	0.4
Female-no/total (%)	38/69 (55)	26/50 (52)	12/19 (63)	
Median age in months-(IQR)	36 (5-84)	28 (4-72)	60 (15-144)	0.03
Median weight in kilograms- (IQR)	14 (6.4-26)	11.5 (5.7-20)	17 (13.5-34.6)	0.04
HIV positive- no/total (%)	3/64 (4.7)	3/50 (6)	0/19 (0)	0.1
Personnel doing the liver biopsy:				
Paeds GI	55/69 (80)	36/50 (72)	19/19 (100)	0.01
Paeds surgery	14/69 (20)	14/50 (28)	0/19 (0)	

Notes: IQR=Interquartile Range

Table 3: Coagulation status and complications

Variables	General population	Native liver	Graft liver	P-value
Median INR- (IQR)	1.1 (1-1.2)	1.14 (1.03-1.18)	1.11 (1.02-1.15)	0.5
Median platelets- (IQR)	352(213-450)	349 (241-452)	249 (199-364)	0.05
Complications:				
Pneumothorax	1/69 (1.4)	1/50 (2)	0/19 (0)	
Major bleeding	1/69 (1.4)	1/50 (2)	0/19 (0)	
Death	1/69 (1.4)	1/50 (2)	0/19 (0)	

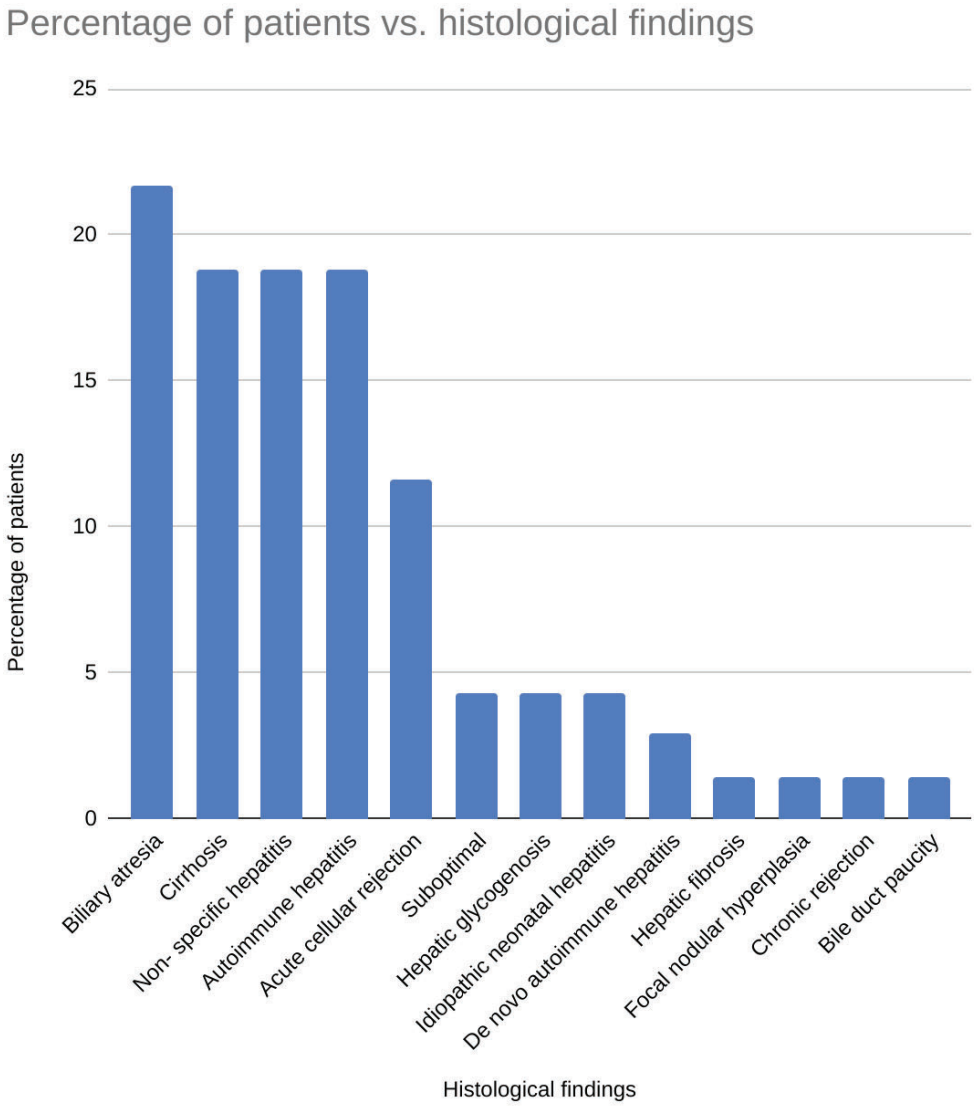

Figure 2: Diagnoses

Table 4:Clinical characteristics

Variables	**General population no/total (%)**	**Native liver no/total (%)**	**Graft liver no/total (%)**	**P-value**
Indications:				
Suspected rejection	19/69 (27.6)	0/50 (0)	19/19 (100)	0
Hyperbilirubinemia	41/69 (59.4)	41/50 (82)	0/19 (0)	
Hepatomegaly	4/69 (5.8)	4/50 (8)	0/19 (0)	
Suspected cirrhosis	3/69 (4.3)	3/50 (6)	0/19 (0)	
Suspected malignancy	2/69 (2.9)	2/50 (4)	0/19 (0)	
Methods:				
Blind percutaneous	10/69 (14.5)	10/50 (20)	0/19 (0)	0.05
Ultrasound guided percutaneous	45/69 (65.2)	26/50 (52)	19/19 (100)	
Laparoscopic	9/69 (13.2)	9/50 (18)	0/19 (0)	
Laparotomy	5/69 (7.2)	5/50 (10)	0/19 (0)	
Approaches:				
Subcostal	68/69 (98.5)	49/50 (98)	19/19 (100)	0.7
Intercostal	1/69 (1.5)	1/50 (2)	0/19 (0)	
Number of passes:				
1	26/69 (37.7)	22/50 (44)	4/19 (21)	0.3
2	32/69 (46.4)	21/50 (42)	11/19 (57)	
3	7/69 (10.2)	5/50 (10)	2/19 (11)	
4	3/69 (4.3)	1/50 (2)	2/19 (11)	
5	1/69 (1.4)	1/50 (2)	0/19 (0)	
Median number of passes-(IQR)	**2 (1-2)**	**2 (1-2)**	**2 (2-2)**	0.1
Number of cores:				
1	25/68 (36.2)	20/50 (40)	4/19 (21)	0.3
2	34/69 (49.3)	24/50 (48)	11/19 (58)	
3	9/69 (13)	5/50 (10)	4/19 (21)	
4	1/69 (1.5)	1/50 (2)	0/19 (0_	
Median number of cores- (IQR)	**2 (1-2)**	**2 (1-2)**	**2 (1-2)**	0.1

Notes: IQR=Interquartile Range

Review of liver biopsies at Red Cross War Memorial Children's Hospital over a six year period

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Synopsis

Introduction:

Liver biopsy is the cornerstone of diagnosis in clinical hepatology. It also plays a vital role in prognostication of liver disease and guiding management. A lot of work has been done at various centers around the world looking at liver biopsies in the work up of liver diseases including indications for liver biopsies, liver biopsy results and complications of the procedure.

Aim:

The aim of this study is to determine the role of liver biopsy in the work up of patients with liver diseases at Red Cross War Memorial Children's Hospital (RCWMCH) over a six year period.

Objectives:

- Primary objectives:
    - To determine the total number of liver biopsies conducted between the 01st/01/2018 and the 31st/06/2023.
    - To determine the indications for liver biopsies in the specified time period
    - To determine the baseline coagulation status of the patients undergoing liver biopsies
    - Identify the personnel performing the liver biopsy, i.e. paediatric gastroenterology or paediatric surgeon.
    - To determine various approaches used to perform liver biopsies (intercostal vs. subcostal)
    - To determine whether the liver biopsy was done under ultrasound guidance or blinded
    - To determine the number of passes and cores attained during the procedure
    - Determine whether the liver was cirrhotic
    - To determine liver biopsy results
- Secondary objectives:
    - To determine minor complications i.e. pain and minor bleeding from the procedure
    - To determine major complications, i.e. major bleeding, pneumothorax and death within 3 days after the liver biopsy.

- To identify possible risk factors associated with complications
- To determine how liver biopsy influenced patient management(eg change of diagnosis or new treatment instituted)

Minor bleeding will be defined as documented bleeding, with a haemoglobin of more than 8 not requiring a blood transfusion and major bleeding will be defined as bleeding that results in a hemoglobin drop to ≤8 and requiring transfusion of blood.

Methodology

- Study design :

  It is a retrospective cross-sectional descriptive study.

- Study setting:

  The study will be conducted at RCWMCH in the paediatric gastroenterology department.

- Study population:

  Participants will be all children who are between the ages of 0 and 15 years who had liver biopsies done at RCWMCH by both the paediatric gastroenterology and paediatric surgical teams between the 01st/01/2018 and the 31st/06/2023.

- Exclusion criteria:

  Missing data and liver biopsies done at other institutions will serve as exclusion criteria.

Study sample and size:

The estimated sample size will be about 70 participants.

- Data collection(see data sheet):
  - Demographics:

    Age, gender and HIV status will be captured

  - Clinical characteristics

    Stratification of liver biopsies into native and transplanted liver

    Identification of the indication for liver biopsy

Baseline bloods (INR and platelets count)

Personnel performing the liver biopsy: Paediatric gastroenterologist vs paediatric surgeon

Presence of liver cirrhosis

Blinded or ultrasound guided biopsy

Intercostal or subcostal approach

Number of passes

Number of liver cores

Liver biopsy results

- Outcomes:
  - Primary outcomes:
    - Minor pain and bleeding that does not require a blood transfusion
  - Secondary outcome:
    - Major bleeding, death within 3 days post liver biopsy and pneumothorax
    - How liver biopsy results influenced the management of patients

Data analysis:

Data will be entered into excel spread sheet, then analysed using Stata (Statacorp, USA). Associations with liver biopsy abnormalities will be tested using Chi square tests (with Fisher exact statistics where appropriate) for categorical variables, and Student t –tests or Mann-Whitney U tests for continuous variables, depending on data distribution. Statistical significance will be set at a p-value< 0.05.

Ethical consideration:

We will apply to both the Hospital Research Review Committee at RCWMCH and the Human Research Ethics Committee (HREC) of the University of Cape Town for consent to conduct research at RCWMCH. Due to the retrospective nature of the research no further consent will be required from participants and their caregivers.

Confidentiality:

Anonymized identifiers will be used to maintain confidentiality, and the study will abide to the POPI act.

Study benefits:

We expect the liver biopsy results in this study to guide our management of patients. We also expect to broaden our knowledge of the common liver biopsy findings and complications specific to our institution.

Contents

Glossary:

1. ESPGHAN: European Society for Paediatric Gastroenterology Hepatology and Nutrition
2. RCWMCH: Red Cross War Memorial Children's Hospital

Introduction:

Liver biopsy is the cornerstone of diagnosis in clinical hepatology worldwide. It also assists in monitoring disease progression, and in therapeutic and prognostic decision making. It is performed on both a native and transplanted liver.

The European Society for Paediatric Gastroenterology Hepatology and Nutrition(ESPGHAN) guidelines of 2015 recognize the following as indications for liver biopsy in a native liver; suspected autoimmune hepatitis, cryptogenic hypertransaminasaemia, diagnostic scoring in Wilson's disease and liver tumours if imaging does not provide the diagnosis, however the risk of tumour seeding should always be considered [1]. In a study done in Mexico the most common indication for a liver biopsy was suspected autoimmune hepatitis closely followed by transaminitis of unknown origin and chronic liver disease [2]. In a single center study done at the British Columbia children's hospital, elevated liver enzymes and cholestasis accounted for most of the indications for liver biopsies [3]. In another study done in Poland hepatitis was found to be the most common indication in children above the age of 6 years, with cholestasis being the top indication in children below the age of 1 year [4]. No published data was found on the same topic in the South African context.

In the transplanted liver indications for biopsy include assessment for rejection, de novo hepatitis, and suspicion of recurrence of the primary disease. There is ongoing debate regarding the significance of protocol liver biopsy in a transplanted liver every 5-10 years in an otherwise healthy recipient. This is thought to subject healthy recipients to invasive procedures and possible morbidity and mortality. The rationale behind this practice is that paediatric liver transplant recipients often have abnormal histopathological findings in 69-97% of cases [5].

Common histopathological findings on a graft liver include fibrosis, chronic hepatitis, and steatosis and all these are associated with late rejection or poor compliance [5, 6]. Reported common findings on histology in the native liver in one study included chronic hepatitis, metabolic liver disease, cirrhosis and neonatal hepatitis [7]. In 2000, Garankuwa Hospital Histopathology Laboratory in Pretoria described neonatal hepatitis and biliary atresia as common findings on histology, with metabolic disease being rare [8].

Complications of liver biopsy can be divided into major and minor. Major complications include massive bleeding that requires blood transfusion, pneumothorax, and death. Minor complications

include pain, sub-capsular bleed not requiring blood transfusion and minor bile leak. In a large systematic review looking at complication rates of all percutaneous liver biopsies done between 2010 and 2020 minor complications were found to occur more frequently than major complications with a prevalence of 9.5% and 2.4% respectively [9].

In a recent study from Japan major complications were found in 0.5% of cases and were said to occur more in the native liver than in the transplanted liver patients (1 % vs. 0.2%). Risk factors for major complications identified in this study include younger age, liver cancers and coagulopathy in a patient with a native liver and younger age alone in a patients with transplanted liver [1, 10]

The risk of bleeding with percutaneous liver biopsy method is estimated to be 10% with less than 2% being major bleeds [11]. In a large retrospective study done in Beijing, low platelets count, young age (<18 years) and increased number of passes were found to be independent risk factors for bleeding [12].The most frequent minor complication is said to be pain and was found to occur in up to 59% of patients who had undergone percutaneous liver biopsy [3]

Pneumothorax has only been described in 1 paediatric study with an incidence rate of 0.2% [13]. Performing liver biopsy under ultrasound guidance and avoiding the subcostal approach ameliorates this complication. Death has been reported to occur in 0.6% of children post liver biopsy and all had a background history of malignancy, and all deaths were related to bleeding [13]. Another author reported a lower death rate of 1 per 10 000 cases, while in another study no death was reported [3, 14].

Aim

The aim of this study is to determine the role of liver biopsy in the work up of patients with liver diseases at Red Cross War Memorial Children's Hospital (RCWMCH) over a six year period.

Objectives

Primary objectives:

- Identify the number of liver biopsies done at RCWMCH done between 01/01/2018 and 31/06/ 2023
- Determine the indications for liver biopsies during the study period

- Determine which personnel performed the liver biopsy(paediatric gastroenterologist vs paediatric surgeon)
- Determine whether the liver was cirrhotic
- Determine baseline INR and platelets count
- Determine the approach used to perform the liver biopsy(subcostal vs intercostal)
- Determine whether the biopsy was done under ultrasound guidance or blinded
- Determine the number of passes and cores attained
- To determine liver biopsy results

Secondary objectives:

- Determine number of minor complications during the study period, i.e. pain and minor bleeding from the procedure
- Determine the number of major complications i.e. major bleeding, pneumothorax and death
- To identify possible risk factors associated with complications
- Determine how biopsy results influenced the management of the patient(eg change of management, or a new diagnosis)

Minor bleeding will be defined as documented bleeding, with a haemoglobin of more than 8 not requiring a blood transfusion and major bleeding will be defined as bleeding that results in a hemoglobin drop to ≤8 and requiring transfusion of blood

Method

It is a retrospective cross sectional descriptive study in children between the ages of 0-15 years at RCWMCH who had liver biopsies between the 01st /01/2018 and 31st /06/2023. The liver biopsies were done in both native and transplanted livers. The liver biopsies were done by both the paediatric surgery and paediatric gastroenterology teams. Inclusion and exclusion criteria are highlighted in table 1 below.

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• All liver biopsies done between 01/01/2018 and 31/06/2023 at RCWMCH • Children between 0-15 years	• Missing files and data • Liver biopsies done in other health care facilities

The sample size will be about 70 patients who had liver biopsies at RCWMCH between the 01/01/2018 and 31/06/2023. Entries will be acquired from the unit liver biopsy checklists and NHLS database. Missing entries will be acquired through records review.

The following data will be collected: (See data sheet)

- Demographics
    - Age
    - Gender
    - HIV status
- Clinical characteristics
    - Native liver vs. transplanted liver
    - Indication for the liver biopsy
    - Personnel performing the liver biopsy: Paediatric gastroenterologist vs paediatric surgeon
    - Baseline bloods (INR and platelets count)
    - Presence of cirrhosis
    - Blinded or ultrasound guided biopsy
    - Intercostal or subcostal approach
    - Number of passes
    - Number of liver cores
    - Liver biopsy results
- Outcomes

- Minor complications:
    - Minor bleeding not requiring blood transfusion
    - Pain
- Major complications:
    - Bleeding that requires blood transfusion
    - Pneumothorax
    - Death within 3 days after the liver biopsy
- How biopsy results influenced patient management(eg change in diagnosis, or new treatment)

Data analysis:

Statistical analysis will be done using Stata (Statacorp, USA). Associations with liver biopsy abnormalities will be tested using Chi square tests (with Fisher exact statistics where appropriate) for categorical variables, and Student t –tests or Mann-Whitney U tests for continuous variables, depending on data distribution. Statistical significance is set at a p-value< 0.05.

Ethics

Consent will be obtained from the Hospital Research Review Committee at RCWMCH and the Human Research Ethics Committee (HREC) of the University of Cape Town. This is a retrospective study involving records review and therefore no further consent will be required from the caregivers.

Confidentiality

Anonymized identifiers will be used to maintain confidentiality. Data management will be compliant with the POPI act.

Funding:

The study will be self-funded.

Challenges:

This a retrospective study and therefore relies on records review. We anticipate missing data and files.

Timeline:

The timeline for this study is illustrated in table 2 below

Table 2: Timeline

	January 2023	**February 2023**	**March 2023**	**April 2023**	**May 2023**	**June 2023**	**July 2023**	**August 2023**	**September 2023**	**October 2023**
Literature review										
Preparing protocol										
Protocol assessment										
Ethics application										
Data collection										
Data analysis										
Write up-thesis										
Write up-paper										

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Data sheet

1. Study number:
2. Age:
3. Gender: Male[ ] Female [ ]
4. HIV status: Positive[ ] Negative[ ] Unknown [ ]
5. Native liver [ ] Graft liver [ ]
6. Indication for liver biopsy:
7. Baseline INR:
8. Baseline platelets count:
9. Paediatric gastroenterologist [ ] or surgeon [ ] performing the biopsy
10. Ultrasound guided [ ] or Blind [ ] liver biopsy
11. Intercostal [ ] or Subcostal [ ] approach
12. Number of passes:
13. Number of cores:
14. Liver cirrhosis present: Yes [ ] No [ ]
15. Liver biopsy findings:
16. Minor complications:

    Pain: Yes [ ] No [ ]

    Minor bleeding not requiring a blood transfusion: Yes [ ] No [ ]
17. Major complications:

    Bleeding that requires a blood transfusion: Yes [ ] No [ ]

    Pneumothorax: Yes [ ] No [ ]

    Death within 3 days post liver biopsy: Yes [ ] No [ ]
18. How biopsy results influenced patient management:

    Change in treatment [ ] New diagnosis [ ]

Data sheet

1. Study number:
2. Age:
3. Gender: Male[ ] Female [ ]
4. HIV status: Positive[ ] Negative[ ] Unknown [ ]
5. Native liver [ ] Graft liver [ ]
6. Indication for liver biopsy:
7. Baseline INR
8. Baseline platelets count
9. Paediatric gastroenterologist [ ] or surgeon [ ] performing the biopsy
10. Ultrasound guided [ ] or Blind [ ]
11. Intercostal [ ] or Subcostal [ ]
12. Number of passes:
13. Number of cores:
14. Cirrhosis present: Yes [ ] No [ ]
15. Liver biopsy findings:
16. Minor complications:

    Pain: Yes [ ] No [ ]

    Minor bleeding not requiring a blood transfusion: Yes [ ] No [ ]
17. Major complications:

    Bleeding that requires a blood transfusion: Yes [ ] No [ ]

    Pneumothorax: Yes [ ] No [ ]

    Death within 3 days post liver biopsy: Yes [ ] No [ ]
18. How biopsy results influenced patient management:

    Change in treatment [ ] New diagnosis [ ]

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If the parents or guardian were unable to be located for their consent, a signed statement from the Chair of the Department or from the Institutional Review Board may be accepted. The statement must read: all attempts have been exhausted in trying to contact the parents or guardian for the purpose of attaining their consent to publish the Case Report, Letter to Editor or Image/Video of the Month.

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patient care (e.g., finding efficacy or lack of efficacy of treatment), and/or if the findings suggest a novel mechanism or understanding of disease process (e.g., new susceptibility gene identification in *H pylori* organism).

Rapid Communications should contain no more than 3000 words, structured abstract with no more than 250 words and no more than four figures and tables combined (for example, a submission may include 4 figures, 1 figure and 3 tables, 4 tables, etc., but not 2 figures and 3 tables) and no more than 50 references. Submissions exceeding these parameters without justification or without a detailed cover letter explaining the rationale for a Rapid Communication will be returned to the author for correction prior to review. Extra material such as very detailed methods, tables or figures that are not needed by most readers may be submitted as Supplemental Digital Content without limitation on length (see below). Articles submitted for Rapid Communication but deemed to be more appropriate for standard submission will be returned for resubmission as an Original Article (below).

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- Methods: 2-3 pages (up to 750-1000 words)
- Results: 2-3 pages (up to 750-1000 words)
- Discussion: 3-5 pages (up to 1000 words)
- References: limited to those critical and relevant to the manuscript (not more than 50)
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searchable, open to prospective registrants, and have not-for-profit management. The following information must be included in the registry: (1) unique identifying number, (2) statement of intervention(s), (3) hypothesis, definition of primary and secondary outcome measurements, eligibility criteria, target number of subjects, funding source, contact information for principal investigator, and dates of registration, start and completion. Authors should provide the URL (website address) and trial identification number on the title page of the manuscript. This information will be published with the article. The length of Clinical Trials should follow the same guidelines as the Original Articles above (Structured Abstract (no more than 250 words); text with no more than 3000 words; no more than 50 references permitted; no more than 4 Tables/Figures; SDC permitted). Clinical trial reports should comply with the Consolidated Standards of Reporting Trials (CONSORT) and the checklist should be submitted at the end of the manuscript.

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All Case Reports, Image of the Month, Video of the Month, and Letters to the Editor will be reviewed and accepted as online only content. Papers in these sections will still be assigned to an issue and listed in the issue's Table of Contents, and will appear in full on the journal website and in all electronic versions of the journal.

Case Reports (online only): Only exceptional cases will be accepted by the JPGN as case reports; thus, the report should present unique case(s) that are deemed important to the health of our patients or the advancement of the knowledge base in our field. It is helpful if you submit a Case Report to please include in your cover letter a brief paragraph that explains why your case meets the above criteria. Alternately, you may submit your report as a Letter to Editor; Letters may include up to one figure or table.

Case Reports will be considered for publication only if they concern a hitherto unrecognized condition or offer new insight into the pathophysiology, diagnosis, or treatment of a disease. Patients should always be referred to as "the patient"; initials or other identification should not be used. Case Reports must not exceed 1,000 words, and may include up to three tables and/or figures, and no more than 8 references. An abstract is not required, and if present, it will be included in the word count.

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Summary of Article Type Parameters

Article Type	Abstract	What is New/ What is Known	Text Limit	Figure/Table Limit	Reference Limit

Rapid Communication	Structured 250 words	Yes	3000 words	4	50
Original Articles	Structured 250 words	Yes	3000 words	4	50
Clinical Trials	Structured 250 words	Yes	3000 words	4	50
Systematic reviews / Meta-analyses	Structured 250 words	Yes	3000 words	4	50
Review Articles	Unstructured 250 words	Yes	3000 words	4	50
Short Communications	Unstructured 150 words	Yes	2000 words	2	20
Invited Commentary	None	Not required	1000 words	1	10
Topic of the Month	Unstructured 150 words	Yes (for social media only)	1500 words	2	30
Societal Papers	Optional 250 words	Yes (for social media only)	n/a	n/a	n/a
Online Only Article Types					
Case Reports*	None	Not required	1000 words	3	8
Image of the Month	None	Not required	200 words	2	8
Video of the Month	None	Not required	200 words	2	8
Letters to the Editor and Response	None	Not required	250 words	1	8

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Sample references:

Journal article

I. Rautava S, Lu L, Nanthakumar NN, et al. TGF-β2 induces maturation of immature human intestinal epithelial cells and inhibits inflammatory cytokine responses induced via the NF-κB pathway. J Pediatr Gastroenterol Nutr 2012;54:630-8.

Book chapter

2. Todd VR. Visual information analysis: frame of reference for visual perception. In: Kramer P, Hinojosa J, eds. Frames of Reference for Pediatric Occupational Therapy. Philadelphia: Lippincott Williams & Wilkins; 1999:205–56.

Entire Book
3. Ming S-C, Goldman H. Pathology of the Gastrointestinal Tract. Philadelphia: Lippincott Williams & Wilkins; 1998.

Software
4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention; 1994.

Online journals
5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol [serial online] January 1988;71: 22-37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database
6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

World Wide Web
7. Sullivan D. Major search engines and directories. SearchEngineWatch Web site. http://www.searchenginewatch.com/links/article.php/2156221. Published May 8, 2011. Accessed July 13, 2012.

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Protocol title	Review of liver biopsies at Red Cross War Memorial Children's Hospital over a six year period		
Principal Investigator	Dr Ronalda De Lacy		
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