THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY IN DIAGNOSIS OF FOCAL LIVER LESIONS IN KHARTOUM STATE

By

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A thesis submitted in partial fulfillment for the requirements of the degree of Clinical MD in Pathology, 2010

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U of K
Chapter one

Introduction

Literature review and objectives
Dedication

To my parents for their life long support, love, and encouragement.

To my wife, daughter, son, sisters, and brothers, whose support made every thing possible.

To my teacher, late Dr: Eltaib Elaasha (1943 – 2002)

Without his support I would not joined the Discipline of pathology .He left a legacy of excellence that will enrich the life of generation of future doctors.

To my faithful teacher Dr: Mohammed Abdel Hameed whose support is the foundation on which pursues my academic aspiration.

To all who strive for improving the health of every one.
Acknowledgement

At the beginning, and at the end all thank, Allah, for helping me in doing and completing this work.

I am very grateful to my supervisor Dr. Mohamed Mohamed Osman, who helped me with his time and his patience. His encouragement and trust help me too much to complete my work.

Special thanks to Dr. Ahmed Omer Almobarak without his help this work would not be possible, hoping that God will bless him with his love that makes every thing a joy to life.

Best regards to my family for their help, support, and encouragement during the period of the research to overcome all the difficulties which faced me.

I'm very grateful to Dr. Mohammed Altaib for his support and advice

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My thanks to all people who helped me, and I may have missed to mention their names.

    Khalid
ABSTRACT

Introduction: Focal liver lesions constitute a major stress to the patient, and challenge for the pathologist in term of diagnosis by fine needle aspiration cytology.

Aim: To evaluate the role of imaging guided fine needle aspiration cytology (FNAC) in diagnosis of focal liver lesions.

Methods: In a retrospective study between 2007 – 2009 in venue of Police Hospital and Khartoum Clinic, on 61 Sudanese patients with unifocal or multifocal liver lesion, diagnosed radiologically. Fine needle aspiration of a liver lesion was done guided by ultrasound in the 57 cases and by computed tomographic scan in 4 cases.

Results: Thirty-two (52.5%) patients from Khartoum State and 29(47.5%) patients from outside Khartoum. Patients’age range from 1-82 years, with preponderance of male (75.4%). Morphologically, malignant lesions constituted 72% and benign lesions 28% for both uni and multifocal liver lesions. For unifocal lesions malignancy was found in 60% and benign lesions in 37% and 3% of cases were suspicious. For multifocal liver lesions 85% were malignant and 15% were benign. Secondary malignancies were more common than primary malignancy, constituting 71% and 77% for unifocal, multifocal liver lesions respectively.

Primary malignant liver tumours were 29% of uni focal and 18% of multifocal lesions. In one patient there was a need for liver biopsy and immunostain to arrive at a final diagnosis of malignancy.

Conclusion: it is concluded that FNAC is useful technique in evaluating focal liver lesions. It is simple, safe, and what made it more useful is the better coordination between radiologist, pathologist, and clinicians.
المسطخـص

١. ال目的地: وتحدي لمرض الكبد بال نسبة لقلق مصدّر للكبد الآفات. تتشكل الدقة بالبر وتشخيصها حيث أن الأعراض العلم الإخصائي.

الأهداف: في الدقة بالبر والشفاء دور وتحقيق تحديد الدراسة هذه أجرية الآفات تشخيص وبحث مرن في إجراءات الكبد الآفات التشخيص.

المنهجية: من فترة في إرتجالية دراسة في 2007 – 2009 بمساندة م، على الخرائط والوعيادة 61 أو وحيدة بالكبد شعاعيًا تشخيصها تم الكبد الآفات أدمامًا، الدقة بالبر والشفاء تم ثم ومنة، فوق بالعاجت في الصوتية 57 في حالات 48 الإصابة، والكبد الآفات توافر كانت للمرضية الكبد الكبد الآفات و االدقيقة بالبر والشفاء 60 % ودقيقة، 28 % ودقيقة بالكبد الكبد الآفات 77 % و71 % ن例子 والأخضر والأمراض الزائدة 18 % و29 % ودقيقة ودقيقة.

النتائج: المرضى عدد 32 (52.5) و 29 (47.5) خارجها ودم مننة 40.6، الأعمار التراوح منها 1 إتي إل 82 ذكور، نسبة ارتفاع مع سنة، 75.4. (1) كان في الآفات من الكبد الكبد الآفات 71 % و77 % ترتيب على الكبد الكبد الآفات 29 % و18 % ترتيب في يد ويد، ويجد 57.6 % في مراض الكبد والكبد الآفات في الكبد الآفات 3.6 % و18 % في الكبد الكبد الآفات 57.6 % و29 % ودقيقة ودقيقة.

التوسيع: لتقييم فائدة الطريقة الإشعاعية بالصور الموجهة الدقة بالبر وتشخيص وسهولة عملية وهم وعمره الكبد الآفات، خاصة بين وتنسيق الدقة بالبر ووجوده في ناجحة عملية هذه و الداء، والمنعاء وآفاق.

يرجى منك أن تبدأ نص أو جزء من النص لكي أتمكن من قراءته.
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<tr>
<td>FNAC</td>
<td>Fine Needle Aspiration Cytology</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular Carcinoma</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>FNA</td>
<td>Find needle aspiration</td>
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<tr>
<td>EUS</td>
<td>Endoscopic ultrasound guidance</td>
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<td>CNB</td>
<td>Core needle biopsy</td>
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<td>AFP</td>
<td>Alpha fetoprotein</td>
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<tr>
<td>EUS-FNA</td>
<td>Endoscopic ultrasound guided FNA</td>
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<tr>
<td>LBC</td>
<td>Liquid based cytology</td>
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<tr>
<td>CEA</td>
<td>Carcinoembryonic antigen</td>
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<tr>
<td>WHO</td>
<td>World health organization</td>
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<tr>
<td>PAP</td>
<td>Papanicolaustain</td>
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<tr>
<td>EMH</td>
<td>Extra medullary haemopoiesis</td>
</tr>
<tr>
<td>EMA</td>
<td>Epithelial membrane antigen</td>
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<tr>
<td>AFB</td>
<td>Acid fast bacilli</td>
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<tr>
<td>IPT</td>
<td>Inflammatory pseudotumour</td>
</tr>
<tr>
<td>PEC</td>
<td>Perivascular epithelial cell</td>
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<tr>
<td>DN</td>
<td>Dysplastic nodule</td>
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<tr>
<td>FNH</td>
<td>Focal nodular hyperplasia</td>
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<tr>
<td>CAMS</td>
<td>Low molecular weight cytokeratin</td>
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<tr>
<td>CK</td>
<td>Cytokeratin</td>
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<tr>
<td>PT</td>
<td>Prothrombin time</td>
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<td>PTT</td>
<td>Partial thrompolastin time</td>
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<td>MRCP</td>
<td>Magnetic resonance cholangiopancreatography</td>
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**Photo 4**
*Well-differentiated hepatocellular carcinoma.*
(A) The peripherally wrapping endothelial cell pattern demonstrates the capillaried endothelial cells of sinusoid wrapping around smooth edged, with nests and thickened hepatic trabeculae of hepatocytes (smear, PAP).

(B) This pathognomonic feature is also demonstrated in cell block preparations (H&E).

**Photo 5**
Moderately-differentiated hepatocellular carcinoma. This high grade hepatocellular carcinoma maintains some hepatic preservation, while displaying obvious malignant features smear.

**Photo 6**
Hepatoblastoma:
(A) The foetal type is composed of cells that resemble the normal hepatocyte but are generally smaller with central round and bland nucleoli (smear, diff-quik)

(B) The embryonal type is composed of more primitive cells with hyperchromatic nucleoli and scant cytoplasm (smear, PAP)
Metastatic colonic carcinoma:

(A) This adenocarcinoma is recognised on smears by the background of dirty necrosis, a non-specific but characteristic feature. Viable aggregates of carcinoma may be few (smear, PAP).

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1-1. INTRODUCTION

Medicine is an ever changing science. As new techniques developed, these will have an impaction on diagnostic and treatment methods.

In recent years advances in radiological investigations cut the way to diagnosis, however this is not enough; it should be supplemented by other investigation in our field of pathology. A number of entities, present as focal liver lesions, including cysts, abscesses, benign liver nodules, primary and metastatic malignancies (HANS popper society symposium MARCH 25, 2007).

Beyond any shadow of doubt focal liver lesions detected by, ultrasound, or C.T scan, constitute a major stress to the patient, and a challenge to pathologist in term of verifying them.
1-2. LITERATURE REVIEW

Find needing aspiration cytology (FNA):

Cytological analysis of the liver involves the aspiration of cells from focal mass lesions or from brushing the lining of strictured intrahepatic ducts primarily for the purpose of determining the presence or absence of malignancy. Fine needle aspiration (FNA) of focal mass lesions can be performed percutaneously or with endoscopic ultrasound guidance.\(^1\)\(^2\) intrahepatic ducts are brushed with the aid of endoscopic retrograde cholangiography.\(^3\)\(^4\)

Needle aspiration of the liver was performed as long ago as 1833 when Roberts and Biett reported its use in the treatment of hepatic abscesses and echinococcal cysts.\(^5\)\(^6\) Although the use of fine needles to obtain tissue for cytological diagnosis was first used by Lucatelio in 1895 [died in Lundquist 1971],\(^7\) it was a technique refined in the 1920s by Martin and Ellis at the Memorial Hospital in New York.\(^8\) Its utility in the liver was highlighted by Lundquist who published his experience using aspiration cytology in the evaluation of neoplastic and non-neoplastic disorders of the liver.\(^7\)\(^8\)\(^9\)\(^10\) FNA biopsy is now the diagnostic procedure of choice for the diagnosis of focal mass lesions and is considered accurate and safe when performed and interpreted by experienced radiologists and pathologists.\(^1\)\(^11\)\(^12\)\(^13\)
The diagnostic accuracy rate of FNA is reported at greater than 85% in most series.\textsuperscript{14} False negative FNAs are most often due to sampling error. Proper specimen processing and staining optimizes the preservation and presentation of the cells and is an important factor in reducing interpretation errors. Routine supplementation of FNA with a cell block of tissue fragments or core biopsy is better than either method alone, especially for benign neoplasms and poorly differentiated neoplasms that require ancillary studies;\textsuperscript{15-17}

**Contraindications**

Contraindications for FNA of the liver are few but include a non-correctable bleeding diathesis, the lack of a safe access route, and an uncooperative patient\textsuperscript{1,11} For EUS guided FNA, gastrointestinal obstruction is an absolute contraindication because of the risk of intestinal perforation.\textsuperscript{18}

**Complications**

With modern-day techniques, complications of FNA are uncommon. The most common complications include pain and haemorrhage\textsuperscript{14} The haemorrhage can be intraperitoneal, subcapsular, or intrahepatic and, if intrahepatic, can lead to haemobilia. There is a low risk of haematogenous dissemination of malignant cells after liver biopsy\textsuperscript{19,30} as
well as 'seeding' of the biopsy tract, complications that tend to be related to larger needle size.\textsuperscript{14,21}

**Pre-biopsy assessment:**

Pre-biopsy assessment of patients undergoing FNA of the liver includes evaluation of the coagulation system to prevent excessive bleeding, generally complete blood count, prothrombin time and partial thromboplastin time.\textsuperscript{1} Patients who are receiving intravenous sedation should not eat at least 6 hours before the biopsy.\textsuperscript{1} Local anaesthetic is generally given down to the liver capsule. Discussing the procedure fully with the patient reduces patient anxiety, improves patient cooperation and increases the likelihood of a successful FNA procedure.

**Guidance systems**

Percutaneous computed tomographic (CT) or ultrasound (US) guidance are the guidance systems used for most liver FNAs.\textsuperscript{122,24} Endoscopic ultrasound guidance (EUS) is increasingly used for those lesions accessible through a transgastric approach, mostly in the left lobe.\textsuperscript{2,25,28} Factors influencing the choice of the guidance system include the size, location and visibility of the mass, in addition to the experience and preference of the operator.\textsuperscript{141} Ultrasound provides real-time needle visualisation, flexible patient positioning, variable imaging of the lesion, and is performed without ionising radiation. CT facilitates biopsy of
small, deep seated lesions not well demonstrated on ultrasound, CT more precisely demonstrates the anatomic relationships of a given lesion compared to US, improves the definition of tissue components and vascularity, and provides for accurate localisation of the needle tip immediately prior to sampling without the transmission of potential impediments such as drains, bone and gas.

**Sampling techniques:**

Percutaneous FNA endoscopic ultrasound-guided FNA and bile duct brushing Percutaneous FNA techniques generally vary depending on the location and size of the mass. Any mass lesion that is palpable can be directly aspirated without guidance in the usual manner for all palpable aspiration biopsies. The most common techniques using guidance include individual puncture, coaxial biopsy, and tandem needle biopsy.\(^1\)\(^1\)\(^1\)\(^2\)\(^9\). The coaxial biopsy technique is most commonly used. This technique uses a coaxial introducer needle through which FNA and core needle biopsy are performed with only one puncture into the lesion and without the need for repeat imaging.

A concomitant core needle biopsy (CNB) following the FNA is recommended when at all possible. CNBs provide the necessary tissue architecture as well as readily available tissue for ancillary studies that aid in providing a more specific diagnosis in many cases. Combined with
a preceding FNA, the accuracy in diagnosing focal mass lesions is significantly greater than that obtained with either method alone.\textsuperscript{15-17,30}

Endoscopic ultrasound-guided FNA \{EUS-FNA\} of liver masses is confined to those lesions visible and accessible via the stomach or duodenum. Lesions in the right lobe of the liver and hilum are assessed via the duodenum and distal stomach while those in the left lobe are accessed via the proximal and mid-stomach.\textsuperscript{31-34}

**Bile duct brushing**

Biliary brushing cytology of suspicious biliary strictures is key to making an early diagnosis. Unfortunately, despite specificity of >95\%, the technique has a low sensitivity ranging from 17-83\%.\textsuperscript{3} Low sensitivity can be attributed to difficult access, desmoplasia, associated inflammation (stents, primary sclerosing cholangitis), scant specimen cellularity and poor cellular preservation and preparation.\textsuperscript{4,35,36} If sufficient cells are present and the cells are properly preserved and prepared for cytological evaluation (direct smears, cytospins or liquid-based cytology (LBC)), the criteria for malignancy are universal: high N/C ratio, prominent nucleoli, nuclear membrane abnormalities and hyperchromasia yielding 100\% specificity for malignancy.\textsuperscript{37} LBC processing alone or in addition to direct smears has improved sensitivity and accuracy in some studies, but cytologists must be familiar with alterations to morphology and
background elements that may make malignancy appear more subtle in some cases.\textsuperscript{38-40}

Elevation in serum tumour markers CEA and CA19-9 have higher sensitivity for detecting carcinoma, but lower specificity (i.e. false positive tests).\textsuperscript{37} Adding digital image analysis\textsuperscript{35} and fluorescence in situ hybridisation (FISH)\textsuperscript{41} show promise in improving the diagnostic value of biliary brush cytology Specimen processing

**Specimen processing:**

It is desirable to obtain both smears and cell block preparations in all FNAs of the liver. Smears are from the aspiration part of the procedure using a small needle (<22 gauge) that provides a rapid means of evaluating the specimen, not only for cellular adequacy but frequently for diagnosis. Multiple FNAs can be performed with minimal morbidity. If well-fixed, adequately smeared slides are difficult to obtain. The aspirate can be expressed into a preservative and submitted to the laboratory as a liquid-based specimen for processing by either the Thin Prep (Cytec Corporation, Marlborough, MA) or Sure Path\textsuperscript{TM} (TriPath Imaging, Burlington, NC) methods. Cell blocks are made from FNA rinsings, any tissue fragments that are obtained, and dedicated CNBs using a spring loaded 18-20 gauge CNB gun such as the ASAP Biopsy System (Meditech/Boston Scientific Corp. Watertown, MA) or the Coaxial
Temno Biopsy System [Allegiance Health-care Corp, McGaw Park, IL). This material provides formalin fixed, paraffin embedded tissue sample from which special stains and immunohistochemical studies can be readily obtained. It also provides, in many cases, the architecture necessary for a specific diagnosis, particularly in benign liver lesions.

CNB specimens can also be used for rapid interpretation by touching the core to a glass slide in a touch prep fashion.\(^{42}\)

Despite the presence of thick, three-dimensional tissue fragments and the probability of some air-drying artefact due to the inherent time delay in preparing the slide, architectural clues may still be readily apparent for rapid diagnosis.

The cytopathologist is an important part of the overall team approach to FNA of the liver. The presence of a cytopathologist at the time of the FNA increases the overall accuracy of the procedure.\(^{13,17,43}\) The time of the actual biopsy, when additional tissue is still readily available, is the time to evaluate the specimen for adequacy and to triage the tissue for special studies such as flow cytometry or electron microscopy studies. If a cytopathologist or cytotechnologist is not available to assist in the preparation of the specimen, it is imperative that the radiologist learn how to make proper smears.
The most cellular specimen is useless if inadequately prepared for optimal interpretation.

**Tumour classification:**

The most recent WHO classification of tumours of the liver is presented in Table JU-44 Correct diagnosis is imperative for proper patient management. Pyogenic abscesses are typically drained, and smaller tumours are being treated with ablation techniques such as alcohol and thermal ablation. Chemotherapy and radiation protocols require a tissue diagnosis and targeted gene therapy is under investigation.

**Normal morphology:**

The liver is a complex organ with functional lobular units of hepatic parenchyma anchored by portal tracts containing branches of the hepatic artery, hepatic portal vein and bile duct. Sinusoids are lined by a discontinuous layer of endothelial cells that separate hepatic plates of 1-2 cells thick and that terminate in the central vein. Sinusoidal endothelial cells differ from vascular endothelial cells, unlike the endothelial cells of true vessels, sinusoidal endothelial cells are not supported by a basement membrane and do not express factor VIII,-Ulex europaeus or CD 34. Transformation of sinusoids with the acquisition of these properties leads to capillarisation or the sinusoids, changes that are exploited in both histological and cytological evaluation for diagnosis.
In order to appreciate an abnormality in liver FNA, it is imperative that the cytopathologist understands the components of the normal liver.

**Table 1: WHO histological classification of tumours of the liver and intrahepatic bile ducts**

1) **Epithelial tumours:**

<table>
<thead>
<tr>
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<tr>
<td>Hepatocellular adenoma (liver cell adenoma).</td>
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<td>Focal, nodular hyperplasia.</td>
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<td>Intrahepatic bile duct adenoma.</td>
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<td>Intrahepatic bile duct cystadenoma.</td>
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<td>Biliary papillomatosis.</td>
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<td>Hepatocellular carcinoma (liver cell carcinoma).</td>
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<tr>
<td>Intrahepatic cholangiocarcinoma (peripheral bile carcinoma).</td>
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<tr>
<td>Bile duct cystadenocarcinoma.</td>
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<td>Combined hepatocellular and cholangiocarcinoma.</td>
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<tr>
<td>Hepatoblastoma.</td>
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<td>Undifferentiated carcinoma.</td>
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2) **Non-epithelial tumours:**

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<td>Lymphangioma and lymphangiomatosis</td>
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<td>Haemangioma.</td>
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<td>Infantile haemangioendothelioma.</td>
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<tr>
<td>Angiosarcoma.</td>
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<tr>
<td>Embryonal sarcoma (undifferentiated sarcoma).</td>
</tr>
<tr>
<td>Rhabdomyosarcoma.</td>
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<tr>
<td>Others.</td>
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3) Miscellaneous tumours

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<thead>
<tr>
<th>Tumour Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary fibrous tumour.</td>
<td></td>
</tr>
<tr>
<td>Teratoma.</td>
<td></td>
</tr>
<tr>
<td>Yolk sac tumour (endodermal sinus tumour).</td>
<td></td>
</tr>
<tr>
<td>Carcinoma.</td>
<td></td>
</tr>
<tr>
<td>Rhabdoid tumour.</td>
<td></td>
</tr>
<tr>
<td>Others.</td>
<td></td>
</tr>
</tbody>
</table>

4) Haemopoietic and lymphoid tumours.

Secondary tumours.

- Epithelial abnormalities:
  - Liver-cell dysplasia (liver cell change).
  - Large cell type (large cell change).
  - Small cell type (small cell change).
    - Dysplastic nodules (adenomatous hyperplasia).
  - Low-grade.
  - High grade [atypical adenomatous hyperplasia].

- Bile duct abnormalities:
  - Hyperplasia (bile duct epithelium and peribiliary glands).
  - Dysplasia (bile duct epithelium and peribiliary glands).
  - Intraepithelial carcinoma (carcinoma in-situ).

5) Miscellaneous lesions:

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchymal hamartoma.</td>
<td></td>
</tr>
<tr>
<td>Nodular transformation (nodular regenerative hyperplasia).</td>
<td></td>
</tr>
<tr>
<td>Inflammatory pseudotumour.</td>
<td></td>
</tr>
</tbody>
</table>
Cytological findings of normal liver:

The normal hepatocyte is a large polygonal cell with:

• Abundant granular cytoplasm

• One or two round to oval, centrally placed nuclei

• Even chromatin pattern and occasionally prominent nucleoli

• Generally appear as small clusters.

• Larger flat sheets with irregular jagged edges without endothelial cell wrapping or Single cells.

Benign bile duct epithelial cells present as:

• Varying sized flat monolayered sheets of epithelial cells

• On-edge or in small acinar structures or groups

• Smaller than benign hepatocytes

• With round regular nuclei

• Inconspicuous nucleoli

• Less abundant cytoplasm than hepatocytes.

Endothelial cells of sinusoidal spaces and Kupffer cells are rarely appreciated in benign lesions.
**Pigments:**

Intracytoplasmic pigments in cytology preparations differ in appearance depending on the stain. Lipofuscin constitutes the debris of intracellular lysosome breakdown, appears as a fine golden, granular, relatively non-refractile pigment in alcohol fixed, Papanicolaou (PAP) stained smears and is typically concentrated around the nucleus. This pigment is generally very common in the FNA of older adults and its absence should increase suspicion of a neoplasm on FNA of a mass lesion. Lipofuscin will stain darkly with a Fontana Mason stain creating a potential diagnostic pitfall with a metastatic melanoma.\(^{51}\)

![Photo 1: Normal hepatocytes. Benign hepatocytes demonstrate a polygonal shape, abundant granular cytoplasm focal steatosis and 1-2 round to oval centrally placed nuclei, with an even chromatin pattern and small nucleoli (Smear, PAP).](image)
Bile pigment is produced by hepatocytes and is virtually pathognomonic of hepatocellular carcinoma when recognised within malignant cells. Bile appears as coarse, irregular, rather amorphous, non-refractile green to golden brown globular intra-cytoplasmic and extra-cytoplasmic deposits on Papanicolaou stain. With Giemsa-Romanowsky stain, bile has a dark green to black hue. The distribution of the pigment is dependent on the degree of cholestasis, with pools of bile within canalicular spaces apparent in cases of extrahepatic obstruction.

Iron or haemosiderin is a coarse, brown-black refractile pigment with Papanicolaou stain. While FNA can confirm the presence of heavy iron overload, it cannot replace CNB for histology and biochemical analysis to answer the clinical question of haemochromatosis. Malignant hepatocytes lose their ability to retain iron, and in the setting of haemachromatosis induced cirrhosis, where reactive hepatocyte atypia may be quite marked, it is helpful to use a special stain for iron like Prussian blue to highlight cells or clusters of cells without staining.\textsuperscript{52}

**Non-neoplastic conditions:**

*Steatosis.*

Fatty change in hepatocytes is common in many conditions in the liver especially toxic/metabolic injury such as with alcohol abuse. Steatosis can result in the radiological appearance of mass lesion leading to FNA.\textsuperscript{53}
Fat may present in the form of macrovesicular steatosis, the most common form in which one or more large fat vacuoles fill the cytoplasm or microvesicular steatosis, in which multiple small lipid vacuoles expand.

**Amyloidosis:**

The deposition of amyloid in the liver can rarely cause the appearance of a mass lesion (amyloidoma) leading to FNA, Amyloid deposition in the liver is most often secondary to systemic diseases such as rheumatoid arthritis and plasma cell dyscriasias (multiple myeloma). Amyloid, regardless of type, is an extracellular amorphous hyaline material that has a pink or green waxy or glassy appearance on Papanicolaou stain, and a magenta.

**Cytological findings amyloidosis:**

- Amorphous glassy or waxy extracellular material
- Rounded dense droplets on LBC
- Pink to green staining on Papanicolaou; magenta on Romanowsky
- Congo red stain is positive
- Apple-green birefringence with polarisation.
**Splenosis:**

Ruptured splenic tissue following trauma or splenectomy can result in implantation or auto-transplantation of splenic tissue in the peritoneal cavity and even within parenchymal organs like the liver mimicking a neoplastic process, including hepatocellular carcinoma and especially metastatic malignancy given the typical multiple implants.\(^{55,56}\) Although most patients are asymptomatic, these mass forming lesions are picked up incidentally and may be investigated by FNA. Smears that are cellular and well-preserved recapitulate the normal spleen demonstrating a mixture of normal haemopoietic cells in a bloody background supported by a reticular network\(^{57}\). Lymphoid dominant FNAs, however, introduce small blue cell tumours into the differential diagnosis including small cell lymphoma and small cell carcinoma. An important clue to the diagnosis is a history of splenectomy and/or trauma.

**Cytological findings hepatic splenosis:**

- Benign appearing haemopoietic cells: lymphocytes and neutrophils
- Lymphoid follicle
- Reticular network holding aggregates of cells together
- Bloody background.
**Extra medullary haemopoiesis:**

Extra-medullary haemopoiesis (EMH) is normal only within the first few weeks of life. After this point, EMH or myeloid metaplasia is an abnormal condition in which the liver attempts to produce the deficient blood cells that results when the normal bone marrow has been replaced by non-functioning tissue such as fibrosis in myeloproliferative diseases. EMH is also commonly associated with hepatoblastomas and hepatic angiosarcomas. EMH is often first considered with the recognition of megakaryocytes, large cells with abundant granular cytoplasm and lobulated nuclei. These cells are associated with normoblasts, red cell precursors, small cells with round, central pyknotic nuclei and dense eosinophilic cytoplasm, and myelocytes, slightly larger cells than normoblasts but smaller than megakaryocytes, a central round nucleus and granular cytoplasm.\(^{58}\)

**Infection:**

Abscess formation in the liver is often suspected radiologically from the characteristic double target sign on computed tomography; however, organised abscesses can mimic hepatic tumours leading to FNA.\(^{53}\) Pyogenic abscess is the most common and percutaneous biopsy is performed for tissue confirmation, culture and drainage.\(^{45,59}\) FNA is an acceptable means of diagnosis for all abscess sizes, but for therapeutic
drainage, FNA is recommended only for small abscesses <50 mm; larger abscesses require percutaneous catheter drainage for complete management. Smears are dominated by nonspecific acute inflammatory cells and cellular debris. Cultures are helpful for identification of bacterial organisms.

Amoebic abscess is 10 times more common in men than in women and is rare in children. The distinction between pyogenic and amoebic abscess is important for both therapy and prognosis. Pyogenic abscesses have a 20-60% morbidity due to an association with systemic infection, the tendency to produce multiple liver abscesses and an association with an older patient age. Amoebic abscesses tend to occur as solitary liver masses in young patients, rarely require drainage and respond to metronidazole therapy. Most patients with hepatic amoebiasis do not demonstrate intestinal symptoms, but hepatomegaly with point tenderness over the liver, below the ribs or in the intercostal spaces are typical presenting symptoms. Identification of organisms in aspiration fluid of hepatic abscesses is uncommon and diagnosis rests on the constellation of presenting symptoms, high risk factors, typical radiological findings of a solitary right lobe mass and positive serum antibodies which are present in 70-80% of patients. The single cell trophozoites of E. histolytica with round nucleus, condensed peripheral chromatin and central small
nucleolus with foamy cytoplasm resemble histiocytes and can be easily overlooked.

Other infectious organisms may be recognised by unique characteristics such as the 'sulphur granules' of actinomyces and 'anchovy paste' smears, echinococcal booklets or scolices or laminated cyst wall in hydatid disease.\(^46\)

Granulomatous inflammation may be related to infection such as with fungal or acid fast organisms, but the presence of granulomata in a liver FNA is in no way diagnostic of an infectious aetiology. The presence of granulomata may be related to many conditions including primary hepatobiliary disorders, sarcoidosis, and tumours such as lymphoma and metastatic carcinoma. Hepatic sarcoidosis is one of the most common causes of non-caseating granulomata of the liver.\(^62\) Cytologically granulomata are composed of clusters of epithelioid histiocytes with oval to elongated, sometimes twisted nuclei and visible but indistinct, non-phagocytic, cytoplasm. Special stains such as Crocott methenamine silver (GMS) and Ziehl Neelsen (ZN) for acid fast bacilli (AFB) can be performed on smears but are easier to perform on cell block preparations.

**Bile duct hamartoma:**

Bile duct hamartoma may present as a mass lesion mimicking a neoplasm, and as a result, may be encountered on FNA.\(^63\)
This lesion is often aspirated at the time of diagnostic EUS-FNA of pancreatic masses, so care must be taken to distinguish gastrointestinal contamination from bile duct epithelium as a means of ensuring the aspirate is representative of the lesion. Smears contain a predominant population of benign appearing bile duct epithelial cells often in the form of flat mono-layered sheets of glandular cells with small uniformly spaced round nuclei. Luminal edges with scant but visible non mucinous cytoplasm are present. Benign hepatocytes are uncharacteristically few or absent as would be expected for a benign hepatic lesion such as focal nodular hyperplasia. Aspirates of bile duct adenoma have a similar appearance. Cell block preparations are helpful in rendering a diagnosis.

**Cytological findings: bile duct hamartoma adenoma:**

- Benign appearing glandular epithelium, often in flat mono-layered sheets.
- Round, uniform, evenly spaced nuclei.
- Scant but visible, non-mucinous cytoplasm.
- Uncharacteristically few to no hepatocytes in the background.

**Mesenchymal hamartoma:**

Hepatic mesenchymal hamartoma is a benign mass forming lesion of malformed bile ducts and myxoid mesenchyme diagnosed in mostly male (70%) infants and children less than 5 years of age with rare cases
reported in adults.64,65 The aetiology is unclear with theories ranging from congenital plate abnormality to true neoplasm. These typically predominantly cystic masses are usually resected in childhood and complete resection is curative. Although serological tumour markers are usually normal, elevated AFP and atypical FNA cytology have been reported to lead to a preoperative diagnosis of hepatoblastoma, the primary tumour in the differential diagnosis.12,66

Reports of the cytological features are few.12,67,68 FNA produces scant smears composed of a loose myxoid mesenchymal stroma with benign-appearing spindle cells and sheets of benign glandular epithelium that reflects the histology of disorganised loose myxoid mesenchymal tissue surrounding variably sized benign bile ducts and occasionally hepatocytes. Pseudocysts occur in the mesenchyme and are not lined by epithelial cells. Extramedullary haematopoesis may be noted. The patient's young age, male gender, cystic radiological appearance and benign-appearing myxoid spindled and glandular cell proliferation should lead to the correct diagnosis.

Cytological findings: mesenchymal hamartoma:

• Benign appearing glandular epithelium, often in flat monolayered sheets
• Myxoid stroma
• Benign appearing spindled cells

• Variable number of benign hepatocytes.

**Inflammatory pseudotumour**

Inflammatory pseudotumour (IPT) of the liver is an uncommon, benign, mass-forming proliferation of mixed inflammatory cells and histiocytes dominated by polyclonal plasma cells infiltrating a stroma of fibroblasts, myofibroblasts and collagen. These lesions are reported in patients of both genders, but mostly males (70%), and all ages from infancy to the eighth decade. The heterogeneity of these proliferations has led to many synonymous names, including plasma cell granuloma, inflammatory myofibrohistiocytic proliferation, fibroxanthoma and inflammatory myofibroblastic tumour. Although some reports of locality and association with the Epstein-Barr virus have supported a neoplastic process in some cases.

The aetiology in most hepatic IPT is inflammatory or infectious. More recently, pseudotumour is considered to be part of the IgG4 related sclerosing disease, often associated with autoimmune pancreatitis. These lesions are benign, but rare cases of biological aggressiveness and malignant transformation have been reported.

Diagnosis by FNA is challenging due to the non-specific and variable nature of the mixed inflammatory sample. Smears are often cellular
composed of cohesive networks of spindle mesenchymal cells enmeshed with mixed inflammatory cells and foamy (xanthomatous) histiocytes that also populate the background.

Plasma cells are usually prominent and neutrophils and eosinophils are minor components. The spindle cell component may also be dominant and single spindle cells are noted. Atypia, especially in the mesenchymal cells and histiocytes, can be a pitfall. The differential diagnosis includes spindle cell lesions of the liver including gastrointestinal stromal tumours and sarcomas. The typical high cellularity of the inflammatory component should preclude a false positive interpretation.

**Cytological findings: inflammatory pseudotumour.**

• Mixed inflammatory proliferation

• Numerous plasma cells and foamy histiocytes

• Benign appearing spindled cells in cohesive groups.

**Ciliated hepatic foregut cyst**

This rare hepatic cyst is an embryological remnant of the foregut that differentiates along bronchial lines to form a cyst lined by pseudostratified columnar epithelium. These cysts are predominantly unilocular, subcapsular and less than 4cm. Except for its hepatic location, the cytology is identical to a bronchogenic cyst.
**Cytological findings: ciliated hepatic foregut cyst:**

- Thin to mucoid cyst fluid.
- Ciliated columnar cells.
- Mucous cells.
- Generally few to no hepatocytes.

**Diagnostic pitfall ciliated hepatic foregut cyst:**

Mucoid contents and mucous cells could lead to diagnosis of hepatobiliary cystadenoma.

**Benign neoplasms:**

*Angiomyolipoma:*

This benign mesenchymal neoplasm is purported to arise from the perivascular epithelioid cell (PEC) and has been classified as a PEComa, one of several benign, neoplasms of the tuberous sclerosis complex. It is composed of varying combinations of fat, smooth muscle and vessels. When the fatty component is readily recognised radiologically, histologically and cytologically, the diagnosis is relatively straightforward, In fact, it is usually the paucity of fat in the neoplasm that leads to a diagnostic dilemma and subsequent FNA the histological and cytological features of this tumour are similar in the liver and the kidney. Diagnostic difficulties on cytology arises when the fatty
component is scant or focal and not sampled, and when solid epithelioid areas predominate. \textsuperscript{85} \textsuperscript{86} Cell block preparations provide not only architectural clues, but tissue for ancillary testing. Positive staining with HMB-45 confirms the diagnosis. \textsuperscript{87} Other immuno-histochemical markers that label this neoplasm include vimentin, desmin, actin and endothelial markers such as Factor V11. \textsuperscript{88}

**Cytological findings: angiomyolipoma:**

- Interlacing complex of smooth muscle, fat and blood vessels.
- Immunocytochemical stain for confirmation: HMB-45.

**Diagnostic pitfalls angiomyolipoma:**

- Smooth muscle may dominate smears and can demonstrate atypia.
- Solid epithelioid areas may be dominant, can produce significant atypia and can lead to false positive interpretations.

**Haemangiomas:**

Haemangiomas constitute the most common benign neoplasm of the liver. They occur in all ages and both genders. These mass lesions are generally small and asymptomatic, but can occasionally be large (>5cm) and cause symptoms. Although radiological diagnosis has improved with enhanced imaging techniques, most are found incidentally during work-up for other conditions, including staging of malignancy, and can be
difficult to distinguish from a metastasis radiologically.\textsuperscript{89,90} Cytologically, FNA smears are frequently considered unsatisfactory or non-diagnostic due to either the aspiration of blood only, or the presence of nonspecific appearing connective tissue. It is this loose, rather than dense fibrous type connective tissue and smooth muscle fragments associated with blood and few to no background hepatocytes that should alert the pathologist to the diagnosis of haemangioma in the proper clinical setting. Cell block preparation of a core needle biopsy is crucial in making a specific diagnosis.

**Cytological findings; haemangioma:**

- Bloody, scantily cellular smears.
- Coils of loose connective tissue and smooth muscle.
- Generally few to no hepatocytes.
- Smears commonly non-diagnostic; specific diagnosis dependent on cell block.

**Hepatobiliary cystadenoma:**

This benign cystic tumor is a solitary, multiloculated cystic neoplasm of the liver that is histologically similar to cysts found in the pancreas and ovary. Women are almost exclusively affected. These neoplasms can become very large and patients usually present with abdominal pain. The neoplasm does not communicate with the biliary system. The locules are
filled with fluid that varies from thin and clear to bloody and turbid. The cyst lining is mucinous but the cells can become attenuated or denuded from fluid pressure. The characteristic subepithelial ovarian type stroma is not typically sampled on FNA which has been rarely reported. The cytology is similar to mucinous cystic neoplasm of the pancreas, often scantily cellular and nondiagnostic on its own. Cyst fluid with foamy histiocytes and mucinous epithelium, even if very scant, are sufficient for diagnosis in the appropriate clinical setting. Recognition of the malignant counterpart, hepatobiliary cystadenocarcinoma, is possible if the cytology is overtly malignant, but less than malignant cytology does not exclude malignancy and, as such, the treatment of choice is total resection.

Cytological finding: hepatobiliary cystadenoma:

- Thin to mucoid cyst fluid.
- Foamy histiocytes.
- Benign appearing mucinous glandular cells.
- Sub-epithelial ovarian type stroma is not aspirated.
- Generally few to no hepatocytes.

Benign hepatocytic nodules or masses:

Benign hepatocytic mass-forming proliferations, including dysplastic nodule (DN), focal nodular hyperplasia (FNH) and hepatocellular
adenoma (HCA), share in common many cytological features and distinction between them on smear cytology alone is difficult if not impossible.\textsuperscript{93} As such, the cytological features of these lesions will be discussed together. In addition, all of these lesions share in common the same differential diagnosis, namely well-differentiated hepatocellular carcinoma.

Taking into consideration the clinical and radiological presentation of the patient in conjunction with the cytohistology will typically lead to the correct interpretation.

Dysplastic nodule is a diagnostic consideration in the clinical setting of a mass in a cirrhotic liver clinically suspicious for hepatocellular carcinoma. The nomenclature of dysplastic nodules has changed over the past decade and now is a term used for macroregenerative nodule, adenomatous hyperplasia and other terms used to describe nodules in the liver that are grossly and histologically larger than the surrounding cirrhotic nodules [usually\textup{1-3cm}].\textsuperscript{94} Dysplastic nodules are sub-classified as low-grade dysplasia (macroregenerative nodule) and high-grade dysplasia (small cell dysplasia), high-grade dysplasia.
Table 8.2 Features typical of benign hepatic nodules versus well-differentiated hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Benign hepatic nodules</th>
<th>HCA</th>
<th>FNH</th>
<th>WDHCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Males and Females</td>
<td>Female &gt; males</td>
<td>Females &gt; males</td>
<td>Males &gt; females</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Adults</td>
<td>15 – 45 years</td>
<td>All ages, mostly adult women</td>
<td>Adults</td>
</tr>
<tr>
<td><strong>Cirrhosis</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Oral contraceptive use; glycogen storage diseases</td>
<td>Hepatic haemangiomas</td>
<td>Alcohol abuse, viral hepatitis</td>
<td></td>
</tr>
<tr>
<td><strong>Radiology</strong></td>
<td><strong>Nodule size</strong></td>
<td>0.8 – 3 cm nodules</td>
<td>5-15cm, solitary mass</td>
<td>&lt; 5 cm mass, stellate scar</td>
</tr>
<tr>
<td><strong>Serology</strong></td>
<td><strong>AFP</strong></td>
<td>Not elevated</td>
<td>Not elevated</td>
<td>Not elevated</td>
</tr>
<tr>
<td><strong>Cytology</strong></td>
<td><strong>Hepatocytes</strong></td>
<td>Irregular shaped clusters without peripheral transgressing endothelial cells; single cells; bland appearing large hepatocytes ± cytoplasmic glycogen and/or fat; round regular nuclear without prominent nucleoli; scattered large dysplastic cells.</td>
<td>Peripherally wrapping and transgressing vessels; monomorphic small hepatocytes nucleoli, often macro; hyaline globules.</td>
<td></td>
</tr>
<tr>
<td><strong>Bile ducts</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Ancillary tests</strong></td>
<td><strong>Reticulin</strong></td>
<td>Present, highlights normal 1-2 cell thick hepatic plates</td>
<td>Decreased to absent; highlights &gt;3 cell thick plates when present</td>
<td></td>
</tr>
<tr>
<td><strong>AFP</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Present (30-40%)</td>
</tr>
<tr>
<td><strong>Glypican-3</strong></td>
<td>Negative</td>
<td>Negative (focal weak positive)</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>CD34</strong></td>
<td>Negative</td>
<td>Positive periportal/septal</td>
<td>Negative</td>
<td>Positive diffuse</td>
</tr>
</tbody>
</table>

Photo 2: Focal nodular hyperplasia hepatocytes bile ducts and fibrous tissue in a
disarray characterize this by endothelial cells. Proliferating or arborising
vessels (transgressing endothelial cells) are focal at best reactive and
proliferative.

Photo 3: Hepatocellular adenoma. Medium sized vessels punctuating hepatic
parenchyma without bile duct epithelial cells characterizes this benign
neoplasm from other benign, hepatocytes nodules (cell block, H&E).
Or in irregularly shaped, jagged edged clusters without associated peripherally wrapping endothelial cells proliferating or arborisig vessels (transgressing endothelial cells are focal at best. 95-97

Bile duct epithelial cells may also be present in FNH or by contamination from the edges of other nodules, and their presence should greatly raise one's threshold for a malignant diagnosis.

Cellular features of reactivity include an apparent hepatocytes pleomorphism rather than monomorphism, an increased number of binucleated cells which tend to be decreased in hepatocellular carcinoma, a relatively low nuclear to cytoplasmic ratio, smooth nuclear membranes and prominent but not large nucleoli. Large cell change seen in low grade dysplastic nodules are dysplastic hepatocytes recognised by their enlarged atypical nuclei and sporadic placement in a background of otherwise typically reactive appearing hepatocytes yielding a polymorphous and pleomorphic smear pattern.

Small cell dysplasia present in high-grade dysplastic nodules is extremely difficult to distinguish from the monomorphism of well-differentiated hepatocellular carcinoma but should be considered with small high N/C ratio hepatocytes-without-an abnormal vascular pattern of peripherally wrapping endothelial - cells or transgressing endothelial cells. 93
Due to the overlap in the components of these various benign entities on smear cytology, cell blocks as well as radiological and clinical correlation are crucial in making a specific diagnosis- The smallest fragment of tissue may be all that is necessary to render a specific diagnosis. This readily available tissue may be used for the few ancillary tests that can aid in the-benign versus malignant differential diagnosis.

**Ancillary tests:**

Cell block preparations of small tissue fragments or CNBs currently offer the best method of distinguishing between benign and malignant hepatocytic proliferations. Not only is the architecture available, but tissue is readily available for ancillary studies. The reticulin stain demonstrates a maintained 1-2 cell hepatic plate framework in all benign lesions even if in disarray and the sinusoids are not apparent\textsuperscript{98}. Alpha-fetoprotein (AFP) staining is only focally and weakly positive at best in benign hepatocytic proliferations, but a negative stain does not exclude HCC. Serum levels of AFP >400ng/mL are highly associated with the presence of HCC.\textsuperscript{87} The immunostain hepatocyte paraffin 1 (HepPar 1)\textsuperscript{99} does not distinguish between benign and malignant hepatocytes. Glypican-3, (GPC3), is an oncofetal protein shown to be over-expressed in HCC and can be detected in the serum and tissue of patients with HCC but not benign hepatic lesions, with the rare exception of weak focal
staining in HCA. Studies with immunohistochemistry staining with anti-GPC3 antibody, have indicated great promise in the distinction between benign and malignant hepatocytes on histological sections, and on cytological material. Immunostains that attempt to highlight capillarisation of the sinusoids such as CD34 factor VIII and laminin can also be helpful. CD34 can show significant sinusoidal positivity in HCA, and this can present a pitfall in the interpretation of small tissue samples in cell block.

Molecular studies and image analysis studies have attempted to discriminate between benign and malignant hepatocytes. Flow cytometry has shown promise, but there is still, significant overlap in the ploidy patterns of benign and malignant processes. Proliferating cell nuclear antigen (PCNA) appears to have a better specificity in separating the two entities, however, there is still overlap in the PCNA positivity of small cell liver dysplasia and grades I and II hepatocellular carcinoma. These methods, as well as polymerase chain reaction, FISH, albumin messenger RNA, and a combination of parameters continue to be investigated.

**Cytological findings:** benign hepatocytic nodules [DN, FNH, HCA]

- Hepatocytes in jagged irregular clusters and singly.
- No peripherally wrapping endothelial cells.
• Clusters may have focal transgressing endothelial cells.

• Mild pleomorphism of cell and nuclear size; sporadically placed large atypical cells (dysplastic hepatocyte large cell change).

• Many binucleated hepatocytes.

• Variably prominent nucleoli but no macroeosinophilic nucleoli.

• Cytoplasm is generally abundant and granular but may show fatty change, lipofuscin pigment or iron deposition.

• Reticulin stain will show retained 1-2 cell layer framework on cell block.

**Diagnostic pitfalls benign hepatocytic nodules (DN, FNH, HCA)**

• Marked steatosis may result in false negative reticulin stain.

• AFP and glypican 3 are negative; weak focal staining may be noted.

• CD34 generally does not stain sinusoids; focal strong staining in HCA.

**Hepatocellular carcinoma:**

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and its incidence is increasing due to the widespread prevalence of hepatitis B and C virus infections, especially in Europe and the USA. HCC almost always develops in the setting of cirrhosis, most often secondary to alcohol but with an incidence about 15% in patients with hepatitis C. The risk in patients with hepatitis C increases
with concomitant metabolic injury from alcohol, obesity, diabetes and hepatitis B virus. The prognosis of patients is poor for large symptomatic lesions, so early detection is key to long-term survival. Early detection of small, non-vascularly invasive tumours can improve survival from <10% to >50%. In that regard, high-risk patients are routinely screened for suspicious nodules (≈ 1 cm) and FNA with CNB is typically used for diagnosis. Treatment options include liver transplantation for good surgical candidates, and percutaneous ablation for non-surgical candidates. Percutaneous ablation techniques include ethanol injection and thermal ablation using radiofrequency, laser or microwave energy.

The cytohistological diagnosis of hepatocellular carcinoma falls into two main categories; low-grade (well-differentiated) and high-grade (moderately and poorly differentiated) tumours.

**Well-differentiated hepatocellular carcinoma:**

Well-differentiated hepatocellular carcinoma is a tumour that looks hepatocytic but does not look obviously malignant. Given that the tumour cells of well-differentiated hepatocellular carcinoma are so similar to normal liver, the smear pattern proves to be a critical feature in evaluating this tumours.
The three basic smear patterns are:

(1) The cohesive, nested and trabecular pattern with peripherally wrapping endothelial cells.

(2) Loosely cohesive sheets with transgressing endothelial cells, or vessels.

(3) The dispersed small cluster-single cell pattern, without a recognizable vascular pattern.

The most specific pattern is the peripherally wrapping endothelial pattern.\textsuperscript{95,96} This pattern is one in which the endothelial cells of the sinusoids wrap around smooth edged, rounded nests and thickened hepatic trabecular. The endothelial cell nuclei may not be apparent in every plane of focus, but the presence of even one or two nuclei at the edge is sufficient. Although this pattern is only found in less than half of tumours,\textsuperscript{95,96} when present, it has been found to be very specific for the diagnosis of hepatocellular carcinoma and proves to be one of the most important diagnostic clues in separating reactive non-neoplastic and benign neoplastic proliferations from well-differentiated hepatocellular carcinoma.\textsuperscript{96,97}

The other pattern of endothelial proliferation has been termed transgressing,\textsuperscript{96} arborising or central.\textsuperscript{95} A complex network of small vessels is present in a loosely cohesive sheet of hepatocytes. The
appearance is similar to proliferating capillaries in other processes, such as granulation tissue, suggesting that the endothelial cells are associated with basement membranes, a feature of abnormal sinusoids as in hepatocellular carcinoma. This pattern is not as specific for hepatocellular carcinoma as the peripherally wrapping endothelial pattern, but is highly associated with the presence of hepatocellular carcinoma. It is rarely seen in cases of cirrhosis and hepatitis.

The dispersed small cluster-single cell pattern is completely non-specific and may be seen in all types of hepatocellular proliferations. The smear pattern will not be helpful in all cases and other features need to be assessed.

Individual cellular features that support a malignant diagnosis include the presence of cellular monotony with a uniformly elevated nuclear to, cytoplasmic ratio, e.g. all of the cells appear to have the same degree of atypia one to the other (Macroeosinophilic nucleoli and intracytoplasmic hyaline globules).

Cell blocks of small tissue fragments and CNBs provide help in assessing the hepatic plate architecture and capillarisation of the sinusoids, either on routine H&E stain or with ancillary tests if necessary.
**Photo 4:** Well-differentiated hepatocellular carcinoma. (A) The peripherally wrapping endothelial cell pattern demonstrates the capillarised endothelial cells of sinusoid wrapping around smooth edged, with nests and thickened hepatic trabeculae of hepatocytes (smear, PAP). (B) This pathognomonic feature is also demonstrated in cell block preparations (H&E).
Moderately to poorly differentiated hepatocellular carcinoma:

High-grade hepatocellular carcinoma displays features of obvious malignancy, for example smears of high cellularity with cellular crowding and nuclear overlapping, nuclear membrane abnormalities, hyperchromasia and macronucleus, which are many of the same features used to assess malignancy in a histological preparation. Moderately differentiated hepatocellular carcinoma is readily recognised as malignant by the nuclear atypia and there is some evidence of hepatic differentiation, such as abundant granular cytoplasm. Poorly differentiated hepatocellular carcinoma is an obviously malignant tumour with little to no hepatic resemblance and as such difficult to distinguish from any other poorly differentiated tumour.

Photo 5: Moderately-differentiated hepatocellular carcinoma. This high grade hepatocellular carcinoma maintains some hepatic preservation while displaying obvious malignant features smear.
Smear patterns are equally important in the diagnosis of high-grade HCC, especially the peripherally wrapping endothelial pattern. The presence of peripherally wrapping endothelial cells around the smooth edged nests and trabeculae of cells supports the diagnosis of HCC. The peripheral pattern is not a feature of metastatic renal cell carcinoma, a morphological mimicker of hepatocellular carcinoma, but the transgressing pattern is the most common pattern of that tumour both in the kidney and in metastatic deposits.\textsuperscript{122}

Although present in less than half of cases, the presence of bile production by malignant tumour cells is a relatively pathognomonic finding for the diagnosis of HCC.\textsuperscript{95,123}

A Hall's stain can be used to confirm the nature of the pigment as bile. The presence of intracytoplasmic mucin generally excludes hepatocellular carcinoma (except in the rare case of a combined hepatocellular-cholangiocarcinoma) and its presence should focus the differential diagnosis on adenocarcinoma.

**Hepatocellular carcinoma variants:**

Fibro-lamellar variant is a variant of hepatocellular carcinoma that generally occurs in young patients as a solitary mass in a non-cirrhotic liver that lends itself more readily to excision, thereby improving the prognostic outlook.\textsuperscript{124} It is a tumour associated with dense broad fibrous
bands and large malignant hepatocytes with an abundance of dense oxyphilic cytoplasm. The presence of abundant dense oxyphilic type cytoplasm often-with intracytoplasmic pale bodies, a deceptively low nuclear to cytoplasmic ratio and intranuclear inclusions are the key characteristic features on FNA cytology. Due to the dense fibrous stromal component of this tumour, smears may be paucicellular and malignant hepatocytes may be individual, single, and widely scattered. Peripherally wrapping endothelium is not a feature of this tumour, but transgressing endothelium has been observed.\textsuperscript{125}

The acinar cell variant is a morphological variant that presents a diagnostic challenge on cell block preparations. The frequently "back to back' acini give the appearance of an adenocarcinoma. There should be no mucin in the lumen of the acini or within the cytoplasm of the cells, and the acini should be separated by similarly malignant cells with no true cribriform architecture. Immunohistochemical stains will be helpful in establishing the correct diagnosis.

The clear cell variant is characterised by the abundance of intracytoplasmic fat and/or glycogen and introduces clear cell tumours metastatic from other sites into the differential diagnosis, especially from the kidney, morphologically, the clear malignant hepatocytes are large polygonal cells with central nuclei, large nucleoli and abundant, clear,
vacuolated cytoplasm. These neoplasms can be impossible to distinguish from clear cell renal cell carcinoma. As mentioned above, the presence of the peripherally wrapping endothelial pattern is a finding that excludes renal cell carcinoma, but the transgressing pattern is not. Immunostains are usually required for diagnosis.

**Ancillary studies:**

As mentioned above under 'Benign hepatocytic nodules', one of the most helpful ancillary tests in the diagnosis of well-differentiated hepatocellular carcinoma includes the use of the reticulum stain.\(^97\,98\,126\,18\)

The reticulum stain can be used on either smears or cell block preparations. An abnormal reticulum staining pattern, usually in the form of an absence of reticulum staining, is highly associated with the presence of hepatocellular carcinoma.\(^98\,126\) Small, very early well-differentiated HCC may retain the reticulum staining pattern and cannot reliably be distinguished from small cell dysplasia.\(^127\) Another pitfall in the interpretation of the reticulum stain is the presence of marked steatosis that can cause a false negative result.\(^98\) Positive staining with GPC3 also supports malignant over benign hepatocytes. An iron stain such as the Prussian blue stain can highlight the normal, haemosiderin laden hepatocytes bright blue leaving malignant hepatocytes that have lost their ability to retain iron unstained.
The basic immunocytochemical panel in the differential diagnosis of hepatocellular carcinoma and metastatic carcinoma, includes anti-hepatocyte antibody HepPar-1 that gives a strong diffuse chunky staining pattern\textsuperscript{123-130} MOC-31,\textsuperscript{131-133} GPC3,\textsuperscript{105,106} AFP, low (CAM 5.2) and high (AE1) molecular weight (MW) cytokeratin (CK), poly-clonal carcinoembryonic' antigen (CEA) and neprilysin (CD 10) staining, the latter two producing a canalicular staining pattern.\textsuperscript{134-136} The hepatocytes markers Hep-Par 1 and GPC3 do not have 100% sensitivity or specificity for the diagnosis of malignancy of hepatic origin, so these markers should be used in a panel of markers to support a the diagnosis of HCC. The endothelial cell marker CD34 stains the sinusoids diffusely indicating capillarisation of the sinusoids and supporting the diagnosis of hepatocellular carcinoma. AFP staining is helpful if positive, but a negative stain does not rule out HCC as only about 40% of HCC are associated with a positive stain.\textsuperscript{137} One must also keep in mind that AFP may occasionally be positive in reactive processes,\textsuperscript{138} so a positive stain does not in itself diagnose malignancy. As mentioned earlier, serum levels of AFP >400ng/mL are strongly associated with the presence of hepatocellular carcinoma, but not all tumours are associated with elevated levels, particularly the fibrolamellar variant.\textsuperscript{124}
**Cytological finding's: well-differentiated hepatocellular carcinoma:**

- Low-power smear pattern with smooth-edged clusters and thickened trabeculae with peripherally wrapping endothelial cells (pathognomonic).
- Low-power smear pattern with more than focal loosely cohesive sheets of hepatocytes with transgressing vessels (highly suspicious finding).
- Monotonous, uniform hepatocytic cell population with subtle malignant features.
- Acinar formation in cell clusters.
- Increased nuclear to cytoplasmic ratio compared to normal hepatocytes.
- Macroeosinophilic nucleoli.
- Reduced number of nucleated cells.
- Background free of bile duct epithelial cells.
- Reticulum stain demonstrates a loss of the normal 1-2 cell thick hepatic plate architecture.
- Iron stain fails to stain tumour in cases of haemachromatosis.
- Positive glypican-3 immunostaining; AFP.

**Cytological findings moderately to poorly differentiated hepatocellular carcinoma:**

- Peripherally wrapping endothelial smear pattern is virtually pathognomonic.
- Transgressing vessels are suggestive but cannot distinguish hepatocellular from renal cell carcinoma.

- Presence of intra-cytoplasmic bile is pathognomonic.

- Polygonal cells with central nuclei and prominent nucleoli.

- with visible, granular to clear cytoplasm in moderately differentiated tumours; scant to no cytoplasm in poorly differentiated tumour.

- Immunopheno type: low MW CK (Cam 5.2), polyclonal CEA, and CD10 (canalicular) HepPar-1 positive; AFP variable; high MWCK(AE1) negative.

**Cytological finding variants:**

*Fibrolamellar hepatocellular carcinoma*

- Population of large hepatocytes singly and in loose clusters.

- Smears may be paucicellular due to fibrosis.

- Transgressing vessels may be seen, but no peripherally wrapping endothelial cells.

- Deceptively low nudearto cytoplasmic ratio.

- Large, variably atypical nuclei with prominent nucleoli and frequent intranuclear inclusions.

- Cytoplasm is characteristically abundant and oncocytic appearing.
**Acinar variant:**

- Back-to-back acini/rosettes of hepatocytes.
- No mucin production.

**Clear cell Variant:**

- Cells with abundant vacuolated, clear cytoplasmic filled with glycogen and/or fat.

**Hepatoblastoma:**

Hepatoblastoma is the most common tumour of children with 75% occurring in males and 90% occurring before the age of 5 years.\(^{69}\) There is a strong association with familial adenomatous polyposis syndrome and Beckwith-Wiedemann syndrome.\(^{133}\) Patients generally present with an enlarging, often palpable mass that is usually single, lobulated and bulky. Serum AFP levels are almost always elevated and serve as a useful marker for recurrence and metastases.\(^{59}\)

Histologically, this tumour recapitulates the developing liver and may contain heterologous mesenchymal or epithelial elements.

Hepatoblastoma are classified as either epithelial or mixed epithelial-mesenchymal. The epithelial type consists of either immature embryonal and/or fetal hepatic epithelial cells; the mixed type typically contains both embryonal and foetal epithelial cells admixed with spindle cell mesen
chyme. Given this heterogeneity, the FNA smears of this tumour can have a varied appearance. Smears of both types of hepato-blastoma are dominated by the epithelial cells. The fetal type cell resembles the normal hepatocyte but is generally smaller.

The nuclei are central, round and bland appearing and the cytoplasm may contain fat, and glycogen. Unlike the embryonal cell type. The embryonic cell is more primitive and undifferentiated with hyper chromatic nuclei and scant cytoplasm that may form rosettes and trabeculae. Rarely the epithelial cells appear undifferentiated or anaplastic and resemble other small round blue cell tumours of childhood and are impossible to distinguish from neuroblastoma or Wilm's tumour on morphology alone.

The mesenchymal component, if present, presents as cellular spindle cell type mesenchyme, but heterologous elements may also be present including osteoid, cartilage, skeletal muscle and, particularly, extra-medullary haematopoiesis. Amyloid matrix has been described. The primary differential diagnosis rests most frequently between the fetal type Hepatoblastoma and hepatocellular carcinoma, and embryonal or anaplastic hepatoblastoma and other paediatric small round blue cell tumours.
Photo 6: Hepatoblastoma (A) The foetal type is composed of cells that resemble the normal hepatocyte but are generally smaller with central round and bland nucleoli (smear, diff-quik) (B) The embryonal type is composed of more primitive cells with hyperchromatic nucleoli and scant cytoplasm (smear, PAP)
Ancillary tests:

Ancillary studies and clinic-pathological correlation are required. Hepatoblastoma will stain with high-molecular weight cytokeratin,\textsuperscript{146} whereas tumour cells of most hepatocellular carcinomas do not.\textsuperscript{147} Both tumours stain with low-molecular-weight cytokeratin (CAM 5.2).\textsuperscript{146} Polydonal CEA. Stains hepatoblastomas with a variable and inconsistent canalicular and/or cytoplasmic pattern depending on the type of hepatoblastoma.\textsuperscript{145} This stain will not distinguish hepatoblastoma from hepatocellular carcinoma but can help in the distinction between a primary and metastatic tumours. HepPar-1 positivity supports a hepatic primary over other small round blue cell tumours of non-hepatic origin,\textsuperscript{128-130-148}

Cytological finding hepatoblastoma:

- Epithelial dominant smears.
- Mesenchymal (spindle cell) component and/or heterologous elements, especially extramedullary haemopoesis and osteoid, relatively scant.
- Epithelial cells are either small hepatocytic (fetal type), smaller immature, pleomorphic cells (embryonal type) or smaller still undifferentiated blue cells (anaplastic type).
- Epithelial cells form cohesive, crowded clusters, cords, ribbons or rosettes
• Immunophenotype: positive for low- and high-MW CK (CAM 5.2 and AE1), pCEA with variable canalicular/cytoplasmic staining, HepPar-1.

**Cholangiocarcinoma:**

Cholangiocarcinoma is an adenocarcinoma of the bile ducts that occurs predominantly in the non cirrhotic liver of the elderly population, most commonly of South-east Asia due to the high infestation with the liver fluke. Patients with primary sclerosing cholangitis (PSC) and cirrhosis due to hepatitis C are also at increased risk. These neoplasms are classified by their location as peripheral (intrahepatic), hilar (Klatskin tumour), extra-hepatic and intraductal types- All but the intraductal type form a firm, white-tan mass that can become quite large when intrahepatic, but is usually small in the hilar or extra-hepatic locations due to obstruction causing early detection. FNA is used to sample obvious masses while brush cytology is used to sample strictured or thickened ducts. EUS-FNA has been shown to add value to the diagnosis of hilar and extra-hepatic cholangiocarcinomas. While the principal criteria for the diagnosis of adenocarcinoma on FNA smears are relatively standard regardless of location, the interpretation of bile duct brushings requires more stringent set of criteria and a higher threshold for the interpretation of malignancy. This is due to the difficulty in obtaining a specimen of quality and quantity sufficient for a confident malignant diagnosis.
Sclerosis and inflammation inherent to the tumour or secondary to an underlying condition (PSC, stent placement, etc.) contribute to a paucicellular sample and preparation artifact from obscuring blood, crush and air drying artifact limit optima Interpretation. Processing bile duct brushings in a liquid-based medium has shown improvement in diagnostic sensitivity and specificity.

The most common histological appearance of cholangiocarcinoma is a low-grade, well- to moderately differentiated adenocarcinoma forming tubules and cribriform glands infiltrating a sclerotic stroma. FNA smears generally produce a readily recognisable adenocarcinoma but with nonspecific features compared to those metastatic from the upper gastrointestinal tract and lung. Smears are variably cellular and demonstrate irregular, variably sized sheets of atypical to malignant appearing glandular cells that resemble bile duct epithelium.

Clusters of tumour cells may show cytoplasmic vacuolisation and focal mucin production. Cell block preparations are particularly helpful in this diagnosis because the characteristic common histological pattern described above may be recognised.

**Ancillary tests:**

A simple mucin stain demonstrating the production of mucin will define the neoplasm as an adenocarcinoma' and, with the rare exception of a
mixed Cholangiocarcinoma-hepatocellular carcinoma, exclude the diagnosis of hepatocellular carcinoma. The basic immunohistochemical panel discussed above under hepatocellular carcinoma will also help in this differential diagnosis. Distinguishing Cholangiocarcinoma from metastatic adenocarcinomas relies primarily on clinical history, but a panel of immunohistochemical markers may be helpful for specific tumours.

**Cytological finding Cholangiocarcinoma:**

- **Glandular cells in flat, angulated sheets:**
  - Low-grade malignant nuclei with nuclear crowding, overlapping, slightly irregular nuclear membrane, par chromatin clearing.
  - A range of atypia may be seen from borderline malignant appearing to obviously malignant looking.
  - Exaggerated honeycombed pattern from uneven nuclear distribution in the sheet.
  - Cell blocks can help by demonstrating sclerotic stroma and cribriforming architecture.
  - Mucin stains positive at least focally in many cases.
  - Immunophenotype: keratin 7+,19+;20-;cytoplasmic.
    - pCEA, LeuM1, B72.3+
**Angiosarcoma:**

Although extremely rare, angiosarcoma is the most common primary sarcoma of the liver occurring in older patients with a 3:1 ratio of men to women. These neoplasms are believed to be secondary to exposure to hepatotoxic agents including vinyl chloride, thorotrast, arsenic and anabolic steroids. The prognosis is poor. Histologically these neoplasms are single or multiple nodules of vascular channels lined by malignant endothelial cells. The malignant cells range from being widely spaced lining dilated sinusoidal channels to more solid growth filling the sinusoids and causing atrophy of the surrounding hepatocytes. An epithelioid appearance to the endothelial cells may also occur creating a pitfall and misdiagnosis of a carcinoma. FNA smears are bloody and may be paucicellular. Malignant endothelial cells can be seen interdigitating among reactive hepatocytes. They have elongated, spindled-shaped hyperchromatic nuclei that are easier to appreciate in small clusters than in large clusters where they tend to blend in with the hepatocytes. Tumour cells should stain positively for factor 8, CD31, CD34, or Ulex europaeus lectin.

**Cytological finding angiosarcoma:**

- Atypical to overtly malignant endothelial cells interspersed with hepatocyte clusters
- Spindle cell malignancy possibly with blood lakes.
- Immunocytochemistry stains: Factor VIII, CD31, CD34, ulex euroopaeus lectin.

**Embryonal, sarcoma:**

Embryonal sarcoma is a rare malignancy of children typically between 6 and 10 years of age patients present with abdominal pain or with an abdominal mass. Histologically these neoplasms are composed of a myxoid stroma embedded with spindled to stellate cells which produces FNA smears composed of large, anaplastic cells, multinucleated tumour giant cells and atypical spindle cells. Intracytoplasmic globules may be seen that are PAS positive but diastase resistant. Tumour cells also stain for vimentin, alpha-1-antitrypsin and alpha-1-antichymotrypsin.153-156.

**Cytological finding: embryonal sarcoma:**

- Hypercellular smears.
- Large, pleomorphic anaplastic cells with multi nucleated giant cells and atypical spindle cells.
- Intracytoplasmic globules that are PAS positive, diastase resistant.
Malignant neoplasms metastatic:

The vast majority of malignancies in the liver are metastases, and the distinction between a primary and metastatic malignancy is of both therapeutic and prognostic significance. Past-medical history is of vital importance as most patients have a known history of a primary malignancy elsewhere. Metastatic tumours tend to recapitulate their appearance in the primary organ, and specific tumour types such as small cell carcinoma and lymphoma generally maintain a consistent cytological appearance. Adenocarcinoma, although frequently recognisable as an entity, presents the most difficulty in making a specific diagnosis as to site of origin. Adenocarcinoma from the colon is the most common metastasis to the liver. The presence of an adenocarcinoma with a 'dirty necrosis' background is sufficient to diagnose adenocarcinoma consistent with colonic primary in a patient with a history of colonic cancer, and should direct the clinician to evaluate the colon first in a patient with an unknown primary. Other metastatic malignances commonly encountered in the liver include those from the pancreas (adenocarcinoma and neuroendocrine tumours), stomach (adenocarcinoma and gastrointestinal stromal tumours), breast, lung (adenocarcinoma, small cell carcinoma and much less commonly squamous cell carcinoma), skin (melanoma) and bladder. Less common but diagnostically more challenging are metastases from the kidney and adrenal gland due to the morphological
overlap with hepatocellular carcinoma. Sarcomas are the least encountered tumour type, the most common type being leiomyosarcoma, generally from the uterus, and it can be challenging but therapeutically important to distinguish metastatic GIST from leiomyosarcoma.\textsuperscript{158,159} Lymphoma may also be seen in the liver, most commonly secondary to systemic disease, but can be a primary malignancy. If lymphoma is suspected on rapid interpretation, request should be made for a dedicated FNA for flow cytometry analysis, e.g. one that is not expressed onto a slide, rather one in which the aspirated tissue is rinsed into either buffered normal saline, cytolyte solution or RPMI. The combination of cytological evaluation and flow cytometry immunophenotyping is very often sufficient for diagnosis and sub-classification of non-Hodgkin lymphoma.\textsuperscript{160-164} The cytological features helpful in the diagnosis of a variety of metastatic tumours are presented below with accompanying supportive ancillary tests and illustrations.

**Cytological finding adenocarcinoma (NOS):**

- Polygonal to columnar glandular cells arranged in flat monolayered sheets, three-dimensional clusters or singly.
- Lumens within clusters may be seen in some cases.
- Nuclei are variably atypical ranging from quite bland in low-grade tumours to extremely atypical and obviously malignant in high-grade tumours.
• Cytoplasm is delicate, frequently vacuolated, and sometimes wispy.

• Intracytoplasmic mucin may be seen; mucicarmine or other mucin stains can help identify focal mucin production.

**Cytological findings; colonic adenocarcinoma:**

• Cigar-shaped, often palisaded nuclei.

• Variably prominent nucleoli but not macroeosinophilic nucleoli.

• Dirty necrosis in the background (KEY).

• Immunocytochemistry: CK20+, CK7-, CEA+, CDX2.

**Cytological findings; breast carcinoma, ductal type:**

• Often low grade with a monomorphic cell population.

• Flat angulated groups.

• Single flame or cone-shaped cells.

• Target cells (cells with intracytoplasmic lumen).

• Cell-in-cell arrangement.

Immunocytochemistry; oestrogen/progesterone ± and supportive if positive, but non-specific, gross cystic disease protein-15 is supportive if positive.
Photo 7: Metastatic colonic carcinoma. (A) This adenocarcinoma is recognised on smears by the background of dirty necrosis, a non-specific but characteristic feature. Viable aggregates of carcinoma may be few (smear, PAP). (3) CI block shows malignant glands composed of tall, crowded columnar cells adjacent to cellular necrosis (HSE).
Cytological finding: squamous cell carcinoma:

- Relatively uncommon metastasis to the liver.
- Large polygonal cells singly and in clusters.
- Usually high grade with large, hyper chromatic nuclei with irregular nuclear membranes.
- Cytoplasm is dense and non-vacuolated as opposed to that of adenocarcinoma.
- Keratinising squamous cells stain orangophilic on Papanicolaou stain but this feature may not be present.

Cytological findings squamous cell carcinoma:

- Small pleomorphic blue cells with little to no cytoplasm in clusters and singly.
- Nuclei are hyperchromatic with coarse, stippled chromatin.
- Nuclear moulding is common and characteristic.
- Necrosis and apoptosis is common.
- Smear or crush artifact is invariably present due to the fragile nature of the cells.
Cytological findings: well differentiated neuroendocrine carcinoma
e.g. metastatic pancreatic endocrine neoplasm (PEN) and carcinoid
tumour:

• Small uniform blue cells with visible cytoplasm that tends to be scant
  and more evenly pronuclear in carcinoid tumours and more abundant
  and eccentric in PEN.

• Nuclei with coarse stippled chromatin, more obvious in carcinoids than
  PEN.

• Nucleoli generally not present in carcinoid tumours but visible in PEN

• No nuclear molding, much less crush artifact, and no significant
  necrosis/apoptosis compared with small cell undifferentiated carcinoma

• Immunocytochemistry: Keratin, synaptophysin and chromogranin
  positive.

Cytological finding large cell lymphoma:

• Mostly diffuse large B cell lymphoma,-predominantly secondary but
  can be primary.

• Discohesive, single cell population; may have pseudogroups, e.g.
  artifactual clustering.

• Lymph glandular bodies (clumps of stripped cytoplasm) in the
  background.
• Coarse, frequently peripherally dumped chromatin.

• Nucleoli may be present.

• Cytoplasm is scant to invisible, but may be abundant in anaplastic large cell lymphoma.

• Immunocytochemistry: leukocyte common antigen, CD20, CD19.

Cytological finding melanoma:

• Large polygonal cells singly and in clusters; may also be spindled or small blue cells with scant cytoplasm.

• Central to eccentric nuclei with large nucleoli.

• Intranuclear inclusions common.

• Cytoplasm is commonly abundant, non-granular and frequently non-granular and frequency non-pigmented.

• Immunocytochemistry: S100, HMB-45, Mart-1 and Melan-A positive; keratin negative.

Diagnostic pitfall melanoma:

Fontana-Masson stain will stain cytoplasmic melanin pigment black but will also stain lipofuscin pigment.

Cytological finding renal cell carcinoma (RCC):

• Large polygonal cells singly and in clusters.
• Transgressing endothelial pattern the most common vascular pattern, but peripherally wrapping endothelial pattern is not a feature.

• Round central nuclei with prominent macro nucleoli ('owl's eye') in typical clear/granular cell type; papillary RCC type does not demonstrate prominent nucleoli.

• Intranuclear inclusions can be seen and are frequent in chromophobe type.

• Cytoplasm is commonly abundant and clear or granular; excessive and 'balloon- like' in chromophobe RCC and scant, often with haemosiderin in papillary RCC.

• Immunocytochemistry: keratin, vimentin and EMA positive;

• CEA negative.

**Cytological finding adrenal carcinoma:**

• Medium-sized polygonal cells singly and in clusters.

• No transgressing endothelial pattern but peripherally wrapping endothelial pattern may be seen on cell block.

• Nuclei are variably atypical with hyperchromasia and pleomorphic nucleoli which do not tend to be macroeosinophilic as in hepatocellular carcinoma.
Cytological findings in gastrointestinal stromal tumor (GIST):

- Prominent vascular pattern.
- Relatively bland nuclei without overt hyperchromasia or pleomorphism.
- Delicate cytoplasmic processes.
- Little to no crush artifact.
- Immunophenotype: c-kit and CD34 positive; smooth muscle actin variably positive; desmin negative.

Cytological findings: leiomyosarcoma:

- Pleomorphic spindle cells in tightly cohesive three-dimensional groups and syncytia; occasional epithelioid features.
- No prominent vascular pattern.
- Hyperchromatic, pleomorphic nuclei with blunted nuclear ends.
- Cytoplasm is more abundant than GIST.
- Immunophenotype: desmin and smooth muscle actin positive; c-kit negative.

Role of liver FNA in the management of liver lesions:

Accurate diagnosis of focal mass lesions in the liver is essential for proper patient management. Assuming that a nodule in the liver in a patient with a known extra hepatic malignancy represents metastatic
disease can lead to patient mismanagement and over-treatment. Even with classic clinical and radiological evidence of metastatic disease from a known primary malignancy, confirmation with tissue diagnosis is essential for patient enrollment in research protocols and for patients to qualify as candidates for new therapies such as ethanol ablation and targeted gene therapy. Infections and lymphomas are treated non-surgically and prompt diagnosis with FNA can expedite appropriate patient triage and treatment.

FNA is the diagnostic procedure of choice for focal liver lesions. When performed by experienced interventional radiologists and interpreted by experienced pathologists, the accuracy rivals that of frozen section. In institutions with considerable experience, the sensitivity of liver FNAB is as high as 90% with specificity approaching 100%. Sampling error is the reason for most false negative results that are most often due to inexact needle localisation. As such, concomitant core biopsy improves accuracy, specificity and sensitivity, and both are better than either alone. The distinction between a primary and metastatic malignancy is also important for proper patient care. Access to the patient's past medical history is of vital importance as most patients have a known history of a primary malignancy elsewhere. Metastatic colon cancer should be high in the differential diagnosis with or without a known primary colon cancer as it is the most common malignancy in the
liver. Recognition of the typical smear pattern of an adenocarcinoma with palisading columnar cells in a background of dirty necrosis is usually sufficient to support the diagnosis in patients with a known history or to suggest clinical evaluation of the colon in patients without a known history. Familiarity of the typical appearance of extra-hepatic malignancies is of benefit as most metastases recapitulate their appearance in the primary organ, and specific tumour types such as small cell carcinoma and lymphoma generally maintain a consistent cytological appearance.

A focal mass lesion in a cirrhotic liver places HCC at the top of the differential diagnosis and prepares the pathologist for the systematic smear evaluation. If not already a routine practice, procurement of tissue for cell block should be requested of the radiologist in anticipation of ancillary studies.

1.2.2. Imaging of the liver:

A. ultrasound:

It is now established as the primary imaging Investigation in hepatobiliary disease particularly in the jaundiced patient. It is useful for determining bile duct dilatation, the presence of gallstones and presence of liver tumors. Also it helps to verify cystic or solid lesions.\textsuperscript{170}

Beyond doubt that ultrasound can also be used to guide the placement of the needle in aspiration cytology or liver biopsy.
Photo 8: Ultrasound scan of focal liver lesions
B. Computerized Tomography scan (C.T scan):

The triple phase spiral C.T provides fine details of liver lesions down to less than 1cm in diameter. Oral contrast enhancement allows visualization of the stomach and duodenum in relation to liver hilum, with intravenous contrast. The early arterial phase shows small tumors owing to their preferential arterial blood supply and the venous phase maps the branches of portal vein within the liver and the drainage via hepatic veins.

Photo 9: CT-Scan of Focal liver lesions.
C. Magnetic Resonance Imaging (MRI):

MRI though effective as C.T scan, but has the advantage in those patients where contrast C.T may not be used due to history of allergy to iodine may be offered MRI. Magnetic Resonance Chollangio Pancreatography (MRCP) can give excellent imaging of the biliary tree non – invasively. Magnetic resonance angiography (MRA) similarly provides high quality images of the hepatic artery and portal vein.

D. Percutaneous Trans Hepatic Cholangiopancreatography

(P T C) indicated where ERCP has failed or is impossible as in patient with polya type gastrectomy or patient with hilar bile duct tumor.170

E. Angiography

Angiography may provide useful information on the blood supply and architecture of the liver. This also may provide information about the nature of a liver nodule and has therapeutic implications as occlusion of arterio-venous malformation and embolization of liver tumor.

F. Laparoscopy and laparoscopic ultrasound:

This allows direct visualization of the liver and may detect other lesions not detected by C.T or MRI.170
1.3. OBJECTIVES

- To correlate between radiological and cytological pattern of focal liver lesion.

- To evaluate focal liver lesion presentation with regard to age group gender, clinical presentation and geographical distribution of patients.

- To determine the role of cytological diagnosis in verifying focal lesion of the liver.

- To determine and evaluate the role of diagnostic radiological technique in diagnosis of focal liver lesion.

- To evaluate role of ultrasound guided FNAC versus computed tomographic guided FNCA as means of guidance procedure.
Chapter 2

MATERIALS AND METHODS

2-1 study design:

This is a retrospective study to evaluate the role of fine needle aspiration cytology (FNAC) in diagnosis of focal liver lesions. FNAC as a diagnostic tool is radiologically guided either by ultrasound or CT-scan. The study was conducted at the Police Hospital and Khartoum Clinic [A joint committee between (Dr. Ahmed Omer pathologist and Dr. Najwa Dongla radiologist)], and the record of patients was from March 2007 to July 2009 at Khartoum state.

2-2: study population:

Sixty one Sudanese’s patients entered this study and were eligible for analysis. Patients were classified according to sex, age, type of radiological investigations for diagnosis and type of radiological tools used to guide FNAC.

The result of fine needle aspiration cytology were stated; labeled as benign or malignant. All mentioned were variable for analysis.

All these (61) patient have liver lesions detected by ultrasound or C.T scan. The aspirations were made in order to either confirm or rule out suspicion of primary or metastatic malignancy in the liver, based on
clinical finding, and supported by the presence of unifocal or multifocal liver lesions on ultrasound or C-T scan.

Informed consent was obtained from each patient when viewed for the initial aspiration.

2-3: Sample collections:

For these sixty one patients many sections taken from every patients with focal liver lesion for cytology, collected from patient using the ultrasound or C-T guided technique, so the entry is to the target lesion.

Photo 10: Equipment used for FNAC.
Photo 11: Procedure of FNAC
Photo 12: Pressing after procedure

Technique:

Specimens for cytologic examination were obtained by direct insertion of a long (0.8 mm, 80 mm) 22-gage needle guided by ultrasound, an attendant cytopathologist, and radiologist are present. The penetrating sites of the liver were subcostal.

After collection the attendant cytopathologist and operator at the same time verify the adequate number of cells of the expected type were present in the sample. In cases of unifocal lesions or multifocal one, demonstrated by imaging methods (with or without enlarged liver) the puncture sites and directions of the needle were directed towards the estimated site of the lesion.
Photo 13: Spreading and preparation of slides
Photo 14: Prepared slides
2.4: Sample processing: For cytology.

Procedure:

- Connection of the Gun with 10 ml syringe.

- Applying negative pressure while lifting & inserting the needle 3-4 times within the mass.

- Pulling-out of the Needle.

- Aspirated tissues are placed on slides.

- Smear preparation.
Fixation:

- Air-dried smears.
- Wet-alcohol fixed smears.

☐ Cell Block preparation.

Exclusion: None is excluded, and criteria for exclusions were the following:

1. A history of marked haemorrhagic tendency.

2. Problems with clotting profiles as:
   - Prolongation of either P-T (prothrombin time) or partial thromboplastin time (PTT).

3. Reduced platelets count.

The precautions were:

- Taking a full history, history of marked haemorrhagic tendency.

- Investigations that include:

  - Complete Blood Count (CBC) platelet count.
  
  - P.T.
  
  - P.T.T.
2-5: Assessment of the results:

Sections were examined first by investigator then by two cytopathologist, and they agree about the results, true positive for those with malignant focal liver lesions, true negative for those with benign disease (including abscess, TB, cyst etc.). Correlations of the results of radiography were labeled as unifocal or multifocal and further cytological analysis for verification of this result of radiography was done.

2-6: Ethical considerations:

The aims of methods and the results and how to get use of results were fully explained to the patient before embarking in aspiration and their consent is obtained.

2-7: Cytopathological interpretations:

Interoperation was done by reviewing of investigator finding by the two cytopathologist. Cytological findings were reported as no malignant cells seen, definitive malignancies were specified whenever possible. The clinical radiological and cytological data were compiled in each patient; in one case liver biopsy and immune stain were recommended to reach a final diagnosis.

In some cases cytologic findings are of use: malignant cells from other organs or from body fluids exhibiting malignant cells similar to
those obtained by FNAC of the liver. Beyond doubt, histological correlation cell block is ready to be prepared.

2-8. **Statistical analysis:**

To determine the sensitivity and specificity of cytological diagnosis, it is necessary to classify cytologic finding for each patient as either benign or malignant. For this purpose, patient with cytologic finding of no malignant or, genuine benign, and a typical reaction were classified as having benign cytological diagnosis, other with definite malignancy or suspicious were diagnosed as having malignancy. The cytological finding of FNA were categorized as true positive, true negative, false positive and false negative.

A cytological diagnosis was defined as true positive if a patient was with malignant cytologic diagnosis had cytologic diagnosis based on finding of malignant cell from other organ or body fluids exhibiting malignant cells similar to those obtained by FNAC of the liver and approved by radiography and cytology so do negative results.
Chapter 3

RESULTS

Table 1: describe gender of patients with focal liver lesion in this table the frequency for males 46(75.4 %) and for female 15 (26.4 %) in our study group hepatic focal lesions are more common in male patient.

Table 2: Age distribution in details for patient with focal liver lesion were between (51- 60) 22 patients 36.1 % followed in descending order by age (61 – 70) 10 patients 16.4% then (41 -50) 9 patients (14.8 %). In the group studied focal liver lesion are more common in middle age (40-60) and elderly subject.

Table 3: this table is for age of male with focal liver lesion (51-60) constitute 30.4% followed by (61-70) which represent 19.6%, so focal liver lesion are common in middle age and elderly.

Table 4: Concerning female patient with focal liver lesion the highest percentage was also as (51-60) 53%.

Figure 1: Describes residence of patient with focal liver lesion patient, were 32(52.5 %) for those from Khartoum and 29 (47.5%). for those from outside Khartoum were 13 from central Sudan, 5 from northern 5 from western,6 eastern Sudan and none from southern Sudan,
and their percentage were, 45%, 17 %, 17 %, 21% and 0%, respectively. As far as those coming out side Khartoum is concerned. In the study group there is an increase in incidence in those coming from central Sudan.

Figure 2: Describes radiography used to diagnose focal liver lesion. C.T scan and ultrasound are used. C.T is the much used radiography for detection of focal liver lesions (superior to ultrasonography) 40 patient (65%) C.T. 21 patients (35%) U/S was the diagnostic radiography to offer diagnosis of focal liver lesion.

Figure 3: showing result of radiography, that were unifocal lesion which constitute 57% and 43% for multifocal lesion.

Table 5: Show clinical presentation of patients with focal liver lesion the most common presentation is with hepatic mass which can be single or multiple.

Table 6 shows radiographic technique used to guide FNAC 57 patients 93.4 % underwent U/S guided aspiration, and four 4 patients 6.6% underwent CT guided aspiration. It is evident that ultrasound as a means of guidance of FNAC is superior to CT guidance.

Table 7: shows results of cytology benign aspiration 17(28%) for malignancy aspiration 44(72%).
Figure 5: showing verification of unifocal lesion. (60%) malignant (37%) benign and (3%) suspicious.

Figure 6: showing verification of malignancy in unifocal lesion by FNA. (71%) secondary malignancy (29%) primary malignancy.

Figure 7: showing verification of benign unifocal lesion by FNA (38.%) Liver abscess (23%) haemoagioma, (15%) cyst, (8%) bile duct hyperplasia (8%) chronic inflammatory (8%) benign with scattered atypical cells.

Figure 8: showing result of cytology of multifocal lesion by FNA (85%) malignant (15%) benign.

Figure 9: showing verification of malignancy in multifocal lesion by FNA. (77%) secondary malignancy (18%) primary (5%) malignant.

Figure 10: showing verification of benign multifocal lesion by FNA (25%) nodular hyperplasia (25%) haemoagioma, (25%) cyst, (25%) TB. This was proved by PCR.
Table 1  Gender distribution of patients with focal liver lesion

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>46</td>
<td>75.4</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>24.6</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
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</table>
Table 2: Age group for patient with focal liver lesion Number: 61

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
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<tr>
<td>0-10</td>
<td>2</td>
<td>3.3</td>
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<tr>
<td>11-20</td>
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<td>4.9</td>
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<tr>
<td>21-30</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
<td>9.8</td>
</tr>
<tr>
<td>41-50</td>
<td>9</td>
<td>14.8</td>
</tr>
<tr>
<td>51-60</td>
<td>22</td>
<td>36.1</td>
</tr>
<tr>
<td>61-70</td>
<td>10</td>
<td>16.4</td>
</tr>
<tr>
<td>71-80</td>
<td>5</td>
<td>8.2</td>
</tr>
<tr>
<td>81-90</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>61</strong></td>
<td><strong>100.0</strong></td>
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</table>
Table 3: Age group for male patients with a focal liver lesion.

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>11-20</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>21-30</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>8.7</td>
</tr>
<tr>
<td>41-50</td>
<td>7</td>
<td>15.2</td>
</tr>
<tr>
<td>51-60</td>
<td>14</td>
<td>30.4</td>
</tr>
<tr>
<td>61-70</td>
<td>9</td>
<td>19.6</td>
</tr>
<tr>
<td>71-80</td>
<td>4</td>
<td>8.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>46</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Table 4: Age group for female patients with a focal liver lesion.

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
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<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>31-40</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>13</td>
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<tr>
<td>51-60</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>61-70</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>71-80</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
<td><strong>100</strong></td>
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</table>
Table 5: Clinical presentations of patients with focal liver lesion.

<table>
<thead>
<tr>
<th>Clinical presentations</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic mass</td>
<td>32</td>
<td>52</td>
</tr>
<tr>
<td>hepatomegaly + Lymphadenophies</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal. mass and hepatomegaly</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Abdominal pain and hepatomegaly</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal distention and hepatomegaly</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Rt. sub costal subcutaneous mass</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Others (renal mass, ascites, pleural effusion, nipple discharge and peritoneal nodule)</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 6: Show residence of patients from outside Khartoum.

<table>
<thead>
<tr>
<th>Residence</th>
<th>Percent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Sudan</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>Western Sudan</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Eastern Sudan</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Northern Sudan</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>29</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Table 7: FNAC guided radiographic technique

<table>
<thead>
<tr>
<th>FNAC guided</th>
<th>Percent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>U/S guided</td>
<td>57</td>
<td>93.4</td>
</tr>
<tr>
<td>C.T scan guided</td>
<td>4</td>
<td>6.6</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 8: Cytology result of patients with focal liver lesions

<table>
<thead>
<tr>
<th>Cytological result</th>
<th>Percent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign aspiration</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>Malignancy</td>
<td>44</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 1:

Residence of patients with focal liver lesions
Figure 2:

Radiograph for technique used for diagnosis of focal liver lesions
Figure 3:

Results of radiographic technique used for diagnosis of focal liver lesions
Figure 4: Verifying of unifocal liver lesions by cytology
Figure 5:

Verification of malignancy in unifocal liver lesions by FNA.
Figure 6:

Verification of benign unifocal lesions by FNA.
Figure 7:

Results of cytology of multifocal lesions by FNA
Figure 8:

Verification of malignant multifocal liver lesions by FNA
Figure 9:

Verification of benign multifocal liver lesions by FNA
Chapter 4

DISCUSSION

FNAC is a procedure available for more than two decades. It is a very useful procedure for the diagnosis of various hepatic lesions. It offers accuracy without major complication and minimal interventions at low cost. The only absolute contraindications are marked haemorrhagic diathesis and suspected vascular lesion.\textsuperscript{171} No complications during this study were found. It is of interest to realize that FNAC of the liver guided by ultrasound or C.T. scan has proved to be a safe and an accurate method for diagnosis of focal hepatic lesions.

The main aim of the study is to evaluate the diagnosis of focal liver lesion by FNA guided by radiography.

In this study patients presented with different clinical features, some with right hypochondrial pain or tenderness other with abdominal pain. Weight loss is a common feature for patients. Examination in most patients revealed spectrum of hepatomegaly, nodular liver, hepatic mass, abdominal mass and some patients with ascites. The most important requirement for such cytodiagnosis is a representative sample from the lesion, and in our case the lesion is located by the guided radiography C.T or U/S, and the operator is expert, so no problem with a representative sample. Patients were subjected to ultrasonography guided FNA which
has been reported to be safe useful and accurate technique for making cytological diagnosis of hepatic masses.\(^{(172-173)}\) Accordingly to imaging scanning hepatic lesions were grouped as unifocal and multifocal, which represent 57% and 43% respectively. Images guidance of FNA yield representative sampling of lesions. Special Imaging scanning suggesting multifocal lesions. It is of interest to realize that, no detection of pathologic findings by imaging liver scans does not preclude the presence of malignancy.

In our study most of the patient came from Khartoum state 32 patients and 29 patients from outside Khartoum state distributed as 13 from central Sudan (area extending from Gezira, white Nile, and blue Nile).6 from eastern Sudan, 5 patients reside northern Sudan, 5 patients from western Sudan.

The patients age range from 1-82 year with male predominance. It was noticed that focal liver lesions are common in middle age group 40-60 years in both sexes, the incidence for male patient is more as compared with female, younger age group are not exempted from focal liver lesion. One case is diagnosed as hepatoblastoma, after examination of its cell block. This case came from Alnohood Western Kordofan, the same area for hepatocellular carcinoma.

The present study was conducted in order to establish the role of fine needle aspiration cytology as a reliable diagnostic method. The cytologic
diagnosis of malignancy was confirmed in 44 patients (72.0%), by matching the result of radiography and the feature suggestive of malignancy.

In addition, to this there is supported evidence in some patients examples matching with cytologic tumor cells obtained from other organs for instance a malignant renal and liver aspirate.

Morphologically, malignant lesions constituted 72% and benign lesions 28% for both uni and multifocal liver lesions. For unifocal lesions malignancy was found in 60% and benign lesions in 37% and 3% of cases were suspicious. For multifocal liver lesions 85% were malignant and 15% were benign. Secondary malignancies were more common than primary malignancy, constituting 71% and 77% for unifocal, multifocal liver lesions respectively.

Primary malignant liver tumours were 29% of unifocal and 18% of multifocal lesions. In one patient there was a need for liver biopsy and immunostain to arrive at a final diagnosis of malignancy.

In order to lower the rate of false negative cytologic findings, we suggest that FNA should be repeated in patient clinically suspected of having malignancy despite negative cytologic findings. Fortunately this pitfall is not found in our studied group. Negative cytologic findings 17(28.0%) were viewed with caution in order to rule out false negative rate that were zero in our study. In the group studied no complication of
the procedure is noted except pain, tenderness at the puncture site that
was not infrequent.

Under the umbrella of focal liver lesion whether unifocal or
multifocal many entities exist including benign and malignant condition

This study highlighted the role played by radiologist, pathologist and
clinician in verifying focal liver lesions.

Clinician in term of providing a full history with regard to the
patient history and a full clinical examination from head to heal. A
competent radiologist understanding patient clinical history and
examination and mastering his job diagnosed competently focal hepatic
lesion and gave advice regarding its verification. Also the radiologist help
in obtaining FNAC by the C.T or U/S. The collaboration between
clinician, radiologist, and pathologist opinion cut the way to diagnosis.
Feedback between the three help in improving the health services in our
beloved country, Sudan.

It is of interest to reflect the role of cell block although not included
in all cases in our study it is among the oldest method for preparing
material for microscopic examination. The method uses histologic
technique for processing and thus offer one major advantages, multiple
sections of the same material may be processed for special stains that may
serve for identification of mucin, melanin or other cell product. The cell
block technique should be used for processing all residential material
remaining after completion of cytologic preparation. This material often contains valuables diagnostic evidence and tissue fragment that cannot be processed by cytology, also this provide ability to perform multiple immunohistochemistry or other special stains if needed. So cell block should be employed to help verifying cytology.

It is a fact that immunohistochemistry has rapidly become an integral part of most diagnostic tool, which confirms histological results. This technique helps to differentiate primary hepatocellular carcinoma and metastatic carcinoma. Positive alpha fetoprotein, strongly support the diagnosis of primary hepatocellular carcinoma. For metastatic carcinoma following help with S100, vimentin, cytokeratin and epithelial membrane antigens.

The result of this retrospective study FNAC guided by U/S or C.T scan is easy and accurate for use, this is not meant to replace U/S guided needle biopsy in major centers outside Sudan. This procedure could be cut-short by early finding of abnormal cells. It allows rapid microscopic diagnosis.

FNAC guided radiography (U/S or C.T scan) is less expensive, with high clinical benefit for both diagnosis and therapy, and this will be the goal for clinicians, health care policy makers and patients.

In the present setup from this study it is felt that USG guided FNAC is very useful in diagnosis of different hepatic lesions as the procedure is
simple and safe. The results are obtained quickly without serious complications related to the procedure. So FNAC is a simple and effective diagnostic tool in our hand.
CONCLUSION AND RECOMMENDATION

- Ultrasound or C.T guided fine needle aspiration cytology (F.N.A.C) is a useful clues test for diagnosis and evaluation of patient with focal liver lesion (unifocal or multifocal).

- With presence of an expert radiologist and cytopathologist it cut the way to making a correct diagnosis of focal liver lesions. The role of correlation between discipline of medicine in diagnosis and management of patients with focal liver lesion should be established to advocate the multidisciplinary approach in handling of patients.

- Not only malignancy can be proved by such a procedure, A number of other conditions as pyogenic liver abscess, tuberculosis and liver hemangioma …etc.

- Radiographic guided FNAC can differentiate benign focal liver lesions against malignancy and also in many cases can verify secondary deposits with help of others features for example finding same cytological pattern in other masses.

Ultrasound guided FNAC is useful diagnostic test for evaluation of patients with discrete hepatic mass and superior to CT guided FNAC except for very small lesion.
-Beyond doubt immunocychimetry is a major tool in the differentiation between primary and secondary liver cancer and its implication is advised to verify secondary.

-It is concluded that fine needle aspiration cytology under image guidance has increasing acceptance as a means of evaluation of focal liver lesions, and in evaluating liver mass, however there are some difficulties which can be over come by more experience in aspiration, and better co-ordination between radiologists, pathologists and clinicians.

Further study in this aspect is recommended with a large number of patients with focal liver lesion with stress on cell block and immunehistochemistry techniques.
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Appendix

QUESTIONNAIRE

Evaluation of the role of FNAC in diagnosis of focal liver lesion

Date: ............................................  Block no : ......................

Patient name : ...................................

1. Residence (Khartoum / outside Khartoum)

2. Age

3. History of liver disease  Yes ☐  No ☐

4. History of previous operation  Yes ☐  No ☐

5. Symptoms of patient:  Yes ☐  No ☐
   - Weight loss
   - Abdominal pain

6. Sign of patients  Yes ☐  No ☐
   - Palpable liver mass
   - Ascites
   - Abdominal mass
   - Others
7. Radiological fool to diagnose liver lesion  
   Yes  
   □  No  
   □
   a. U/S  
   Yes  
   □  No  
   □
   b. C/T  
   Yes  
   □  No  
   □

8. Result of radiography image  
   Yes  
   □  No  
   □
   a. multiple liver lesion  (Unifocal)  
   Yes  
   □  No  
   □
   b. single liver lesion  (Multifocal)  
   Yes  
   □  No  
   □

9. cytological result  
   Primary malignancy  
   Yes  
   □  No  
   □
   Secondary malignancy  
   Yes  
   □  No  
   □
   Benign aspirate  
   Yes  
   □  No  
   □

10. Verification of unifocal lesion  
   -Benign  
   Yes  
   □  No  
   □
   - Malignant  
   Yes  
   □  No  
   □

11. Verification of multifocal lesion  
   Yes  
   □  No  
   □
   -Benign  
   Yes  
   □  No  
   □
   - Malignant  
   Yes  
   □  No  
   □