Pathology Section

Unravelling the Significance of Immunohistochemistry in Differentiating Neoplastic Lesions of Liver Biopsy: A Cross-sectional Study

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ABSTRACT

Introduction: Liver cancer globally remains the fourth-leading cause of cancer-related mortality. Metastatic lesions are more common in the liver tissue due to its rich portal and systemic venous blood supply. Even though an accurate histopathological diagnosis of tumours is possible in some cases. Distinguishing the hepatocellular carcinoma from metastatic lesion with similar morphology, poor differentiation and sub classification of carcinomas with unknown primary tumours always remains a problem. A multidisciplinary approach of histomorphological evaluation combined with systematic Immunohistochemistry (IHC) study is essential in differentiating neoplastic lesions.

Aim: To evaluate the role of IHC in differentiating the neoplastic lesions of liver biopsy to hepatic origin and metastatic lesion.

Materials and Methods: The present cross-sectional study of 50 neoplastic liver biopsy reported in the Departments of Pathology in oncquest laboratory for 3 years from march 2021 and February 2023, Bangalore, Karnataka, India. Clinical details were retrieved by request forms. All the tissue blocks were processed for Haematoxylin and Eosin (H&E) sections and for IHC. Primary panel includes HepPar1, CK7,CK20,CK19 and KI67. The second

panel includes arginase, glypican 3, CEA, CDX2 and SATB2. The third panel was decided further based on results of second panel, clinical details and radiological findings. A known positive and negative controls were used for each batch of slides. The slides were reviewed by two Pathologist and results were analysed.

Results: Of the total 50 cases 37 were male (74%). The primary panel classified and second panel confirmed the liver biopsy into hepatic origin and metastatic lesion. The lesions classified were Hepatocellular Carcinoma (HCC) 7 (14%) cases, cholangiocarcinoma 2 (4%) cases, hepatic adenoma 6 (12%) cases and metastatic deposits 35 (70%) cases by the end of secondary panel. The third panel determines the origin of metastatic deposits. Metastatic lesions were the most common lesions in liver biopsy 35 (70%) cases. Colorectal adenocarcinoma was the most common lesion in metastatic deposits (74%).

Conclusion: Diagnosing liver tumours on core biopsy specimen can be challenging. Accurate diagnosis is essential to establish the optimal treatment strategy. A systematic approach of initial differentiation by primary panel, confirmation by secondary panel and narrowing down the primary site of metastasis using third panel is highly recommended.

Keywords: Hepatocellular carcinoma, Hepatocellular adenoma, Cholangocarcinoma, Metastsicdeposis

INTRODUCTION

Liver cancer globally remains the fourth-leading cause of cancer-related mortality after lung, breast and colorectal cancer. According to GLOBOCAN estimates, there were nearly 905,677 newly diagnosed cases and 830,180 deaths related to HCC in 2020 [1]. The rates of incidence and mortality were 2-3 times more prevalent in males than in females. The Age-Standardised Incidence Rate (ASIR) and Age-Standardised Mortality Rate (ASMR) are highest in Sub-Saharan Africa (SSA) and certain regions of Asia (Southeast, East, and Central) as well as high-income areas of the Asia Pacific. Approximately 75% of liver cancers occur in Asia. The highest rates of incidence are found in Mongolia, but China accounts for the largest number of cases due to its high incidence and large population. The rates of incidence and mortality associated with HCC are quite comparable, implying a significant case fatality rate. Around 40% of HCC cases can be attributed to hepatitis B, 40% to hepatitis C, 11% to alcohol consumption, and about 10% to other factors; however, the underlying causes are expected to evolve with a growing prevalence of Non-alcoholic Steatohepatitis) related cirrhosis [1]. The Surveillance, Epidemiology, and End Results (SEER) program reports that the 5-year survival rate for liver and intrahepatic duct cancers was 20.8% (data accessed in August 2022); 44% of patients presented with localised disease, which had a 5-year survival rate of 36.1%, in contrast to just 3.1% for those

with distant metastases. In the United States, HCC accounts for 2.2% of all new cancer cases [1].

In comparison with primary liver tumours, metastatic lesions are more common in the liver tissue due to its rich portal and systemic venous blood supply. Metastases to the liver account for approximately 25% of all metastases to solid organs. Adenocarcinoma is the most common metastatic tumor followed by neuroendocrine tumours. Other tumours that commonly metastasize to the liver include melanoma, lymphoma, and, rarely, soft tissue sarcoma (Khadim MT et al., 2001) [2]. Even though an accurate histopathological diagnosis of tumours is possible in some cases. Distinguishing the hepatocellular carcinoma from metastatic lesion with similar morphology and sub classification of carcinomas with unknown primary tumours always remains a problem. A multidisciplinary approach of using morphological evaluation of H and E stained sections combined with immunohistochemistry is much essential in differentiating the primary hepatic neoplasm to secondary tumours in liver biopsy. The aim of this study was to evaluate immunohistochemistry (IHC) to distinguish between primary hepatic neoplasms and metastatic lesions in liver biopsy specimens.

MATERIALS AND METHODS

The present cross-sectional study conducted for 3 years from march 2021 and February 2023. All neoplastic liver biopsy reported

between March 2021 and February 2023 were retrieved from the archives of the Departments of Pathology in oncquest laboratory, Bangalore, Karnataka, India. Clinical data related to all the cases were retrieved from the request forms. All the cases were reviewed before including in the study.

Inclusion and Exclusion criteria: All the neoplastic lesions of liver biopsy were included. The biopsy with inadequate tumor cells, scant tissue exhausted during routine processing and cases without adequate clinical details were excluded from study.

All the tissue blocks were subjected to further processing which involved cutting of serial sections of 3 μm thickness and staining of sections with routine H&E.

Study Procedure
The primary panel included CK7, CK20, HepPar1, CK19 and KI67. The secondary panel includes arginase, glypican 3, CEA, CDX2 and SATB2. The third panel included markers like MUC5, MUC2, UROPLAKIN, GATA 3, AMACR, TTFI, NAPSIN, PAX8, ER/PR, HER2, WTI, CHROMOGRANIN A, SYNAPTOPHYSIN, PANCK, P63, CK5/6, CALRETININ, P40, S100 ,CD34, CD45, CD138, CD117, CD30, CD117, DESMIN, VIMENTIN and CD99 was applied to all metastatic lesions which were decided further based on results of second panel, clinical details and radiological findings for determining the cell origin.

The primary panel were applied on all the cases. The primary panel was used to differentiate all cases into two group, group 1 as HepPar1 positive cases as hepatic origin cases and group 2 as HepPar1 negative cases as non hepatic origin cases. The second panel helped in further confirming the classification of cases into group 1 (hepatic origin) and group 2 (Non hepatic origin).

The technical aspects of the immunohistochemistry procedures included antigen retrieval by microwave heating, and the streptavidin-biotin-horse radish peroxidase-DAB technique for antibody application and signal detection. A known positive and negative control was used for each batch of slides.

STATISTICAL ANALYSIS
Descriptive statistics were used to analyse the data.The values were presented in the format of frequency and percentages

RESULTS
A total of 50 biopsies were retrieved and analysed. Out of 50 cases, 37 (74%) were male. The primary panel of HepPar1, CK7,CK20,CK19 and KI67 classified the liver biopsy into group 1which is Heppar1 positive and hence hepatic origin. Group 1 included HCC and hepatic adenoma/ dysplastic nodule. Group 2 which is heppar1 negative and classified as otherwise included cholangiocarcinoma and metastatic neoplasm [Table/Fig-1]. CK19 and CK7 expression were significantly higher in cholangiocarcinoma. 13(26%) cases were hepatic origin cases and 37 (74%) cases were non hepatic origin cases.

| HepPar1 results	CK7	CK20	CK19	KI67
Group 1: Hepatic origin (13) -HepPar1: POSITIVE (13/50).	Positive (3/13)	Negative (13/13)	Negative (13/13)	30-50%
Group 2: Cholangiocarcinoma or Metastatic origin (37) -HepPar1: NEGATIVE; Cholangiocarcinoma or Metastatic origin (37/50).	Positive (20/37)	Positive (14/37)	Positive (7/37)	15-30%
[Table/Fig-1]: Primary panel.				

The further confirmation of hepatic origin of lesion was done using the second panel of arginase, glypican 3, CEA, CDX2 and SATB2. Arginase differentiated HCC including poorly differentiated and scirrhous HCC from metastatic deposits .Glypican3 differentiate HCC from benign liver lesions. CK7, CK20, CEA, CDX2 and

STAB2 helped in differentiating metastatic neoplasm. A total of 7 (14%) cases were hepatocellular carcinoma and 6 (12%) cases were hepatic adenoma/dysplastic nodule, 35 (70%) cases were metastatic lesions and 2 (4%) cases were cholangiocarcinoma. [Table/Fig-2-4].

Second panel results	Arginase	Glypican 3	CEA	CDX2	SATB2
HCC (7)	Positive (7/7)	Positive (7/7)	Positive (2/7)	Negative (7/7)	Negative (7/7)
Hepatic adenoma/ Dysplastic nodule (6)	Positive (5/6)	Negative (6/6)	Negative (6/6)	Negative (6/6)	Negative (6/6)
Cholangiocarcinoma (2)	Negative (2/2)	Negative (2/2)	Positive (2/2)	Negative (2/2)	Negative (2/2)
Metastatic deposits (35)	Negative (35/35)	Negative (35/35)	Positive (26/35)	Positive (10/35)	Positive (9/35)
[Table/Fig-2]: Secondary panel.					
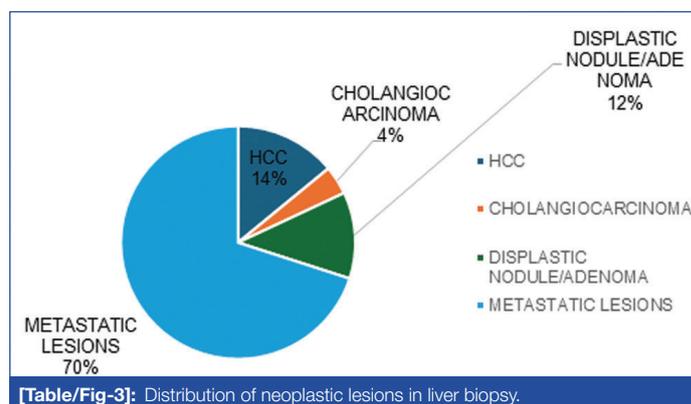
**[Table/Fig-3]:** Distribution of neoplastic lesions in liver biopsy.

The third panel was selected based on the results of primary and second IHC panel with clinical details and radiological findings on the metastatic lesions for the confirmation of the primary site of origin. MUC5, MUC2, UROPLAKIN, GATA 3, AMACR, TTFI, NAPSIN, PAX8, ER/PR, HER2, WTI, CHROMOGRANIN A, SYNAPTOPHYSIN, PANCK , P63, CK5/6, CALRETININ, P40, S100 ,CD34, CD45, CD138, CD117, CD30, CD117, DESMIN, VIMENTIN and CD99 were used in determining the primary site.

Out of 35 metastatic deposits, 26 cases were adenocarcinoma, 3 cases were neuroendocrine tumor,1 was squamous cell carcinoma and 5 were poorly differentiated as the IHC markers were inconclusive and the tissue exhausted. Out of 26 cases of adenocarcinoma, colon was the most common primary site followed by pancreas. [Table/Fig-5].

DISCUSSION
The second most common site of metastatic lesions after lymph node is liver. It is frequently biopsied for evaluating the site of primary. The identification of primary is important for the management of metastatic liver disease. Often, the distinction between HCC between the cholangiocarcinoma or metastatic deposits can be made on the basis of clinicoradiological studies and histopathological examination. The diagnostic dilemma arises in cases of poorly differentiated tumours and without clinical details. There have been several studies on establishing the role of IHC in differentiating the HCC and adenocarcinoma. The primary panel is selected to make a initial impression and classification. Additional antibodies are added once the diagnosis has been narrowed.

In this study HepPar1 showed cytoplasmic positivity in all the 7 cases of HCC which is simillar to that of Wennerberg, et al., [3] [37 of 38 (97.37%) HCC were positive for HepPar1], S-Y Leong A et al., [4], [30 of 32 (93.75%) HCC were positive for HepPar1] and Al-Muhannadi, et al., [5] [18 of 22 (81.8%) HCC].Thus establishing HepPar1 as a high sensitivity marker in HCC. Similar to previous
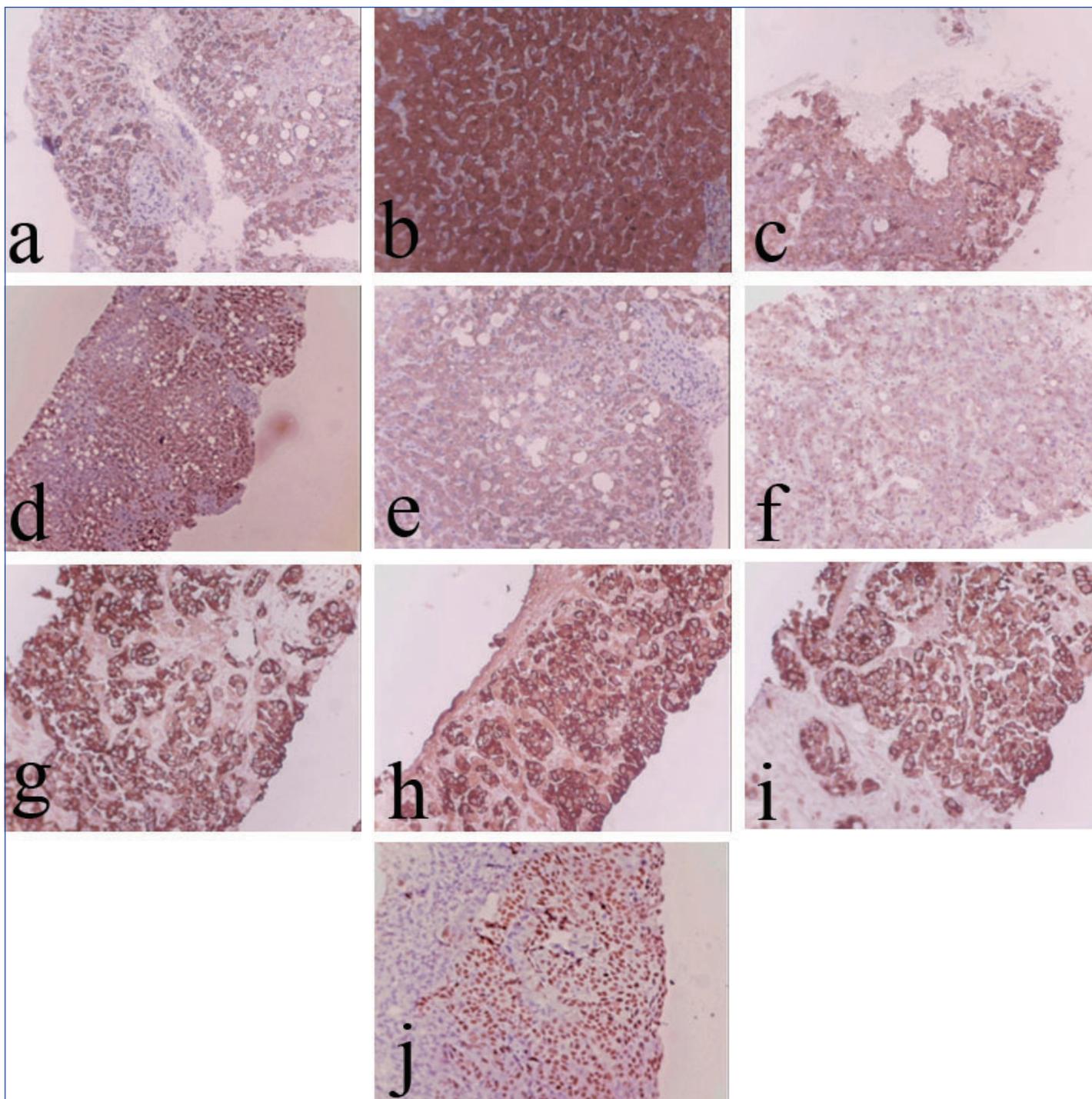
[Table/Fig-4]: a) Cytoplasmic positivity of HepPar1 (10x); b) Cytoplasmic positivity of arginase (40x) and; c) Cytoplasmic positivity in glypican (10x); d) Cytoplasmic positivity of HEPPAR 1 (10x); e) Cytoplasmic positivity of arginase (40x) and; f) Negative glypican (40x); g) Menbranous positivity of CK7 in cholangiocarcinoma (40x); h) Membranous positivity of CK19 in Cholangiocarcinoma (10x); i) Membranous positivity of CK20 in metastatic deposits from colorectal carcinoma (10x); j) Nuclear positivity of CDX2 in metastatic deposits from colorectal carcinoma (10x).
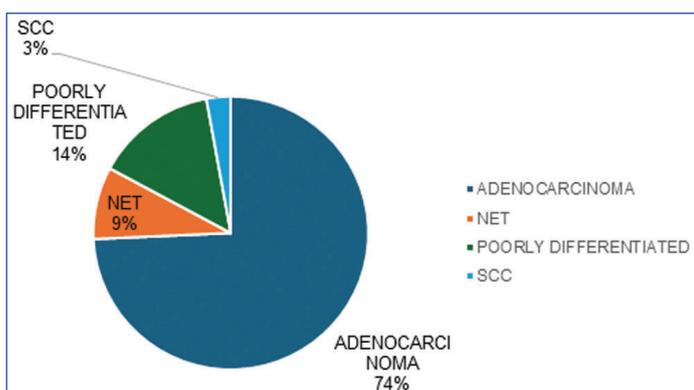
[Table/Fig-5]: Distribution of metastatic lesions.

studies, HepPar1 immunoreactivity was not observed in any of the cholangiocarcinoma or metaststic deposits evaluated in this study,

and this is consistent with the lack of staining in normal bile ducts and nonparenchymal liver cells.

CK7 is present in bile duct epithelium, but not in normal hepatocytes. CK7 cytoplasmic and membranous positivity is seen predominantly in cholangiocarcinoma (100%) whereas CK7 expression was absent in most HCC (4/7 were positive), and metastatic deposits (18/35 positive). Maeda T et al., [6] [29 out of 30 cases of cholangiocarcinoma (97%) whereas only two out of 30 cases (7%) of HCCs and one out of 30 cases (3%) Metastatic deposits were diffusely positive for CK7] and Al-Muhannadi N et al., [5] reported that the positivity of CK7 expression in cholangiocarcinoma was significantly higher than that in HCC or metastatic deposits. Inthere study CK7 stained 6 of 22 (27.3%) HCC, and 5 of 15 (33.3%) metastatic deposits and all 5 of 5 Cholangiocarcinoma carcinoma (100%). Hence CK7 would appear to be a useful member of the diagnostic panel.

The authors found CK20 to be cytoplasmic and membranous positive in most of the metastic deposits from the colorectal carcinoma to the liver and negative in HCC and cholangiocarcinoma. Simillar results were seen in the studies done by Ramya V et al., [7] [In their study, CK20 was positive only in 87.50% cases of metastatic adenocarcinomatous deposit in liver from colorectal region, negative in 100% cases of Hepatocellular carcinoma and Cholangiocarcinoma] and Shimonishi T et al., [8] [Cytokeratin 20 expression was observed in 17 (81%) of metastatic adenocarcinomas in liver from colorectal regions].

CK19 cytoplasmic and membranous expression appears relatively more specific for cholangiocarcinoma (2/2) than HCC (2/7)and metastatic deposits (5/35). Simillar results were seen in study done by Al -Muhannadi N, et al., [5]. In there study CK19 was diffusely positive in 5 of 5 (100%) CC, only 3 of 22 (13.6%) HCC were and 13 of 15 (86.7%) metastatic deposits.

The primary panel of HepPar1, CK 7, CK20, CK19 and KI67 helps in differentiating the HCC from cholangiocarcinoma and metastatic deposits. However the authors used the second panel of IHC for confirmation.

Arginase and glypican 3 are yet another markers differentiating HCC from adenocarcinoma. The arginase and glypican 3 show diffuse cytoplasmic positivity in the (7/7) HCC incuding poorly differentiated and scirrhous HCC. In addtion to that glypican3 differentiate HCC from benign liver lesions. The glypican3 is negative in (6/6) hepatic adenoma. The present study results coincides with the results of Choi Won-Tak et al., [9].

The CEA gives a canalicular pattern of staining in HCC in 2 cases where as diffuse cytoplasmic staining in the adenocarcinomas. However the negative staining was noted in the poorly differentiated adenocarcinoma. These findings were simillar to the study done by Al -Muhannadi N, et al., [5] and Tanaka S, et al., [10].

CDX2 serve as a marker for intestinal differentiation. The study by Mocan LP et al., [11] shows CDX2 diffuse and strong nuclear positivity in all metastases with colorectal primaries and 70% of pancreatic ductal adenocarcinoma cases which is similar to the present study.

In addition to CDX2, SATB2 is also a specific immunohistochemical marker for epithelial tumours of the small and large intestines and the appendix. Thus, according to a previous study [12] the combination of CDX2 and SATB2 can be used as a specific marker panel for the diagnosis of metastatic colorectal carcinoma in the liver which is similar to the results of this study.

After excluding the HCC and cholangiocarcinoma by using primary and second panel, the primaries of metastatic deposits were evaluated by third panel. The most common metastatic deposits are appears to be colorectal adenocarcinoma which is similar to the study done by Ramya V et al., [7].

Limitation(s)

Only diagnostic markers were used in the study, further study of prognostic markers would help improving the study.

CONCLUSION(S)

Diagnosing liver tumours on core biopsy specimen can be challenging. A constellation of clinical details, radiologic findings and laboratory results is very important for the correct diagnosis of neoplastic lesions in the liver. Proper identification and classification is essential to establish the optimal treatment strategy. Immunohistochemistry plays an important role in differentiating the neoplastic lesions in liver biopsy. A systematic approach of differentiating HCC from adenocarcinoma by primary panel, confirmation by secondary panel and narrowing down the primary site of metastasis in third panel is highly recommended.

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AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was Ethics Committee Approval obtained for this study?   Yes
• Was informed consent obtained from the subjects involved in the study?   Yes
• For any images presented appropriate consent has been obtained from the subjects.   NA