Received: 24 July 2022 | Revised: 14 October 2022 | Accepted: 24 October 2022

DOI: 10.1002/dc.25072

BRIEF REPORT

WILEY

A case of bone metastasis of hepatocellular carcinoma: Mallory hyaline bodies can lead to the correct cytological diagnosis

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Abstract

Hepatocellular carcinoma (HCC) accounts for most primary tumors of the liver. Although bone metastasis does not occur in a high percentage of patients, bone metastasis is often found first, which leads to the diagnosis of HCC. In this report, we describe a case of bone metastasis from HCC in which bone lesions were detected incidentally, and in which a cytological diagnosis was difficult to make. The patient was a 78-year-old man with a history of renal dysfunction after orthopedic surgery. He underwent a thorough examination after a bone tumor was incidentally found on abdominal CT. Plasmacytoma was suspected. Fine needle aspiration cytology revealed irregular clusters of medium-to-large atypical epithelioid polygonal cells with relatively abundant eosinophilic, somewhat granular cytoplasm, and indistinct cell borders, which led to a diagnosis of malignancy. Histologically and immunohistochemically, the tumor was diagnosed as bone metastasis of HCC. Re-examination of the cytological specimen revealed characteristic Mallory hyaline bodies (MHBs). Immunohistochemistry using a cell transfer method revealed that they were positive for low molecular weight cytokeratin, Cam5.2, in a densely granular fashion. In this case, the cytological diagnosis of HCC was difficult to make due to the unclear cytoplasmic borders and absence of bile pigment. However, the identification of MHBs can potentially guide me to the correct cytological diagnosis.

KEYWORDS
bone metastasis, Cam5.2, cell transfer method, cytopathology, hepatocellular carcinoma, Mallory hyaline body

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 90% of tumors originating from the liver and is usually seen in males in their 60 s. Bone metastasis occurs in 13.5% of patients[1]; the most common sites are the vertebrae, ribs, and sternum.[2] According to the National Bone Tumor Registry (1971–1996), metastatic tumors account for approximately 30% of all tumors that occur in bone, with HCC accounting for 5.3%.[1,2] In cases where HCC arises in a non-cirrhotic background, it might have few symptoms in the primary site of the liver. The first symptoms are often due to metastasis. For this reason, it is not uncommon for HCC metastasis to be detected initially.[3] In addition, distant metastasis has become an increasing problem, since the

The abstract of this paper was presented at the 38th Annual Meeting of the Society of Ishikawa Prefectural Clinical Cytology (in 2022).

prognosis of HCC has improved with recent advances in its diagnosis and treatment.[1,2,4] We describe a case of HCC in which a metastatic bone tumor was detected incidentally, and the cytological diagnosis of the primary site was difficult. However, the hallmark of Mallory hyaline bodies (MHBs) could have potentially guided us to the correct cytological diagnosis.

2 | CASE PRESENTATION

A 78-year-old man with a history of left shoulder tendon plate rupture, presented with a sudden decrease in his renal function after shoulder joint replacement surgery at our hospital. A subsequent abdominal CT scan incidentally revealed a mass in the left 10th rib, which prompted a thorough examination (Figure 1A). Bone and Tl scintillation showed no abnormal accumulation in the rib tumor area. He had stopped drinking and smoking since 50 years of age, but before that he had smoked 100 cigarettes and drink two bottles of beer per day. He was positive for HBc antibodies and was considered to have been infected with hepatitis B virus. The patient's laboratory studies showed mild chronic liver injury: aspartate aminotransferase (AST), 52 U/L; alanine aminotransferase (ALT), 49 U/L; alkaline phosphatase (ALP), 145 U/L; gamma-glutamyl transpeptidase (γ-GTP), 279 U/L; lactate dehydrogenase (LD), 256 U/L; total bilirubin, 0.5 mg/dl; hemoglobin (Hb), 10.0 g/dl; and creatinine, 1.19 mg/dl. His serum tumor markers such as alpha fetoprotein (AFP) and protein induced in vitamin K absence II (PIVKA II) were significantly increased to 55.9 ng/ml (upper normal value of AFP: 10 ng/ml) and 621 mAU/ml (upper normal value of PIVKA II: 40 mAU/ml), respectively.

The patient was referred to the Department of Hematology and Immunology, where plasmacytoma was suspected due to high IL-2R levels and a solitary tumor. Subsequently, fine needle aspiration (FNA) cytology and biopsy of the rib tumor were performed. Later, bone metastasis of HCC was diagnosed based on the examination of the bone tumor biopsy specimen. Liver biopsy was not performed. A contrast-enhanced CT scan performed at a later date (Figure 1B) showed diffuse early staining with non-rim-like arterial phase hyperenhancement in the posterior right lobe of the liver. A tumor shadow (diameter, >10 cm) showed non-peripheral washout in the late phase. These findings fulfilled the diagnostic criteria for HCC (LR-5 according to the Liver Imaging Reporting and Data System). The background of the liver was not apparently cirrhotic. There was a tumor plug in the portal vein from the main branch to the right branch, and small granular shadows were seen in other areas as well (Figure 1B). A tumor shadow was also observed in the left 10th rib, which was a metastatic lesion. The patient had stage IV HCC with a good hepatic reserve. Treatment was started with a combination of atezolizumab and bevacizumab was begun without surgical resection. However, the patient was lost to follow-up after 3 months of chemotherapy.

Four 1-cm^2 FNA smears were obtained from the rib tumor. At low magnification, medium-to-large atypical epithelioid polygonal cells with indistinct cell borders and seemingly naked nuclei appeared in irregular clusters or partially in a pseudoglandular luminal fashion along with small lymphocytes (Figure 2A). The nuclei were round and mildly irregular, and large nuclei were also observed. The chromatin was granular with distinct nucleoli and intranuclear vacuoles, which were considered to be intranuclear glycogen. The cytoplasm was relatively abundant, eosinophilic, and somewhat granular. FNA of the left 10th rib lesion initially diagnosed as suspicious for malignant cells, not further specified. Careful re-examination of the cytological specimen revealed characteristic intracellular MHBs, which appeared as brightly eosinophilic, irregular rope-like structures along with tumor cells (Figure 2B). No cells were suspicious for plasmacytoma. The cytological differential diagnoses included—but were not limited to—neuroendocrine tumor, adrenocortical tumor, pancreatic acinar cell carcinoma, HCC, hepatoid adenocarcinoma, and granular cell tumor.

A needle biopsy specimen from the rib tumor was examined. Under a low-power view, eosinophilic atypical cells were arranged in a cord-like or pseudoglandular-like fashion. Skeletal muscle and connective tissue were seen in the background, but no bone tissue was observed (Figure 3A). As in the cytological examination, the nuclei were mildly, irregularly shaped, with prominent nucleoli and intranuclear vacuoles that appeared to be intranuclear glycogen. Characteristic MHBs frequently appeared as eosinophilic, irregular rope-like material

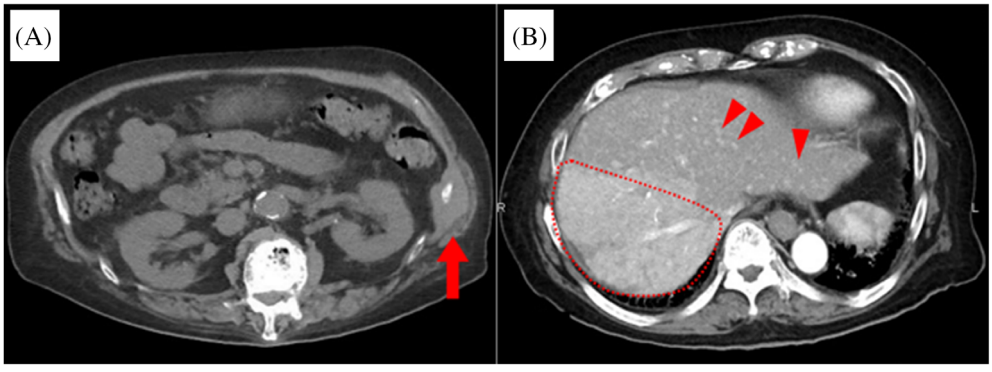

FIGURE 1 The findings of abdominal CT. (A) CT image of the abdomen. A mass was seen in the left 10th rib (arrow). (B) Contrast-enhanced CT image. Diffuse tumor shadows (diameter > 10 cm) were identified in the posterior right lobe of the liver (dotted line). The background of the liver was not apparently cirrhotic. Tumor shadows in the portal vein and a small granular tumor in the left lobe (arrowheads) were also observed.

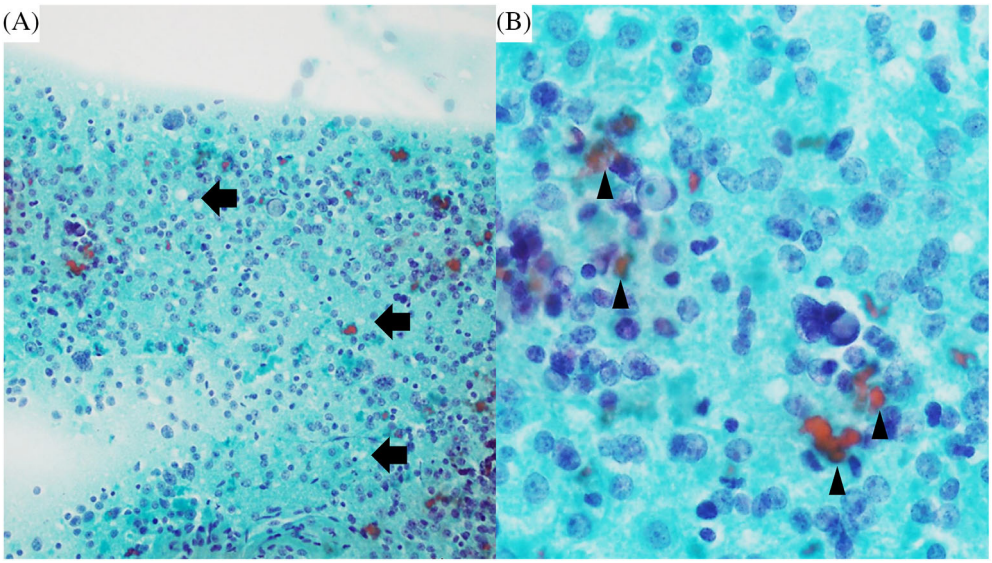

FIGURE 2 The cytological/cytomorphological findings of fine needle aspiration (FNA) specimens. (A) Atypical medium to large, epithelioid polygonal cells with indistinct cell borders and seemingly naked nuclei appeared in irregular clusters or partially in a pseudoglandular luminal fashion (arrows) along with small lymphocytes. (Direct smear, Papanicolaou staining; ×200). (B) The nuclei were round, with mildly irregular nuclei, and large nuclei were also observed. The chromatin was granular with distinct nucleoli and intranuclear vacuoles. The cytoplasm was relatively abundant, eosinophilic, and somewhat granular. Re-examination recognized intracellular Mallory hyaline bodies (MHBs) (arrowheads) appearing as brightly eosinophilic, irregular rope-like structures along with tumor cells (direct smear, Papanicolaou staining; ×600).

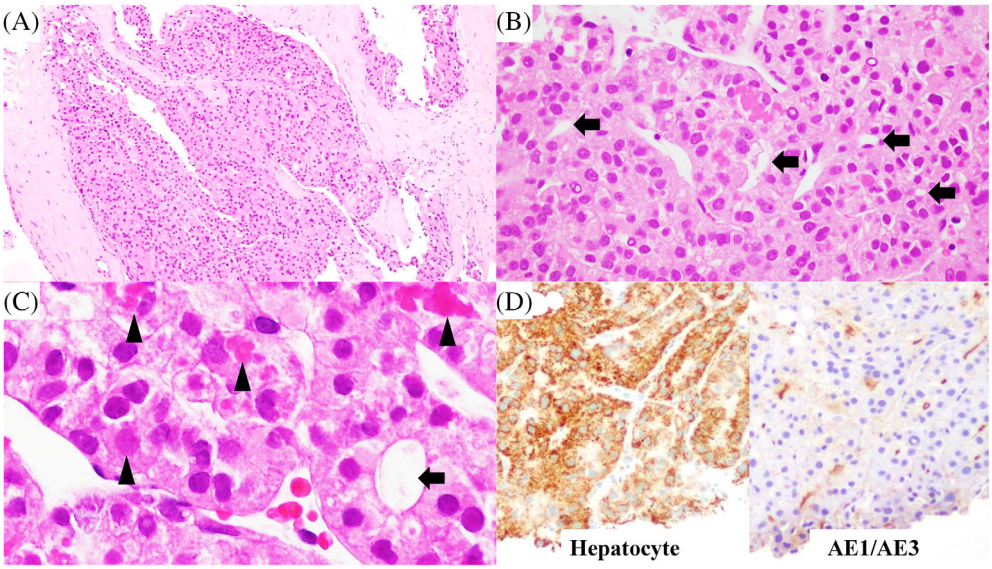

FIGURE 3 The histological/immunohistochemical findings. (A) Eosinophilic atypical cells were arranged in a cord-like pattern. No bone tissue was seen in the background. (H&E staining; ×100). (B) The atypical hepatocyte-like epithelial cells showed a pseudoglandular-like appearance (arrows). Prominent nucleoli and intranuclear vacuoles were identified with mild nuclear irregularities. (H&E staining; ×400). (C) In addition to prominent nucleoli and mild nuclear atypia, pseudoglandular-like arrangements (arrow) and many Mallory hyaline bodies (MHBs) (arrowheads) were present. They appeared as eosinophilic, irregular rope-like material in the cytoplasm. Moreover, a pseudoglandular-like appearance (arrow) was readily identified (H&E staining; ×600). (D) The metastatic hepatocellular carcinoma (HCC) cells were specifically and diffusely positive for Human Hepatocyte (left) and weakly positive for pan-cytokeratin (e.g., AE1/AE3) (right) (Immunohistochemical staining; ×200).

in the cytoplasm (Figure 3B,C). On immunohistochemistry, the tumor cells were negative for CK7, CK20, and AFP, but were weakly positive for pan-cytokeratin (AE1/AE3) and polyclonal CEA, and positive for Hepatocyte and CD10 (Figure 3D). The tumor cells were negative for CD38, CD45, ISH-κ, and ISH-λ. These findings were not consistent with plasmacytoma. Although liver biopsy was not performed in this case, based on the features above, a conclusive diagnosis of metastatic HCC was made on the left 10th rib lesion.

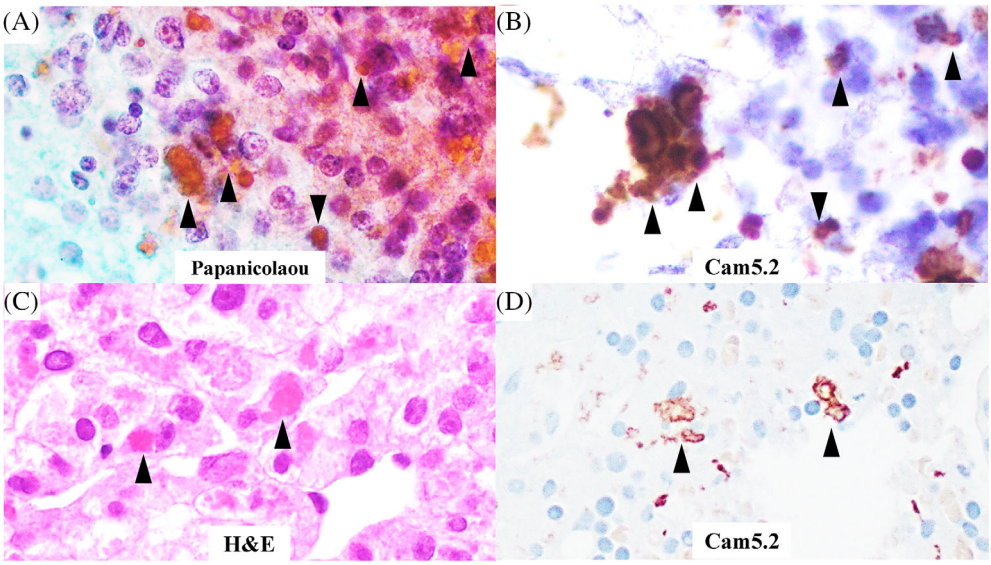

FIGURE 4 Mallory hyaline bodies (MHBs) in cytology, histology and immunostained material. (A, B) Characteristic MHBs (arrowheads) (A: Direct smear, Papanicolaou stain; ×400) were strongly immunopositive for low molecular weight cytokeratin, Cam5.2 (B), in a densely granular fashion with variable density (arrowheads). (C, D) Characteristic intracellular MHBs (arrowheads) (C: H&E stain; ×400) were characteristically immunopositive for Cam5.2 in a coarsely granular fashion (arrowheads) (D).

We performed a careful cytological, histological and immunohistochemical re-examination with regard to the MHBs. In a cytological examination using a cell transfer method (Figure 4A), the characteristic MHBs were strongly immunopositive for low molecular weight cytokeratin, Cam5.2, in a densely granular fashion with variable density (Figure 4B). Furthermore, in the histological examination (Figure 4C), these intracellular MHBs were characteristically immunopositive for Cam5.2 in a coarsely granular fashion (Figure 4D).

The methodology of our cell transfer technique and immunocytochemistry on the transferred cells was as follows:

1. Mark the target cells with a glass pen from the back of the glass slide;
2. Remove cover glass by soaking in xylene;
3. Apply a thick layer of the mounting medium, Marinol, to a glass slide and allow it to dry overnight on a stretching plate;
4. Mark the target cells again on the dried Marinol;
5. Soften Marinol by soaking in a warm bath at 50–60°C for about 1 h;
6. Remove Marinol from the glass slide, cut out the marked area, affix it to the anti-peel coated glass slide and allow it to dry;
7. Remove Marinol by soaking in xylene, decolorize with 1% hydrochloric alcohol, and then immunostain.

3 | DISCUSSION

Because the patient developed renal dysfunction after orthopedic surgery and had no significant symptoms or history of cirrhosis, his liver function and tumor marker levels had not been examined prior to the current admission. The hematologist-immunologist suspected the rib lesion was a plasmacytoma. HCC was not listed as a differential diagnosis at the time of the FNA. However, after the histological diagnosis of metastatic HCC was made on biopsy of the rib, the FNA was re-evaluated. Comparing the cytology to typical cytological images of moderately to well differentiated HCC,[5,6] we were still unable to identify the characteristics of HCC, since the cell borders were indistinct, the nuclei appeared naked, and bile pigment was not seen. According to a cytological/cytomorphological study of HCC by Hayashida et al.,[7] HCC is classified into grade I-IV according to the degree of cellular atypia. They reported that grade II is the most typical, and grade III, which shows sparse cell cohesiveness and marked atypia, is more likely to show "melting-like, liquefacient cytoplasm". The cytological features in our case show pseudo-glandular groups of cells with less conspicuous nuclear atypia. Based on the nuclear features, it is considered our case grade II.

Re-examination of the case allowed us to focus on MHBs, which were not appreciated at the time of the initial diagnosis. MHBs are known to occur as eosinophilic, irregularly-shaped structures in the cytoplasm of tissues from patients with alcoholic and non-alcoholic steatohepatitis, chronic cholestatic and metabolic diseases, and/or HCC.[8] In a case report describing HCC presenting with multiple bone and soft tissue metastases, Rastogi et al. cytologically identified not only bile pigments but also abundant eosinophilic dense intracytoplasmic hyaline bodies (IHBs).[9] In contrast to MHBs, IHBs are morphologically defined as well-circumscribed homogeneous cytoplasmic eosinophilic globules and masses.[8] A very close pathogenic relationship between their IHBs and our MHBs was indicated as follow: both IHBs and MHBs were immunohistochemically positive for p62 and ubiquitin.[8,9] However, MHBs are characteristically immunopositive for low molecular weight cytokeratins, such as Cam5.2 (as in the current case), whereas IHBs are not.[8] In our case, the cytology, histology, and immunohistochemistry showed structures and expression of characteristic MHBs.

There have been several reports of bone metastasis from HCC.[5,6,9–12] But only a few cases in which cytological examination was reported.[9,10] We did not find any case reports in which MHB was identified by cytological examination. When MHBs are identified by cytology, HCC should be strongly considered as one of the differential diagnoses and immunohistochemical staining to detect Cam5.2 should be performed with the use of a cell transfer method.

4 | CONCLUSION

We report a case of HCC in which a rib metastasis was incidentally detected, but in which a cytological diagnosis was very difficult to make. The difficulties in the diagnosis arose due to a lack of clinical information, cytoplasmic findings that differed from the typical findings, and the absence of bile pigment. However, we identified MHBs, which can potentially guide cytologists to a correct diagnosis of HCC. In the diagnosis of bone tumors, it is important to consider the possibility of metastatic HCC and to recognize MHB when present.

AUTHOR CONTRIBUTIONS

Sohsuke Yamada and Makoto Kawasaki participated in the conception of the study and writing of the manuscript. Sohsuke Yamada, Makoto Kawasaki, Akihiro Shioya, Mao Takata, Yumi Tsubata, Yoshiiku Okanemasa, Michiho Takenaka, Toshie Terauchi, Manabu Yamashita, and Makoto Kawasaki performed the clinical imaging and/or cytological/pathological/immunohistochemical interpretation of this tumor lesion. All of the authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

We would like to thank native English-speaking scientific doctor, Mr. Brian Quinn, as an Official English Editor of Japan Medical Communication, and Clinical Research Support Office, Kanazawa Medical University, Japan, for their expert English editing, technical assistance and helpful comments.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The dataset supporting the findings and conclusions of this case report is included within the article.

INFORMED CONSENT

Written informed consent was obtained from the patient for their anonymized information to be published in this article.

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How to cite this article: Kawasaki M, Shioya A, Takata M, et al. A case of bone metastasis of hepatocellular carcinoma: Mallory hyaline bodies can lead to the correct cytological diagnosis. *Diagnostic Cytopathology*. 2022;1-5. doi:10.1002/dc.25072