

Case Report

A Molecular Diagnosis of Cholangiocarcinoma suggested using Cell-free DNA following Multiple Unsuccessful Attempts at Obtaining a Tissue Diagnosis

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Abstract

A tissue diagnosis is fundamental for the evaluation and care for most malignancies. However, for malignancies arising from certain tissues, obtaining a tissue diagnosis can occasionally be problematic. Here a patient is described where multiple attempts failed to confirm a tissue diagnosis, despite clinical, radiographic and biochemical evidence of localized cholangiocarcinoma. Circulating cell-free DNA next generation sequencing (cfDNA NGS) testing was suggestive of an underlying cholangiocarcinoma.

Although cfDNA NGS testing is not validated or approved as a method to diagnose cholangiocarcinoma, this case illustrates the potential utility of cfDNA NGS testing in order to suggest a genomic diagnosis of cholangiocarcinoma when attempts at a tissue diagnosis are not feasible or were not successful.

Keywords

Cell-free DNA; Cholangiocarcinoma; Genomic diagnosis; Liquid biopsy

Introduction

Despite the variety of diagnostic procedures and accompanying laboratory tools available, obtaining tissue confirmation of apparent cholangiocarcinoma is often challenging. Here, a patient with clinical, radiographic and biochemical evidence of localized cholangiocarcinoma is described. Over several months, Endoscopic retrograde cholangiopancreatography (ERCP)-obtained specimens were evaluated but failed to establish a tissue diagnosis of cholangiocarcinoma.

The results of circulating cell-free DNA next generation sequencing (cfDNA NGS) testing suggested a genomic diagnosis of chol-

angiocarcinoma. The same results were inconsistent with either clonal hematopoiesis of indeterminate potential (CHIP) or with the identified mutations being of germline origin. Although cfDNA NGS testing is not validated or approved as a method to diagnose cholangiocarcinoma, this case illustrates the potential utility of cfDNA NGS testing in order to suggest a genomic diagnosis of cholangiocarcinoma when attempts at a tissue diagnosis are not feasible or were not successful.

Case Report

A 74-year-old man presented in April 2017 with jaundice and left upper quadrant pain. Laboratory studies included a normal complete blood count (CBC) and differential, bilirubin 5.8 mg/dL (0.0-1.2), alkaline phosphatase 631 IU/L (39-117), AST 85 IU/L (0-40), ALT 68 IU/L (0-44) and albumin 3-4 gm/dL. CA19-9 was 444 U/mL (0-35). Hepatitis BS Antigen, Hepatitis C virus antibody and HIV rapid screen were all negative. There was a reported history of COPD but no history of gastrointestinal disease. There was no relevant family history reported.

A computerized tomography (CT) scan (abdomen/pelvis) showed marked intrahepatic ductal dilatation without obvious stone or mass involving the common bile duct, while a chest x-ray demonstrated no active disease. Endoscopic retrograde cholangiopancreatography (ERCP) with biliary sphincterotomy, spyglass cholangioscopy with spy bite microbiopsies (Boston Scientific, Natick, MA), biliary brushings, and plastic biliary stent placement showed tight stricturing of the common hepatic duct (4 cm in length) and intrahepatic ductal dilatation. Multiple cytology specimens were interpreted as showing "ductal cells with mild atypia."

Over the next three months he underwent two subsequent ERCPs that included biliary stent exchanges, with cytologic examination of the brushings again showing only mild atypia. Af-

ter an admission for cholangitis, bilateral percutaneous biliary drains were placed.

In August 2018, a magnetic resonance cholangiopancreatography (MRCP) showed bilateral percutaneous drainage tubes, minimal intrahepatic ductal dilatation with marked wall thickening of the common hepatic duct and central intrahepatic ducts, particularly the posterior division of the right intrahepatic bile duct. There was no extrahepatic biliary ductal dilatation. Radiology reported that the thickening of the ducts “raises the concern for infiltrative tumor, although exact tumor extent is difficult to interpret due to superimposed reactive changes related to indwelling biliary drains.” At that time the patient’s bilirubin decreased to 3.0 mg/dL, while the CA19-9 increased to 1,652 U/mL and other laboratory studies included AST 36 IU/L (0-40), ALT 25 IU/L (0-44).

A whole-body PET/CT scan demonstrated increased activity at the distal common bile duct and along the course of the biliary catheters and no clear evidence of distance FDG hypermetabolic activity.

In late 2017, he experienced and recovered from multiple episodes of apparent infectious cholangitis. Due to clinical “debilitation”, surgical oncology felt he was not eligible for resection of the presumed cholangiocarcinoma. The infectious disease service recommended indefinite use of antibiotics to prevent further episodes of cholangitis and hospice was recommended.

The patient was first seen by medical oncology in January 2018. At that time, the patient was uncertain as to why he had only been referred to a surgical oncologist months after he presented with hyperbilirubinemia. He reported that the surgical oncologist told him that there was no effective therapy for what he considered likely to be unresectable cholangiocarcinoma. Nonetheless his family later encouraged him to pursue a visit with medical oncology. At that first medical oncology visit he expressed a strong interest in pursuing any testing that might suggest the presumed diagnosis of cholangiocarcinoma. At this time his CA19-9 was 18,712 U/mL and bilirubin 1.3 mg/dL. His gastroenterologist was consulted and felt that additional invasive procedures to obtain a tissue diagnosis as well as molecular profiling of supernatant specimen DNA would not be worthwhile. Similarly, Pathology did not recommend testing prior specimens for molecular markers of cholangiocarcinoma. He was offered the option of a liquid biopsy (blood test) interrogating circulating cell-free DNA by next generation sequencing testing (cfDNA NGS). The patient agreed with the understanding that this testing is not a validated and an approved basis for cholangiocarcinoma diagnosis.

The cfDNA NGS testing included complete sequencing of covered exons of 73 malignancy-associated genes and demonstrated four pathogenic somatic alterations. The alterations identified were classified as driver mutations based on the resulting functional consequence in these alterations when associated with biliary or other cancers (Guardant 360, Guardant Health, Inc, Redford City, CA 94063). In the Guardant360 NGS assay,

cfDNA is extracted from plasma and genomic alterations are analyzed by massive parallel sequencing of amplified target genes using a variety of platforms, and hg19 is used as the reference genome. Guardant360 is validated to detect gene alterations in the gene or promoter region of the gene and/or amplifications for the following genes [1]:

AKT1, ALK, APC, AR, ARAF, ARD1A, ATM, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCNE, CDH1, CDK4, CDK6, CDKN2A, CTNNB1, DDR2, EGFR, ERBB2, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3,

GATA3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MAPK1, MAPK3, MET, MLH1, MPL, MTOR, MYC, NF1, NFE2L2, NOTCH1, NPM1, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, PTPN11, RAF1, RB1, RET, RHEB, RHOA, RIT1, ROS1, SMAD4, SMO, STK11, TERT, TP53, TSC1, and VHL.

The relevant somatic alterations detected in the patient’s sample were *TP53* (C141fs), *IDH2* (R140Q), *NF1* (Q83*) and *ATM* (R3008H) at reported frequencies of 1.3%, 0.3%, 0.2%, 0.1% respectively.

Discussion

The incidence of cholangiocarcinoma is rising while the survival rate remains roughly 10% in the United States. As with other malignancies, a tissue diagnosis is highly recommended once cholangiocarcinoma is part of the differential diagnosis based on the clinical presentation, radiographic or biochemical findings [2].

However, establishing a definitive tissue diagnosis can be challenging despite a variety of methods commonly used including ERCP with brushings and biopsy (including forceps biopsies and ERCP with endoscopic ultrasound), cholangioscopy (e.g. percutaneous cholangioscopy or the spyglass used in the case described), fluorescence in situ hybridization (FISH) analysis of the cytology cell blocks of specimens obtained and repeated brushings or use of stiffer bristles in obtaining tissue. A worldwide study of resection for “presumed” cholangiocarcinoma underscores the challenge as 8-22% of patients “turned out to have benign disease on microscopic examination of the resection specimens [3].”

cfDNA NGS testing is increasingly used to identify actionable molecular alterations in DNA shed from tumor cells, as therapies aimed at counteracting the consequences of specific alterations may offer effective therapeutic options for these patients. Indeed, cfDNA NGS is endorsed as a method to establish tumor *EGFR* status in patients with presumed non-small-cell-lung cancer where a tissue diagnosis has not or cannot be obtained [4]. Recently, an expert review panel of the American Society of Clinical Oncology and the College of American Pathologists concluded that there is little evidence to support the use of cfDNA to diagnose early stage cancer or monitor for a treatment response or to detect residual disease after treatment [5]. However, the authors

did not specifically address cfDNA use to suggest a diagnosis of cholangiocarcinoma for a patient with clinical and laboratory findings consistent with cholangiocarcinoma, as in the described patient.

Molecular profiling studies of tumor tissue or cfDNA from patients with cholangiocarcinoma have reported a variety of molecular alterations (2,6,7), including somatic *KRAS* driver mutations. Other frequently mutated genes in cholangiocarcinomas were also studied (see above), although *BAP1* (which is commonly mutated in cholangiocarcinoma) was not (8,9,10,11). In this case, the *TP53* (C141fs), *IDH2* (R140Q), *NF1* (Q83*) and *ATM* (R3008) alterations were detected using cfDNA NGS testing. These alterations are thought to be somatic driver mutations, given the known oncogenic or lost tumor suppressor gene activity resulting from the mutated gene. While detection of these alterations suggests malignancy, there remains the possibility that one or more of these variants are a result of clonal hematopoiesis of indeterminate potential (CHIP). For example, in a study of 17,182 normal subjects without a diagnosis of malignancy, 9.5% of individuals age 70-75 years harbored somatic mutations identified by cfDNA NGS testing, but the majority of mutations identified were in one of three CHIP-associated genes: *DNMT3*, *TET2* and *ASXL1* [12]. In another study of 12,380 Swedish individuals, somatic driver mutations were detected in 308 samples with only 18 subjects harboring more than one of the screened 370 driver mutations and none of these samples showed both *TP53* and *IDH2* driver somatic mutations (13).

While *TP53* alterations can be attributed to a germline event, the extremely low cfDNA percentages favors that that alteration is not present in the germline. Given the allelic frequency and association of *TP53* (C141fs) and *IDH2* mutations in cholangiocarcinomas as well as the lack of radiographic abnormalities indicating the presence of other malignancies, the most likely conclusion is that this patient does indeed have cholangiocarcinoma. Of note, the *IDH2* mutation identified (R140) has been commonly reported as a somatic mutation seen in acute myelogenous leukemia, not cholangiocarcinoma [14]. However, there was no laboratory or other evidence of acute myelogenous leukemia in the patient described. Confirmatory NGS testing of non-hematopoietic tissue (e.g. skin) showing a lack of these altered genes would further exclude the possibility of germline alterations or CHIP.

In summary, cfDNA NGS testing is increasingly used to identify actionable molecular abnormalities that represent potential therapeutic targets. This case serves as an example of how molecular alterations identified using cfDNA NGS testing can be used to suggest a diagnosis of cholangiocarcinoma. Future reports of additional patients diagnosed based on cfDNA NGS testing will lend credibility to cfDNA NGS testing as a valid tool for diagnosing cholangiocarcinoma, particularly when combined with resected specimens that are histologically confirmed as cholangiocarcinoma. In cases where a tissue biopsy is not an option or multiple attempts have failed to yield a tumor diagnosis, perhaps

one can consider cfDNA NGS testing to suggest a genomic diagnosis of malignancy.

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