Beyond Standard Practice in Liquid Biopsy: Selective Venous Sampling

Bruno Damascelli, MD, Vladimira Tichà, MD, Elena Repetti, MD, and Tshering Dorji, MD

ABSTRACT

Liquid biopsy is a molecular diagnostic procedure that aims to provide readily accessible genetic profiling of tumors for primary diagnosis, detection of minimal residual or metastatic disease, and therapeutic decision-making, especially for molecularly targeted treatments. Cancers release various biological markers into the circulation, although the most widely used are cell-free tumor DNA and circulating tumor cells. The paucity of biological material means that laboratory methods mainly based on genetic sequencing expose this innovative diagnostic method to a considerable incidence of false negatives. The 3 cases presented here show how the sensitivity and specificity of liquid biopsy may be improved through selective venous sampling.

ABBREVIATIONS

CTC = circulating tumor cell, ctDNA = circulating tumor DNA

The term liquid biopsy refers to methods for the molecular analysis of biological markers released into bodily fluids by tumors (1). Circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and other elements, such as exosomes and the new class of RNA, microRNA, are extracted from peripheral blood samples for tumor characterization. Liquid biopsy is based on the detection of cancer-related mutations that have diagnostic, prognostic, and therapeutic implications. A major challenge in the clinical application of liquid biopsy is the paucity of ctDNA and CTCs in peripheral blood samples, wherein CTCs account for only 1 in 600,000 nucleated cells (2). Detection can be increased by drawing a considerably larger volume of blood or by implementing experimental enrichment methods based on costly technologies for active CTC recovery. Both solutions are challenging to put into practice. A simpler way to increase ctDNA extraction and CTC capture might be selective venous sampling from the veins draining blood from one or more of the body districts invaded by the tumor. This technique is directly borrowed from that long in use for hormone assays and in the diagnosis of neuroendocrine tumors, such as insulinomas (3,4). Percutaneous venous catheterization via the peripheral veins allows access to all body districts, enabling significant amounts of ctDNA and a larger number of CTCs to be detected by avoiding the dilution of these biological markers in the total blood volume that occurs with peripheral sampling. We report 3 cases in which this method was successfully applied prior to tissue biopsy in patients referred to us by their attending oncologists. In each case, selective venous samples were compared with contemporaneous peripheral samples drawn from an arm vein. In 2 patients, sampling was repeated after resection surgery. The volume of blood drawn from each site was 8 mL.

All procedures involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was requested and signed by all patients.

Case 1: A 74-year-old asymptomatic female, non-smoker, with no significant personal or family history, presented with left supraclavicular and laterocervical adenopathy, along with elevated levels of carcinoembryonic antigen and cancer antigen 15.3. Contrast-enhanced computed tomography (CT) revealed supra- and subdiaphragmatic adenopathy, which was positron emission tomography-positive, with no significant uptake in the lungs. Mammography and

From the Departments of Interventional Oncology (B.D., V.T.) EMO GVM Centrocuore Columbus, Milano, Italy, Chief Geneticist (E.R.), and Chief Pathologist (T.D.), TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio (VA), Italy. Received October 13, 2020; final version accepted February 13, 2021. **Address correspondence and reprint requests to** B.D., Via Edoardo Bassini 17/2, 20133 Milan, Italy.; E-mail: b.damascelli@gmail.com

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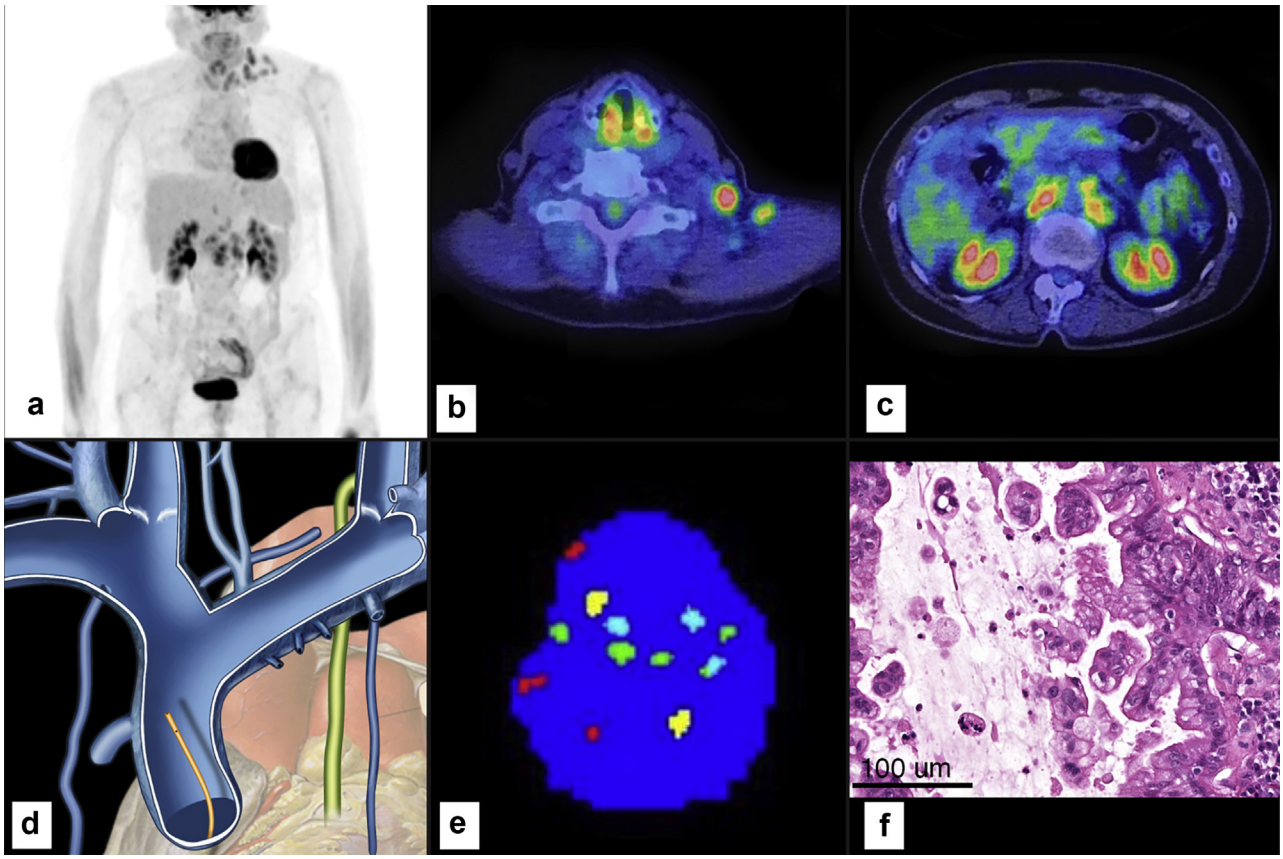

Figure 1. Lung cancer evidenced only by lymph node metastases. (a) PET-18FDG scan showing uptake in multiple supra- and subdiaphragmatic lymph nodes. (b) Enlarged left supraclavicular lymph node. (c) Enlarged retroperitoneal lymph nodes. (d) Superior vena cava catheterization via the femoral vein for selective liquid biopsy sampling. (e) Fluorescent in situ hybridization (FISH) of the abnormally irregular outline of a CTC isolated from the superior vena cava blood sample. (f) Supraclavicular lymph node histology suggestive of lung adenocarcinoma metastasis.

gynecological examination results were negative for tumors. Liquid biopsy yielded 2.15 ng/μL of fragmented DNA and KRAS gene mutation in the superior vena cava, 1.75 ng/μL of DNA in the inferior vena cava, and 1.55 ng/μL of DNA in the peripheral blood sample. Three CTCs were detected in the superior vena cava, although none in the inferior vena cava or in the peripheral sample. Histologic examination of the supraclavicular lymph node resulted in the diagnosis of adenocarcinoma of pulmonary origin (Fig 1). Selective sampling provided information that would have been missed by peripheral sampling alone in this patient with a negative pulmonary imaging result.

Case 2: A 79-year-old male presented with a nevus on the upper third of the left arm suspicious for melanoma. Liquid biopsy with sampling from the left subclavian vein yielded a 1.5-ng/μL of fragmented DNA with NRAS gene mutation, related to melanoma. A peripheral blood sample collected simultaneously yielded 0.6 ng/μL of DNA without mutations. Radical resection confirmed the diagnosis of T1a superficially spreading melanoma (Fig 2). Tissue-based molecular diagnosis confirmed the NRAS gene mutation. Repeat liquid biopsy with similar procedure 6 months after surgery yielded a negative result for the NRAS mutation, and ctDNA decreased to 0.46 ng/μL. The diagnostic difference between the liquid biopsy on a peripheral sample (ctDNA, 0.6 ng/μL) and that on a selective sample (ctDNA, 1.5 ng/μL) was remarkable. The NRAS gene mutation was expressed by a superficial melanoma in the absence of mitosis or local invasion.

Case 3: A 72-year-old male with a paternal family history of prostate cancer presented with a prostate-specific antigen of 4.55 ng/mL and a Prostate Imaging Reporting and Data System score of 4. Liquid biopsy with sampling from the peripheral vein (DNA, 0.69 ng/μL), right hypogastric vein (DNA, 0.50 ng/μL), and left hypogastric vein (DNA, 0.62 ng/μL) revealed an HNF-1A gene mutation related to prostate cancer in all samples. CTC counts in the same samples were 6, 4, and 5 in the right hypogastric vein, left hypogastric vein, and peripheral blood, respectively. After confirmation by percutaneous biopsy, radical prostatectomy was performed, and bilateral grade 7 prostate adenocarcinoma (Gleason 4+3) without lymph node metastasis was found (Fig 3). Repeat liquid biopsy 6 months after yielded a negative finding for the HNF-1A gene mutation. No CTCs were detected at this time, and ctDNA deceased to 0.1 ng/μL. In this case, the peripheral blood sample was also diagnostic, although selective sampling from the 2 hypogastric veins yielded a larger total amount of ctDNA.

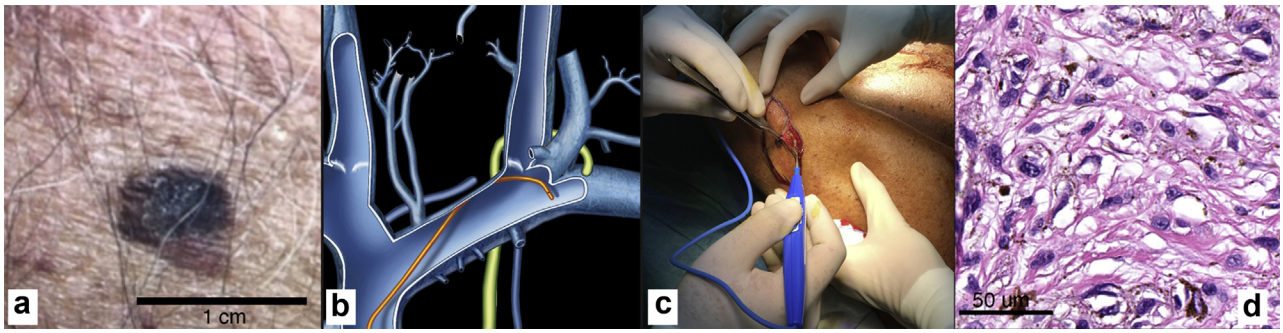

Figure 2. Left arm melanoma. (a) Appearance of melanotic nevus at presentation. (b) Venous sampling from the left subclavian vein with contemporaneous peripheral sampling. Following the finding of a melanoma-related mutation only in the subclavian vein sample, one-time treatment by radical resection was decided. (c) Melanoma excision. (d) Histology showing a superficially spreading melanoma with Breslow 0.9 mm, Clark III, 0 mitosis, horizontal growth phase present, and ulceration absent.

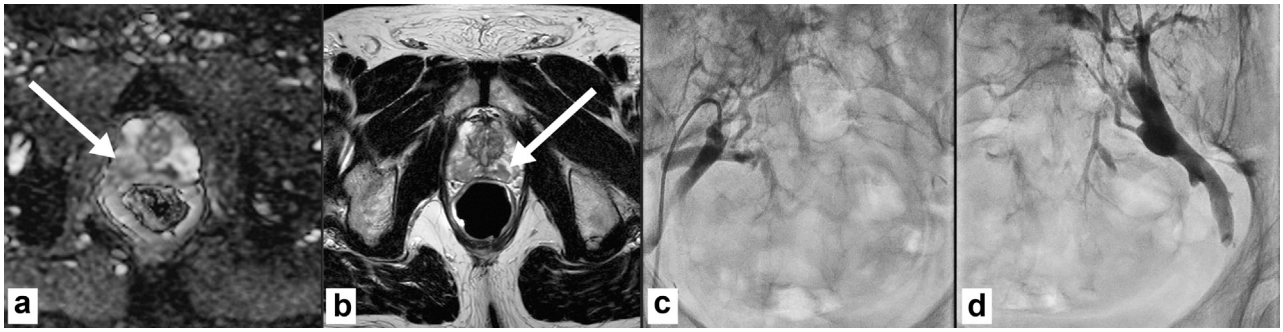

Figure 3. Prostate cancer. (a) Magnetic resonance showing a PIRADS 3 lesion (arrow). (b) PIRADS 4 lesion (arrow). (c, d) Phlebography check with a contrast agent.

DISCUSSION

The potential uses of liquid biopsy are manifold, ranging from the early diagnosis of cancer to the detection of minimal residual disease, recurrence, or metastasis not detectable by imaging. In particular, liquid biopsy can be repeated during cancer monitoring more easily than tissue biopsy, which is not always risk-free (4). Even heterogeneous genome mutations expressed by a tumor during its evolution can be detected by ctDNA extraction, whereas tissue-based biopsy reflects the mutation status at the point of sampling. This information is of therapeutic interest because it can reveal acquired resistance to treatment or provide an early confirmation of sensitivity to treatment. The ctDNA is mixed with cell-free DNA, which is physiologically released by the tissues through a similar mechanism as tumor cell apoptosis and necrosis (5). The minimal quantity of ctDNA in the peripheral blood samples limits the potential application of liquid biopsy owing to the significant number of false-negative results; moreover, the diagnostically effective moment of shedding of ctDNA is unpredictable. Despite these limitations, liquid biopsy is used as a diagnostic adjunct in lung, colon, breast, and prostate cancer as well as in melanoma (6).

The cases reported here appear to support the possibility of improving the diagnostic accuracy of liquid biopsy; thanks to selective venous sampling, which theoretically captures a higher concentration of ctDNA and a larger number of CTCs than peripheral blood sampling. This approach is based on an intuitive concept that is by no means new. Selective sampling has already been applied for the topographical identification of endocrine tumors (7), and it has recently been extended to pelvic prostate-specific antigen sampling with a good correlation to the site of prostate cancer (8). Access to all body districts through percutaneous catheterization via a peripheral vein is certainly more practical than that of venous sampling during open surgery, which has confirmed the greater sensitivity and relevance of selective sampling (9,10).

The analysis of biological markers released by a tumor is rapidly evolving; therefore, it is likely that in the near future, the sensitivity of liquid biopsy may reach levels that no longer require the greater efficacy provided by the selective venous sampling method that we propose. At present, this procedure could be used in appropriate cases, for example, when a result of peripheral liquid biopsy or tissue biopsy is negative. It is a minimally invasive outpatient procedure that can have significant diagnostic and therapeutic repercussions.

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