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Letter to the Editor

HPV-circulating tumoural DNA by droplet-based digital polymerase chain reaction, a new molecular tool for early detection of HPV metastatic anal cancer? A case report

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Dear Editor,

Incidence of anal cancer has been increasing dramatically since 10 years, especially in HIV-positive men who have sex with men (HIV+MSM) [1,2]. Most anal cancers are due to human papillomavirus (HPV)infection [3], and the natural history of anal dysplasia is poorly known. Recent retrospective data suggested that untreated high-grade anal intraepithelial neoplasia (HGAIN) progression to anal cancer approximates 1–2% per patient-year [4,5]. HGAIN follow-up and treatment are not standardised, ranging from simple surveillance to destruction

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but with a limited efficacy. New tools to identify lesions at highest risk of progress to cancer are needed.

2. Case report

Herein, we report the case of a 59-year-old HIV+MSM patient who presented with vertebral metastasis of an HPV16-induced anal neoplasia.

Diagnosed with HIV infection in 1990, his HIV RNA plasma levels were undetectable under antiretroviral treatment since 2006.

In 2016, during his recommended anal screening, HPV16 was detected in his anal swab, whereas normal cytology and the standard anoscopy did not reveal any abnormality. High-resolution anoscopy (HRA) was further performed according to our screening strategy in European Georges Pompidou hospital [6]. According to the proctologist, condyloma was observed associated with presumed HGAIN and confirmed by the pathologist who performed biopsy. In parallel, HPV16, HPV11 and HPV6, the last two being the major cause of condyloma, were also detected by polymerase chain reaction (PCR). In October 2016, this HGAIN was surgically resected and definitely confirmed on the surgical tissue sample with free resection margins.

In April 2018, the patient was hospitalised for back pains associated with fever and recent weight loss (4 kg), a CT scan showed abscessed lesion of the psoas with L5 spondylitis. An extended microbiology screening and puncture of the abscess mass remained strictly negative. Bone biopsy found aspecific inflammation. However, besides recurrence of fever at the end of July, a second CT scan showed progression of the spondylitis and increased size of the psoas abscess. On a new bone biopsy, the pathologist concluded to a metastasis of squamous cell carcinoma (SCC). Immunohistochemical assay showed p16 expression, and HPV16 DNA was detected by PCR. The diagnosis of anal SCC with advanced local extension and metastasis at the L5 vertebra was suspected and finally retained considering the HPV16 HGAIN history, despite the lack of proof of infiltrative SCC after proctologic examination and anal biopsy sample at the time of metastasis diagnosis.

Faced with the difficulty to diagnose this particular metastatic anal cancer, we investigated whether HPV16-circulating tumour DNA (HPV16ctDNA) could have been detected earlier in plasmas collected for HIV follow-up. With patient consent, HPV16ctDNA detection was retrospectively performed by droplet-based digital PCR (ddPCR) on nine plasma samples collected before the first diagnosis of HGAIN, at the time of HGAIN diagnosis and just after HGAIN resection up to the metastatic cancer diagnosis.

DdPCR of HPV16 E6 gene was performed on a RainDrop Digital PCR System (RainDance Technologies, Bio-Rad). Simultaneously, albumin was detected as an internal control.

HPV16ctDNA was undetectable in the two samples in 2015 and at the time of HGAIN diagnosis. In contrast, HPV16ctDNA became weakly positive in December 2016 (1.07 log cp/mL), just after the surgical treatment, and kept on increasing on subsequent samples from 1.97 log cp/mL in November 2017 to 4.63 log cp/mL in May 2018. Finally, HPV16ctDNA rose up to 5.21 log cp/ml in July 2018, just before the metastatic anal cancer diagnosis (Fig. 1).

3. Discussion

Different authors have recently already shown the feasibility of detecting HPVctDNA using ddPCR in the plasma of patients with HPV-related cancer [7,8]. DdPCR has been showed to be highly efficient for the detection of rare events and particularly relevant for ctDNA detection [9,10]. It has already been shown that women with cervical cancer have detectable HPVctDNA by ddPCR even in early-stage cases, whereas it is strictly negative in patients with high-grade cervical intraepithelial lesion. Moreover, other studies performed in advanced anal cancer suggested that HPVctDNA is an interesting biomarker to monitor the efficacy of cancer therapy [11–13] and could also represent a new prognostic biomarker in such cancers [12]. However, no studies of HPVctDNA have been carried out during follow-up of HGAIN to perform early detection of invasive or metastatic anal cancer.

To the best of our knowledge, it is the first time that early detection of HPV-induced cancer invasion by ddPCR and HPVctDNA increase in a patient from HPV16 HGAIN to metastatic cancer have been demonstrated. It is tempting to claim that HPV16ctDNA monitoring in this patient could have potentially allowed a better medical management thanks to an earlier metastasis diagnosis.

The physiopathology and rapid progression of this vertebral metastasis remain unclear. Other origins of HPV-induced metastasis were ruled out by intensive diagnosis procedures. It could also be suggested that the pathological diagnosis of an invasive anal cancer was initially missed. However, this hypothesis is unlikely as the diagnosis of HGAIN was confirmed twice on independent anal tissue samples by different pathologists. Similarly, HRA clinical observation and HPV16ctDNA negativity at the time of HGAIN do not support this hypothesis. Therefore, the kinetics of HPV16ctDNA increase and the metastasis localisation near the anal canal could lead us to consider an eventual impact of the surgical resection in the vertebral metastasis genesis. Indeed, it could be postulated that pretumoural cells, released in the circulation after surgical resection and early detected by HPV16ctDNA, could have consecutively colonised vertebral bone at the origin of the

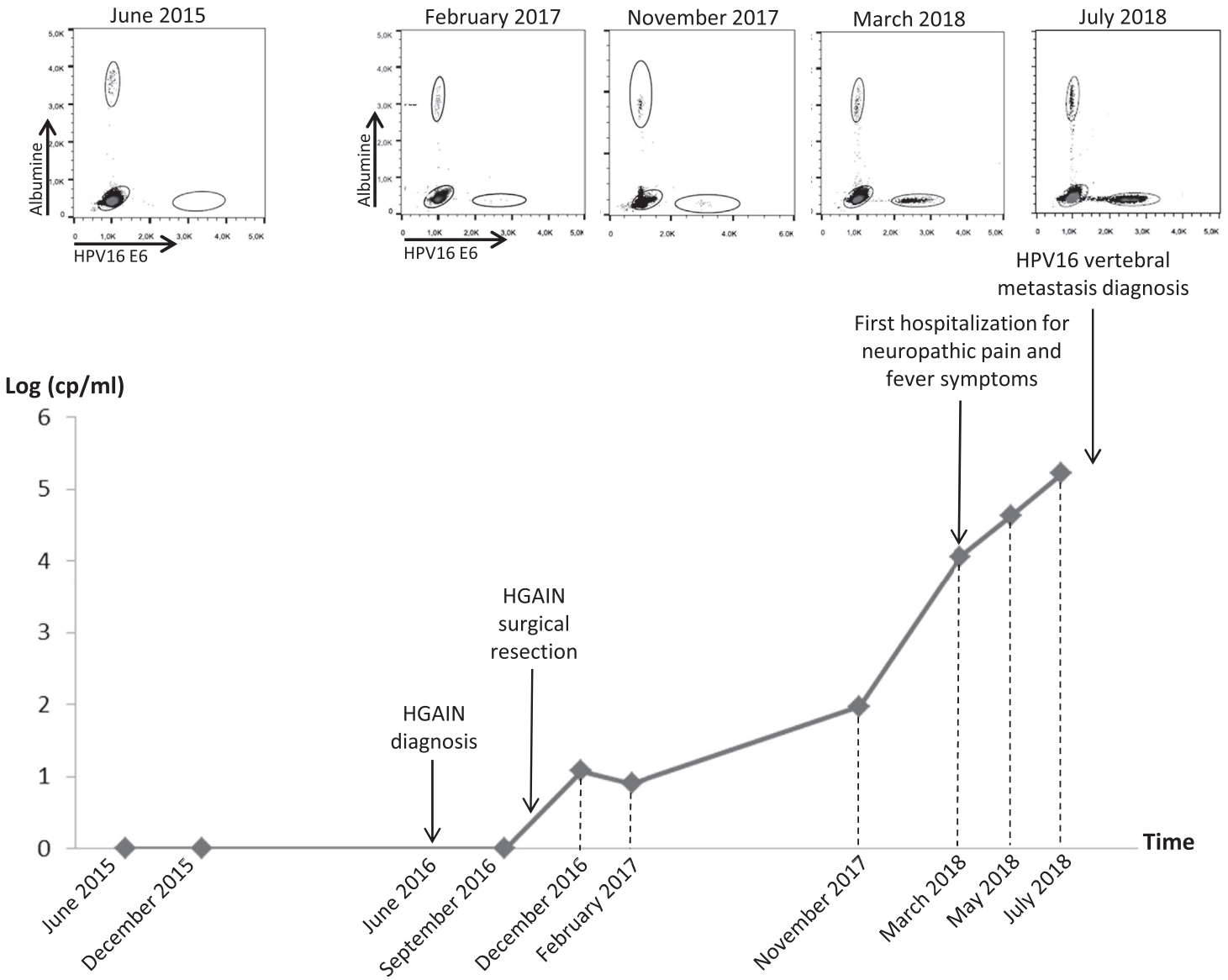

Fig. 1. Kinetics of HPV16-circulating tumoural DNA plasma concentrations monitored by ddPCR between June 2015 (1 year before high-grade anal intraepithelial neoplasia diagnosis) and July 2018 (1 month before HPV16 vertebral metastasis diagnosis. ddPCR, droplet-based digital PCR.

metastasis. To our knowledge, cancer metastasis genesis in these surgical conditions has never been reported, and this case can only raise the question without providing definitive confirmation. Finally, although this clinical case is atypical, the contribution of ddPCR to early monitoring of anal metastatic cancer genesis confirms its strong potential. It will be interesting to perform prospective studies on HGAIN cohorts to assess whether HPVctDNA could be used as an easy and non-invasive early dynamic biomarker to monitor and manage HGAIN progression. Such studies could be firstly conducted in HIV+MSM patients at higher risk of invasive anal cancer and annually followed up for HPV-related anal lesions.

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Conflict of interest statement

None declared.

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