Liquid Biopsy for Ophthalmic Malignancies

Subjects: Oncology

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Intraocular solid malignancy biopsy is usually not performed due to the risk and fear of extraocular extension. Recently, liquid biopsy has gained in popularity in this field. Liquid biopsy allows the diagnosis of intraocular malignancies as well as its monitoring in retinoblastoma. Liquid biopsy may help the clinican to better understand the metastatic spread, especially in uveal melanoma.

Keywords: liquid biopsy ; circulating tumor cells ; circulating tumoral DNA ; aqueous humor ; choroidal metastases ; uveal melanoma ; retinoblastoma

1. Introduction

Tissue biopsy is still considered to be the gold standard for establishing the diagnosis of cancer. However, a tissue biopsy is rarely repeatable over time [1], sometimes associated with significant morbidity, and contraindicated in several malignancies [2]. Recently, the Food and Drug Administration (FDA) approved the use of liquid biopsy (LB) as a pertinent diagnosis, prognosis, and monitoring tool [3][4] in non-small cell lung carcinoma (NSCLC) to avoid invasive tissue biopsy in selected cases [5]. Compared to tissue biopsy, LB has indeed the advantages of being non-invasive, collected from multiple body fluids such as blood, urine, saliva, cerebrospinal fluid, and aqueous humor [6]. At molecular diagnosis, LB detects of several biomarkers such as circulating tumor cells (CTC), circulating tumoral DNA (ct-DNA), circulating tumoral RNA (ct-RNA), micro RNA (miRNA), tumor-related exosomes (TREs), and tumor-educated platelets (TEP) [5].

Retinoblastoma (RB) and uveal melanoma (UM) are the most common primary intraocular tumors in childhood and adulthood, respectively [2][7]. Conversely to other cancers, a tissue biopsy is usually not readily available in intraocular malignancies. Intraocular biopsy carries the risks of irreversible intraocular damages, tumoral dissemination, and low sensitivity rates, due to the low amount of tissue harvested [8][9]. Therefore, RB and UM are usually treated based on clinical and radiological findings without histological tissue confirmation [10]. This could explain why LB has gained in clinical practice in intraocular malignancies.

Interestingly, RB and UM highlight two radically opposed LB paradigms. RB is an intraocular tumor encountered in children that develops intraocularly even in advanced stages. Metastasis and relapse are exceptional, and the prognosis is currently excellent [2]. UM is encountered in adult patients, develops intraocularly but early disseminates in the bloodstream. It is estimated that about half of the patients will develop further metastases and will die in the next 24 months despite achieving local control of the tumor [7]. Based on these clinical features, RB is more eligible to an "ocular LB", whereas UM is more eligible to a "systemic LB".

2. Liquid Biopsy in Uveal Melanoma (UM)

UM is the most common primary intraocular tumor encountered in adulthood [7]. UM diagnosis is based on clinical and B-scan ultrasonography findings. Transscleral and intravitreal UM biopsies have proven to be challenging, associated with low positivity rates, intraocular complications, and extraocular tumor spread [11]. To date, the main indication for performing a tissue biopsy is to assess whether the patient is at low or high metastatic risk [12]. Local treatments include proton beam therapy and brachytherapy for small to medium-sized tumors, and enucleation of the eyeball for larger tumors. Local control is achieved in up to 95% of cases, even in larger UM [10]. Synchronous metastatic spread at the time of the primary tumor treatment is an exceptional finding [7]. Despite this, it is established that around one-third to 50% of the patients will experience a metastatic spread in the ten years following the diagnosis highlighting the concept that UM cells escape from the primary tumor very early and remain in dormancy for a while [13][14]. It is still unclear whether the liver is the primary metastatic site of UM. Despite a better molecular understanding, no treatment has shown efficacy for treating UM metastases [15]. When metastatic spread occurs, the overall survival (OS) does not usually exceed 24 months [16].

For a few years, attention has been directed to classify UM patients as low or high metastatic risk based on chromosomic and genetic abnormalities. The aim is to detect as early as possible the metastatic spread to include patients in ongoing clinical trials [17].

Identifying a reliable biomarker in UM would be of high clinical relevance. The ideal biomarker should be sensitive, specific, non-invasive, and reproducible [5]. Several pathophysiological factors highlight the fact that LB is a very appealing tool in UM [18]: (i) UM disseminates through the bloodstream many months to years before the local treatment, (ii) LB may provide the missing tumor genetic insights of the primary tumor and in-transit metastases, and (iii) given the lack of effective treatment on UM liver metastases, early LB detection of UM spread may improve patient management with a quicker referral of the patient to clinical trials.

After summarizing the current knowledge on the molecular characteristics of UM, we will develop the different components of venous LB: CTCs, ct-DNA or ct-RNA), non-coding miRNA, TREs and TEPs.

3. Retinoblastoma (RB)

RB is the most common primary intraocular malignancy in childhood. RB arises from the photoreceptors located in the inner retinal layers. RB is the result of a mutation of the tumor suppressor gene RB1 located on chromosome 13q [19]. Non-inherited forms are usually unilateral, whereas bilateral or trilateral (pineal gland involvement) RBs are mainly encountered in inherited forms. RB is usually diagnosed when the patient is approximately two years old [2]. Leukocoria and strabismus are the most common clinical signs leading to the diagnosis. Tissue biopsy is usually contraindicated since it is thought to favor extraocular dissemination. Several differential diagnoses such as Coats disease, persistent fetal vasculature, retinopathy of prematurity, coloboma, and toxocariasis may be misdiagnosed as an RB despite using optical coherence tomography and B scan ultrasonography [2]. Studies on enucleated eyes also found significant somatic copy number alterations such as gains on 1q, 2p, 6p, and losses on 13q and 16q [20]. Treatment is not consensual and is based on the laterality involvement and the TNM classification. Localized intraocular RB can be treated by cryotherapy or laser therapy. Intravitreal seeding can now be safely treated by intravitreal injection of chemotherapy medications [21]. More advanced cases may be treated by intraarterial chemoembolization or enucleation. Rarely, retinoblastoma may spread locally into the orbit and the brain through the optic nerve and disseminate to the bone marrow and later to visceral organs [2]. Despite this, the prognosis is usually excellent [22].

Tissue biopsy in RB is relevant for two reasons. Firstly, the biopsy allows for diagnosis confirmation. Differencing RB from Coats disease appears as particularly challenging, and some infants may undergo an enucleation for diagnosis purposes [23]. Secondly, the tissue sample allows the assessment of Rb1 mutational status for prognostic counseling [22]. However, RB biopsy is contraindicated, due to the fear of extraocular tumor seeding [2], and the rate of enucleation has been dramatically reduced thanks to eye-sparing strategies [21]. Therefore, LB has emerged as a possible useful diagnostic and monitoring tool.

In contrast to UM, the hematogenous spread is rarely encountered in RB, and the aqueous humor (AH) sample has gained interest [20]. AH may provide diagnosis, genetic, prognosis, and treatment response data. AH puncture is an easy, relatively non-invasive, and safe procedure performed under general anesthesia in infants. AH sampling may be combined with eye examination performed under general anesthesia in infants, as well as in combination with intravitreal delivery of chemotherapy. Berry et al. demonstrated that a higher AH somatic chromosomal copy number alteration, including 6p gain, was predictive of more advanced and aggressive RBs [24][25]. They found that AH ct-DNA was concordant with ct-DNA providing from a tissue sample of enucleated patients. The same team demonstrated that AH provided a higher ct-DNA sensitivity compared to the blood sample [6]. Gerrish et al. also identified ct-DNA in AH samples of 12 RB patients [26]. AH ct-DNA profile was identical to this found in enucleation samples. Interestingly, a lower quantity of AH ct-DNA was found in patients treated by intravitreal injection of chemotherapy. In recent years, the rate of enucleation for the treatment of RB has been dramatically reduced, limiting tissue analysis to establish the mutational status of the RB1 gene. Kothari et al. recently demonstrated that circulating plasma DNA was able to assess RB1 mutation status non-invasively without the need for biopsy or enucleation [27].

Other AH contents have been investigated, but most of them are limited by their lack of specificity and the lack of genetic status assessment. AH LDH was found to be increased in locally advanced retinoblastoma compared to healthy controls. However, AH LDH did not correlate with the clinical features, the treatments underwent, and serum LDH. In addition, LDH is not specific and may be elevated in glaucoma (sometimes encountered in locally advanced RB) and Coats disease, which is a challenging differential diagnosis [28]. Some studies investigated the AH detection of Neuron Specific Enolase (NSE) secreted by numerous neuroendocrine tumors. These studies found that NSE was increased in enucleated RB eyes compared to controls but failed to demonstrate a correlation with clinical and pathological features [28]. Survivin and

TGF-β were found to be elevated in AH and serum of patients with RB with high sensitivity and specificity rates [20]. Unlike the previous biomarkers, they were positively correlated with the clinical and pathological features, especially optic nerve invasion [20][28]. Further studies on these biomarkers are warranted.

Serum biomarkers have been less investigated. Beta et al. demonstrated that several serum miRNAs were up and downregulated in 14 RB patients [29]. They found that miRNA-17, miRNA-18a, and miRNA-20a were upregulated and could be considered as potential biomarkers [29].

The development of AH puncture has led to reconsider the rule, which stated that an RB eye should never be violated. Since tissue biopsy is still contraindicated, retinoblastoma research is now directed to AH analysis for establishing RB diagnosis, prognosis, and treatment response monitoring. Relevant and large sample sized studies are currently ongoing to assess the indications of AH puncture and to determine the best biomarker.

4. LB in Conjunctival Malignancies

Conjunctival melanoma and squamous cell carcinoma are rare ocular neoplasms. Contrary to intraocular malignancies, the biopsy is routinely performed in conjunctival tumors to confirm the diagnosis and identifying key mutational status [30]. Taken together, this could explain why LB has been little investigated in conjunctival tumors.

Conjunctival melanoma accounts for less than 5% of ocular tumors but is associated with a mortality rate of around 30% [31]. Despite its location, conjunctival melanoma behavior is more related to cutaneous melanoma rather than uveal melanoma [32]. Unlike UM, conjunctival melanomas disseminate through lymphatics and hematogenous routes, harbor *NF1, BRAF, NRAS*, and *KRAS* mutations [30], and may be treated with targeted therapies [33] and immunotherapies [34][35]. LB has not been studied specifically in conjunctival melanoma. However, one might hypothesize that LB techniques developed in cutaneous melanoma may be relevant in conjunctival melanoma [36].

Conjunctival squamous cell carcinoma is an exceptional ocular surface malignancy with an incidence of 2–35 per million [37]. Conjunctival carcinoma is strongly related to UV exposition and HIV. Recently, HPV infection has been incriminated in conjunctival carcinoma development [38]. Dissemination may be local to the orbit, lymphatic, and/or hematogenous. Treatment involves surgery sometimes associated with topical chemotherapy and/or radiation beam therapy [39]. To date, there is no standard of care for metastatic conjunctival carcinoma.

5. LB in Choroidal Metastases

Although being often asymptomatic, choroidal metastases are the most common intraocular malignancies in adulthood [40]. Breast and lung carcinomas are the most common primary tumor encountered [40]. The increasing overall survival seen in several malignancies, including breast and lung cancers, has led to a higher detection rate of choroidal metastases [40]. In most cases, choroidal metastases occur at a late stage of the disease, and LB does not appear to be useful. LB may be relevant in the case of (i) unknown primary malignancy, (ii) difficulty for performing the biopsy of the primary tumor, and (iii) if the patient underwent multiple malignancies. Choroidal metastases may be the first clinical manifestation of the underlying malignancy in about one-third of patients [41][42]. In their study conducted in 420 and 96 patients experiencing choroidal metastases, Shields et al. [42] and Konstantidinis et al. [41] did not know the primary tumor in 34% and 28% of their patients, respectively. Several patients benefited from an intraocular biopsy to identify the primary tumor. This biopsy may be risked and associated with a lack of sensitivity, given the low amount of tissue available [8]. LB may be a particularly useful and non-invasive method for identifying the primary tumor. Our team recently reported a patient with bilateral choroidal metastases without a known primary tumor. Plasma ct-DNA providing from a Non-Small Cell Lung Carcinoma (NSCLC), was found. In addition, EGFR mutation was identified, and targeted therapy was successfully initiated [43]. Aqueous humor analysis may also provide new insights. Daxecker et al. reported 40 years ago, a case of elevated anterior chamber CEA in a patient with bilateral choroidal metastases from breast carcinoma [44].

6. Conclusions

LB is a non-invasive and promising technique for diagnosing and monitoring intraocular malignancies. LB may be useful in daily clinical practice to (i) confirm the cancer diagnosis without tissue biopsy, (ii) to establish a reliable prognostication, (iii) to allow early detection of metastatic spread, and (iv) monitoring treatment response. LB may also provide new pathophysiological insights concerning tumor dissemination and dormancy. Although very promising, LB suffers from

several inherent limitations. To date, there is a lack of consensus regarding the ideal biomarker. Pre- and post-analytic processes differ widely from a study to another, limiting their reproducibility. The biggest challenge will be to establish an international consensus among the ocular oncology centers.

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