The ROYAL COLLEGE of OPHTHALMOLOGISTS

REVIEW ARTICLE

Choroidal biopsies; a review and optimised approach

R. N. Hussain[1]✉, B. Damato[2,3] and H. Heimann[1]

The majority of choroidal tumours are diagnosed accurately with clinical examination and the additional data obtained from non-invasive imaging techniques. Choroidal biopsies may be undertaken for diagnostic clarity in cases such as small melanocytic or indeterminate lesions, identifying the primary tumour in the case of choroidal metastases or the subclassification of rarer conditions such as uveal lymphoma. There is however an increasing use of biopsy techniques for prognostication in uveal melanoma. This review explores the main indications and surgical techniques for tumour acquisition, and the optimised approach utilised by the current authors to improve successful yield for histological and genetic analysis.

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INTRODUCTION

Unlike most oncological specialities, the majority of choroidal tumour diagnoses are made combining clinical features with non-invasive technology aimed at imaging in detail the deeper structures of the chorioretinal boundary. There have been comprehensive clinical descriptions of risk factors associated with melanocytic lesions and risk for malignant transformation [1–4] including symptomatology, overlying lipofuscin (a sign of cellular turnover), lesion dimensions (basal diameter and height), ultrasound characteristics (internal reflectivity, visible vascular pulsations), subretinal fluid, proximity to the optic nerve head and documented growth. Cumulative information from clinical examination, ocular coherence tomography (OCT), ultrasound biomicroscopy and fundus autofluorescence are usually sufficient to confirm the diagnosis [5–10]; other more invasive techniques such as fundus fluorescein angiography and indocyanine green, as well as radiation emitting imaging such as positron emission tomography and computerised tomography may assist in ruling out less common pathologies, or be utilised in individualised treatment planning [11–18]. The diagnostic accuracy in medium-sized and large tumours utilising clinical and imaging methods is >99% in experienced hands [19]. As such, histopathological verification is predominantly unnecessary. In this review, we describe the indications and considerations of choroidal biopsy.

INDICATIONS

Diagnostic biopsies

There are three main indications for a choroidal biopsy for diagnostic purposes.

1. Diagnosis of small melanocytic or indeterminate lesions for observation or treatment. It was historically considered that micrometastases would have disseminated by the time of presentation of uveal melanoma, awaiting a positive environment for the development of macrometastases [20]; as such, traditional management of such patients was almost universally observation to monitor for progression. However, increasing evidence suggests that earlier diagnosis and treatment of these small but cancerous tumours may improve overall survival [21–24]. As such, there is a trend towards treating small melanomas earlier, with the aim to reduce the risk of metastatic disease. With the advent of more abundant imaging in the community and the wide availability of OCT scanning, more small lesions are being identified [25]. Although definitive small melanomas are often now treated at an earlier stage, the options for indeterminate melanocytic lesions (such as those with multiple risk factors for the diagnosis of choroidal melanoma but the absence of others) includes a period of observation, treatment with radiotherapy (or alternatives such as photodynamic therapy (PDT)) or diagnostic biopsy. The relative risks and advantages to each of these options can be discussed with the patient to enable a decision. Biopsy of these smaller lesions is therefore becoming more common. Should such biopsies demonstrate a malignant lesion, rapid and prompt treatment should be planned and recommended. In Liverpool, should there be a high suspicion of melanoma and the patient wishes for a diagnostic biopsy to confirm, we may put tantalum markers on at the time of biopsy to expedite proton radiation treatment following the histopathological analysis.
2. Choroidal metastases are identifiable from clinical and imaging features such as the creamy colour, presence of subretinal fluid, often multiple/bilateral and classical uneven appearance on the OCT and on ultrasound biomicroscopy [26]. Although the most common causative primary tumours include those originating from the breast and lung, approximately 30% of patients with choroidal metastatic lesions have no known primary tumour at the time of presentation. Systemic screening to identify the primary tumour is common, however in Liverpool the trend is to undertake a choroidal biopsy to allow identification of the location of the

[1]Liverpool Ocular Oncology Centre, Royal Liverpool Hospital, Liverpool L7 8XP, UK. [2]Ocular Oncology Service, Moorfields Eye Hospital, London EC1V 2PD, UK. [3]NIHR Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London EC1V 2PD, UK. ✉email: rumanahussain@hotmail.com

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primary tumour (within a few days) to enable quick and targeted investigation and treatment by the medical oncologists subsequently involved [27]. Larger tumour samples are required to enable multiple immunostaining, targeting various organ systems.

3. The clinical diagnosis of uveal lymphoma is often challenging due to the classic masquerading nature of the disease. Biopsy is often required to enable subclassification [28–32]. Haemato-oncologists will base systemic therapies on the pathological subtype of lymphoma which can only be described on a histopathological level. Primary uveal lymphoma tends to be low-grade B-cell non-Hodgkin lymphoma [33–39]. Secondary manifestations of systemic lymphoma in the eye can also involve the uveal tract [38, 39]. Multiple samples are required with a high cellular yield to enable processing for cytology, immunocytology, immunohistochemistry and PCR [31]. In addition, the fragility of lymphocytic cells adds another dimension to the difficulty in obtaining and transporting biopsy samples, which need to be processed rapidly in the laboratory [40].

Prognostic biopsies

Due to the diagnostic accuracy with clinical and imaging features, the vast majority of choroidal biopsies are obtained for the process of prognostication for uveal melanoma. Although clinical features such as age, sex, tumour diameter, thickness and location (involvement of the ciliary body) and extraocular extension all enable a degree of prognostic estimation, cytogenetic analysis considerably increases the accuracy of these predictions [41–43]. Spindle-cell tumours grow in a compact cohesive fashion surrounded by a dense reticulin framework; epithelioid cells grow less cohesively and are not surrounded by a network of reticulin. Spindle cell melanomas have the most favourable prognosis [44–46]. Aberrations in chromosomes 1, 3, 6 and 8, in particular deletion of one copy of chromosome 3 (termed monosomy 3), have certainly been shown as the strongest predictors for metastatic disease. Not only is monosomy 3 associated with BAP-1 loss, monosomy 3 UM are more likely to be associated with increased inflammatory cell infiltration (macrophages and T-cells) and larger tumour sizes. When using transcriptional profiling based on 12 signature genes, UM can be classified into class 1 and class 2 categories [47–51], with class 1 tumours having a better prognosis and less risk of metastatic disease. Since the development of this classification, they have been subdivided into class 1 A and B and class 2 A and B, based on the presence or absence of a preferentially expressed antigen in melanoma (PRAME) mutation, which is also considered to be a negative prognostic indicator [52]. Individualised risk stratification based on all the molecular characteristics of the tumour better correlates with mortality when combined with all clinical, histological and genetic risk factors. With the analysis of a large number of tumours and the development of increasing numbers of prognostic indicators, it is clear that although there are clear high and low risk UM, there are also many with a combination of high and low risk characteristics that are more difficult to prognosticate. The Liverpool Uveal Melanoma Prognosticator Online (LUMPO) has been internally and externally validated as a very effective and accurate predictor of metastatic risk, and combines a number of key clinical, histopathological and genetic risk factors to produce an individualised outcome predictor [53, 54].

Processing these small samples requires analysis that can obtain information via amplification methods. A number of methods are practised at present including fluorescence in situ hybridization, comparative genomic hybridization, spectral karyotyping, microsatellite analysis, multiplex ligation-dependent probe amplification, gene expression profiling and single-nucleotide polymorphism arrays [42, 49, 55–89]. Next generation sequencing processes created in Liverpool are likely to become more established with the need for smaller tumour samples and a targeted array panel [90, 91]. All of these methods require experienced Pathologist and technician support in the handling and processing of these often very scanty cellular samples.

METHODS OF TUMOUR SAMPLE ACQUISITION

Intraocular biopsies may be taken either via a trans-scleral or a trans-retinal route. Both run risks of complications from the procedure and, in the case of uveal melanoma, the risk of tumour seeding on to the surface of the eye [92–96].

Trans scleral approach

Biopsies taken via the trans-scleral approach tend to be for tumours more anteriorly located (pre-equatorial). This procedure involves creating a scleral flap and cut down to the choroid and obtaining a sample blindly with a small gauge needle (fine needle aspirate, FNAB) usually 25–30 g [97–99]. The technique pioneered in Liverpool can also be undertaken with sampling under direct visualisation with Essen forceps pushed through the scleral cut withdrawing segments of tumour; this gives a much higher yield and therefore higher success rate of both cytological and genetic analysis (99% and 96%) [95]. The scleral tract is then closed with surgical glue, negating the risk of suture tract migration of cells (video supplementary file 1). For uveal melanoma, this technique is most commonly performed at the time of plaque brachytherapy treatment [97], where the plaque (either iodine^{121} or ruthenium^{106}) is placed over the tumour base and therefore over the biopsy site, sterilising any potentially seeded cells and the biopsy tract. This can also be undertaken at the time of plaque removal to negate the risk of local seeding, although the tissues tend to be more oedematous and bleed more during the surgery.

Trans retinal approach

Choroidal samples taken via the trans-retinal route is much more variable in technique. FNAB (25–27 g needle) is also utilised for this approach with the needle inserted via the pars plana into the choroidal via the retina [98–100]. This process may involve no vitrectomy, partial (core) vitrectomy or full vitrectomy prior to sampling [101], usually via 25/27 g ports, to try and minimise non clearing vitreous haemorrhage and vitreous incarceration/traction. Some groups promote maintaining the intraocular pressure (IOP) with an infusion to reduce bleeding whilst others advise keeping the IOP relatively low to prevent egress of fluid and consequent tumour cell surface seeding. It is thought that bending the needle [60–90°] may increase yield due to the oblique nature of the needle track, and also reduce the risk of scleral perforation beneath the tumour base [102, 103]. Viewing techniques also vary from use of the indirect ophthalmoscope to the newer microscope wide field viewing systems.

FNAB has a low rate of complications but a notoriously low yield [97, 104–108] often giving rise to just a cytological result with insufficient sample for genetic analysis. This is certainly related to the tumours size (diameter <5 mm and thickness <2.5 mm) [98, 107, 109]. The Shields group however have reported a high yield with FNAB (84%) in tumours <3 mm in thickness [99].

Additional surgical methods to obtain a higher yield of tumour sample have since been developed. The use of the vitreous cutter has been pioneered in Liverpool [110] (video supplementary file 2) with good yield for both cytology and genetic analysis [25, 92–94, 96, 111, 112]. Although there are some centres that advocate this approach with full vitrectomy and tamponade to reduce the risk of rhegmatogenous retinal detachment from the biopsy site by reducing vitreous traction and incarceration [109, 113] the non-vitrectomy technique with no retinopexy seems to have a low rate of such secondary complications [93, 95], with reduced procedure time and procedure related patient morbidity.

Vitrectomy seems to bear little benefit for this procedure, and it is likely that the formation of a small iatrogenic round hole, with no vitreous traction, supported by a posterior buckling effect of the choroidal tumour, closed by any blood at the time of sampling and a chorioretinal scar formed by any radiation treatment would be protective against such complications.

More invasive methods include retinal incisions with tissue acquisition with an Essen forceps [114]. This gives rise to a high yield of tissue but has a risk of the sample getting caught in the port, increasing the risk of tumour dissemination. In addition, haemostasis in the case of sudden subretinal and/or preretinal haemorrhage during tissue extraction can only be controlled by raising the intraocular pressure, quickest with a vitrectomy infusion set up. Tumour en bloc incisional biopsy has also been described following 20/23 g vitrectomy with a diamond knife retinotomy and direct excision of a 1–3 mm block of tumour extracted with forceps and a subsequent sulphur hexafluoride 20% tamponade [115]. This technique has a higher risk of retinal detachment than others described, but only in cases with a pre-existing exudative detachment. A multicentre study [116] demonstrated that the choice of biopsy technique by individual surgeons was determined by tumour location (anterior vs posterior), tumour size, and the experience of the surgeon in vitreoretinal procedures (more likely to use a direct viewing system and sample via a trans-retinal approach with a cutter).

Whether utilising FNAB or the cutter technique, the treatment of ports also demonstrates variability between techniques, with some suturing sclerotomies, and some advocating cryotherapy in the sclerotomy region to eliminate any seeded cells. There is theoretically less risk of seeding with newer smaller gauge and valved ports.

CONSIDERATIONS OF CHOROIDAL BIOPSIES

Complications

Most disappointing for patients is the failure to obtain a diagnosis/prognostic result due to a poor yield of tumour tissue. The trans-scleral Essen forceps and the trans-retinal vitreous cutter techniques certainly provide more tissue with higher rates of success for histology, immunohistochemistry and cytogenetics than FNAB. The success is of course related to tumour size, although small tumour biopsies can reach high levels of success with experience [95].

The most common complication of choroidal biopsy is vitreous haemorrhage, especially in those utilising vitrectors as a sampling instrument in comparison to FNAB. [93, 94, 96, 107, 112, 117]. Rates of vitreous haemorrhage on day 1 have been reported up to >90%; the majority spontaneously resolve however, and the incidence of persistent haemorrhage requiring further surgery is low (1–3%) with both techniques, but particularly low with FNAB.

Retinal detachment is relatively uncommon via all methods, particularly with FNAB. When retinal detachments do occur, the breaks tend to be away from the biopsy site, suggesting either a mechanism of vitreous base traction during the sampling process, or the promotion of posterior vitreous detachment/vitreous base contraction following the procedure.

Other complications are rare, but include hyphaema, haemophthalmos, subretinal haemorrhage, cataract, endophthalmitis and tumour seeding. The more invasive methods have a higher rate of such complications. Another described complication is the development of scleral thinning/necrosis post plaque brachytherapy at the site of the biopsy [118, 119]. The risk of scleral compromise was higher in larger tumours, likely due to the higher scleral dose at the base.

Need for prognostication

Although the current systemic treatments for metastatic disease are suboptimal in this patient cohort with uveal melanoma, multiple clinical trials are underway and newer drugs are in development, targeting various points along the oncogenic and cell cycle pathways, as well as harnessing and manipulating the tumour microenvironment targeting inflammatory and complement pathways. Tebentafusp has recently been approved for the treatment of systemic dissemination of uveal melanoma [120] targeting patients with a HLA-A*02:01 subtype. It is clear that the future of medical oncology is patient tailored care and it is likely that tumour analysis will be at the forefront of that. Currently, inclusion into clinical trials would not be possible for patients with no cytogenetic evidence of their risk profile for metastatic development.

The prognostication of uveal melanoma into fairly definitive high and low risk groups is uncommon in other forms of malignancy. Despite the lack of definitive treatments, this information is often desired by patients, particularly in the younger age group, to enable ownership of their disease and plan for the future. Psychological analyses have demonstrated that the primary concerns of patients diagnosed with uveal melanoma are prognosis related; there do not seem to be any significant symptoms of anxiety or depression in patients who have undergone a prognostic biopsy in comparison to others also diagnosed with uveal melanoma [121–127]. Prognostication does not, however, completely resolve the level of uncertainty which accompanies a diagnosis of cancer, even for those with a 'good' result.

Prognostic biopsies also enable patient tailored screening and monitoring for systemic disease. There is no consensus on the screening regime for detection of metastatic disease in uveal melanoma patients; traditionally, all patients would undergo liver scans (ultrasound or MRI) twice annually for an indefinite period of time. The predictability of the onset of metastatic disease by the LUMPO system can be utilised with prognostic information to determine individualised screening protocols and duration [22]. This could lead up to £500,000/year of saved costs for unnecessary liver screening for patients at low risk in the UK (where the incidence of uveal melanoma is approximately 600/year [128]).

Tumour heterogeneity

The genetic instability of uveal melanoma cells encourages the accumulation of aberrant mutations. As such, as the tumour progresses and divides, it is likely that amongst the clonal cells, a non clonal subgroup arises with acquired genetic heterogeneity. The presence of genetic variation within a uveal melanoma has been demonstrated [25, 129]. Bagger et all demonstrated up to 13% of enucleated eyes for large uveal melanoma had heterogeneity of chromosome 3, and 46% for chromosome 8. The accuracy of detection will depend on the sample analysis utilised [72] with MLPA being able to detect monosomy 3 populations more frequently than FISH. The higher the tumour cell yield, the more likely any heterogeneity will be detected; this is also less likely with FNAB due to the low yield, and the single pass through a single track (resulting in a lower spatial diversity of sample than with alternate methods). Heterogeneity seems to also occur to a greater extent at the base of the tumour; as such, this would be the optimal place for tumour sampling. Trans retinal FNAB is unlikely to access this region due to the risk of perforation through the sclera adjacent to the tumour base. Heterogeneity is less important in the biopsy of small tumours where the proportion of representative cells in the sample is greater, and the chance for multiple cumulative mutations is less likely.

Ocular surface tumour seeding

Intravascular spread of tumour cells during biopsy has been theorised due to the disruption of tumoural blood vessels [130] thereby increasing the risk of systemic tumour spread. Bagger et al however could find no difference in mortality between uveal melanoma patients who have had various techniques of tumour biopsy (FNAB, vitreous cutter, Essen forceps) and those that have never underwent a biopsy procedure [25, 117].

Although ocular surface seeding does not occur with choroidal metastases or lymphoma, a serious consideration for surgeons undertaking melanoma tumour sampling is the risk of seeding of active tumour cells into the vitreous cavity, along the scleral tract, into the anterior chamber or on to the ocular surface. It is these areas where subsequent conservative treatments such as proton beam or plaque radiotherapy would not be involved in the treatment field. Extra ocular spread of tumour cells is in itself an independent risk factor for increased metastatic related mortality [131]. For this reason, measures have been suggested to reduce the risk of seeding; maintaining a low IOP to prevent fluid egress containing tumours cells via the scleral track/port, use of valved and smaller gauge ports, and suturing ± cryotherapy to the port sites. There seems to be less cells along the needle track via a trans retinal FNAB; however a trans-scleral FNAB is likely to have the protection of involvement in the field of subsequent radiotherapy [105, 132]. Extra ocular seeding has been described following both FNAB and trans-retinal 25 g cutter sampling [92, 133–135]. Interestingly, histological examination of subsequently enucleated eyes showed no evidence of tumour cells along the scleral track following FNAB [105]. Some authors advocate choroidal melanoma tumour sampling for prognostication only post radiotherapy, to reduce the risk of active tumour cell migration. This has been shown to be accurate and successful [136], but there may a time limiting factor before the extent of tumour cell DNA damage causes an alteration in the cytogenetic analysis [137].

LIVERPOOL EXPERIENCE OF PROGNOSTIC BIOPSIES

The Liverpool Ocular Oncology Centre is the first to routinely offer prognostic biopsies to all choroidal melanoma patients at presentation, regardless of tumour size or location. As such, our practice is to offer patients prognostic uveal melanoma biopsies during the insertion of their brachytherapy plaque via a trans-scleral route utilising a scleral flap and cut down and sample acquisition with Essen forceps and closure with tissue glue, enabling us to place the plaque over the biopsy site, with the aim to treat any cells inadvertently seeded onto the ocular surface. The yield of both cytology and genetics has increased following change to this methodology [95]. However, if the lesion is too posterior to safely access via this surgical method, or proton beam radiotherapy was the method of radiotherapy treatment, we offer prognostic biopsy after completion of their radiotherapy, ideally on the last day or within 4 weeks of their radiotherapy [136]. For this predominantly trans-retinal approach, 25–27 g valved sutureless ports are utilised and the vitrector passed through the tumour, a full vitrectomy, tamponade or retinopexy is not required in these cases with an intact posterior vitreous face. Ports are not sutured or treated with cryotherapy. Complication rates are low with this method, with no cases of tumour seeding onto the ocular surface in a recently analysed series [93]. Our experience in small tumours has increased over the years. We have recently undertaken a retrospective analysis of prospectively collected data on all patients who underwent a prognostic biopsy for a choroidal melanoma of <2 mm thickness over a 5-year period between January 2016-December 2021. During the study period, 320 prognostic choroidal biopsies were undertaken; 68 cases were of a tumour thickness ≤2 mm (0.7–2.0 mm, mean 1.4 mm). Longitudinal base diameter ranged from 4.1 to 15.6 mm (AJCC classification 64 cases T1a, 4 cases T2a). Treatment included ruthenium-106 plaque brachytherapy in 21 patients (31%) and proton beam radiotherapy in 47 (69%). Biopsies were taken 0-98 days post treatment. All cases were May Grunewald Giemsa stained and histologically confirmed as melanoma (8 epithelioid, 4 mixed cell, 56 spindle); chromosome 3 status was determined by multiplex ligation-dependent probe amplification (MLPA) or microsatellite analysis (MSA). One case had insufficient material for genetic analysis; of the remaining 67 cases, 19 (28%) had complete or partial loss of a copy of chromosome 3, 48 (72%) were disomy 3. There were no cases of non-clearing vitreous haemorrhage or retinal detachment.

CONCLUSION

Choroidal biopsies are necessary where additional histopathological information may influence patient care and outcome. The ability to locate the primary tumour in the case of choroidal metastases influences patient onward care and management without the need for prolonged systemic screening. The concept of early treatment of small melanomas to reduce the risk of metastatic disease has also increased the use of these biopsy techniques in small melanocytic lesions. However, in Liverpool, most choroidal biopsies are undertaken for prognostic purposes in the context of a clinically diagnosed uveal melanoma. Although this procedure remains controversial, the familiarity with newer and more controlled equipment, and the increasing yield from these small samples with various techniques is enabling an exponential rise in the use of prognostication in the standard treatment and counselling of choroidal melanoma patients. Certainly, in our experience, Essen forceps trans scleral samples and 25 g trans retinal biopsies result in an excellent yield sufficient for prognostic analysis of chromosome 3 status (predominantly with MSA) in 99% of cases, even in small (<2 mm) tumours. Despite the clinically low risk by TNM staging, over one quarter of these demonstrated high risk monosomy 3 mutations, challenging the concept of low risk 'small benign melanomas'. An increasing understanding of patient tailored care and its potential in the future will drive prognostication biopsies further, and it likely that any development in systemic treatments will rely on an individualised approach based on these parameters.

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COMPETING INTERESTS

The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to R. N. Hussain.

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