



Bone metastasis of retinoblastoma five years after primary treatment

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ABSTRACT

Purpose: Histopathological, immunohistochemistry- and molecular pathology-based diagnostics to distinguish metastasis of retinoblastoma from subsequent primary malignancy in patients with heritable retinoblastoma.

Observations: An eight year-old girl presented with tibial pain and bone lesion five years after multimodal treatment of bilateral retinoblastoma, initially clinically suspicious of osteomyelitis. Histopathological examination of bone biopsy specimen revealed a highly proliferative small blue round cell tumor mimicking Ewing's sarcoma of bone. Immunohistochemistry confirmed the diagnosis of a distant metastasis of the previous retinoblastoma. Other subsequent primary malignancies presenting as small blue round cell tumors, such as sarcomas or leukemia, were excluded by immunohistochemistry and molecular methods.

Conclusions and importance: In countries with early diagnosis of retinoblastoma, distant metastases of retinoblastoma are extremely rare, whereas subsequent primary malignancies are common in survivors of heritable retinoblastoma. Immunohistochemistry and molecular pathology are essential components of diagnostic pathway. In retinoblastoma patients, distant metastases including osseous lesions should be included in the differential diagnosis of small blue round cell tumors.

1. Introduction

Retinoblastoma (RB) is a rare malignancy, but represents the most common intraocular malignant tumor in childhood. Most patients are younger than five years at initial diagnosis. The annual incidence rates are 13.2 diagnosed RB per million population for male and 11.2 per million population for female patients in the U.S.¹ Five year-overall survival is higher than 95% in developed countries, although RB is a lethal disease if left untreated. Tumor spread of RB is mostly confined to the ocular bulb or infrequently locally to intracranial sites. Distant metastases of retinoblastomas are exceedingly rare, but basically known also in developed countries.^{2,3} Most affected site of distant metastases is the bone marrow, infiltrated by tumor cells spread with the blood flow.^{4,5} These metastases may present as bone lesions and have to be carefully distinguished from other primary bone tumors.

Retinoblastoma develops from early retinal progenitor cells.

Molecular tumorigenesis is caused by a functional loss of the retinoblastoma protein, subsequent to inactivating mutational events in both RB1 alleles. There are heritable and non-heritable forms, and children can develop unilateral or bilateral tumor manifestation.⁶

Children with heritable retinoblastoma carry a constitutional pathogenic RB1 variant. These children are not only at risk to develop multifocal retinoblastomas in both eyes during childhood, but also other malignancies outside the eye later in life, often referred to as second or subsequent primary malignancies (SPM). SPM are the major cause of death among survivors of retinoblastoma treatment. Most SPM are soft tissue sarcomas or osteosarcomas. However, there appears to be an increased risk also for melanoma, lung cancer, leukemia and other cancers. The incidence rate of SPM depends strongly on the previous treatment for retinoblastoma. External beam radiotherapy (EBRT) significantly increases the risk of SPM compared to survivors of bilateral retinoblastoma without eye-preserving radiotherapy. Chemotherapy

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especially with alkylating agents contributes to an increased incidence of SPM, too.⁷⁻¹⁰

2. Case report

We present the case of an eight year-old girl, who was diagnosed with bilateral sporadic retinoblastoma (bilateral ICRB D) at the age of 22 months. Further genetic background is unknown.

In the local hospital, she had received 9 cycles of polychemotherapy combined with multiple intravitreal melphalan injections, subsequent cryotherapy and plaque brachytherapy in both eyes. At the German RB referral center in Essen she presented for second opinion at the age of two years. The right eye was enucleated due to a subtotal retinal detachment without secure tumor control in a blind eye. The left eye showed a prominent viable tumor close to the posterior pole. Therefore, her left eye was treated with eye-preserving EBRT according to the Schipper technique as salvage therapy at the age of two years. Treatment data are summarized in Table 1; MRI scans are shown in Fig. 1.

In regular ophthalmological follow-up examination no further recurrence or new tumor developed in the left eye. The best corrected visual acuity was 20/200.

Five years after completion of EBRT the patient presented with pain in the right leg. She was diagnosed with osteomyelitis in the local hospital, but swelling and pain remained progressive under antibiotic treatment. At presentation at the referral center in Essen, MRI (Fig. 1) showed an expansive intramedullary lesion in the right tibia with wide surrounding tissue reaction. A core biopsy was taken to differentiate the diagnosis of metastatic RB from subsequent primary malignancy. After confirmation of the diagnosis of metastatic RB, the patient received multimodal treatment including high-dose chemotherapy and autologous stem cell transplant according to the national consensus recommendations. Therapy was well tolerated, at the time of this report she is still under treatment.

3. Histopathology, immunohistochemistry and molecular findings

3.1. Primary tumor/intraocular retinoblastoma

On the surgical enucleation specimen of the right eye, the tumor presented macroscopically as a grey-white, partially calcified mass inside the vitreous body without affection of the anterior chamber. Inside the vitreous body the tumor showed a small-blue-round cell pattern with dark dense chromatin and nearly no detectable cytoplasm. Necrosis and focal calcifications as a sign of regression after therapy were present. There was no infiltration of the sclera or optic nerve, but focal infiltrative growth into the choroid. No extraocular growth was detected, the resection margin of the optic nerve was free of tumor cells (Fig. 2).

Immunohistochemical stainings showed a complete positive nuclear reaction for CRX (cone rod homeobox protein) as well as nearly 100% proliferating cells in viable tumor areas, as demonstrated with positive staining for Ki67.

Table 1

Overview over local treatment courses of the primary tumor.

Right Eye (ICRB D)	Left Eye (ICRB D)
Nine cycles of intravenous chemotherapy within five months	Synchronous treatment for vitreous seeding: seven intravitreal melphalan injections
8 weeks later: plaque brachytherapy and cryocoagulation	5 months later: cryotherapy
9 months after initial diagnosis: secondary enucleation	9 months after initial diagnosis: external beam radiation therapy

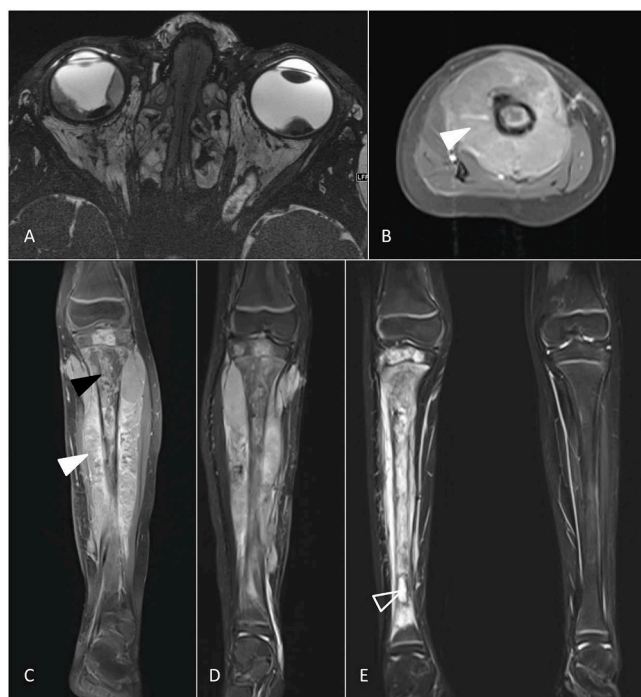


Fig. 1. Representative MR images. (A) CISS sequence axial of the orbit after chemotherapy and local therapy: tumor manifestations with calcifications bilateral and retinal detachment right. (B–D) 5 years later extended tumor manifestation of the right tibia (black arrow head) with broad tumorsleef in the surrounding tissue (white arrowhead): (B) T1 sequence + contrast enhancement fat-saturated axial, (C) coronal, (D) STIR sequence coronal. (E) Follow-up MR 2 months later: significantly regressive surrounding tumor tissue and mainly posttherapeutic changes in the tibia with residual tumor, edema and cyst (open arrowhead).

3.2. Distant metastasis in the bone

The tibial biopsy contained several solidly growing tumor formations, extending between the trabecular bone into the marrow spaces and arrodng trabecules. Nests of tumor cells showed a small blue round cell appearance with lots of chromatin smearing and streaming as crushing artifacts of vulnerable tumor cells.¹¹

Proliferative activity of tumor cells was extremely high with Ki67 being nearly 100% (Fig. 3). Immunohistochemistry exhibited negative staining for TdT (terminal deoxynucleotidyl transferase), FLI1, CD99 and myogenin. These results made acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma, Ewing's sarcoma and alveolar rhabdomyosarcoma extremely unlikely. In contrast, CRX immunohistochemistry was positive.

We performed fluorescence *in situ* hybridization with an *EWSR1* probe, which showed no break apart signals. This negative result helped to exclude Ewing's sarcoma as a differential diagnosis. Additionally, an anchored multiplex RNA based sequencing assay (Archer FusionPlex Sarcoma panel, including 26 sarcoma-related genes, capable to detect any of their genetic fusion partners) was carried out which presented neither *EWSR1* fusion nor any other gene fusion which are known to occur in small blue round cell sarcomas (data not shown). Therefore, we could exclude the vast majority of sarcoma subtypes with small blue round cell morphology.

Based on the patient's history, the comparison of both tumor morphologies, and ancillary findings in the bone lesion by immunohistochemistry and molecular techniques, we established the diagnosis of a distant metastasis of the retinoblastoma to the tibia.

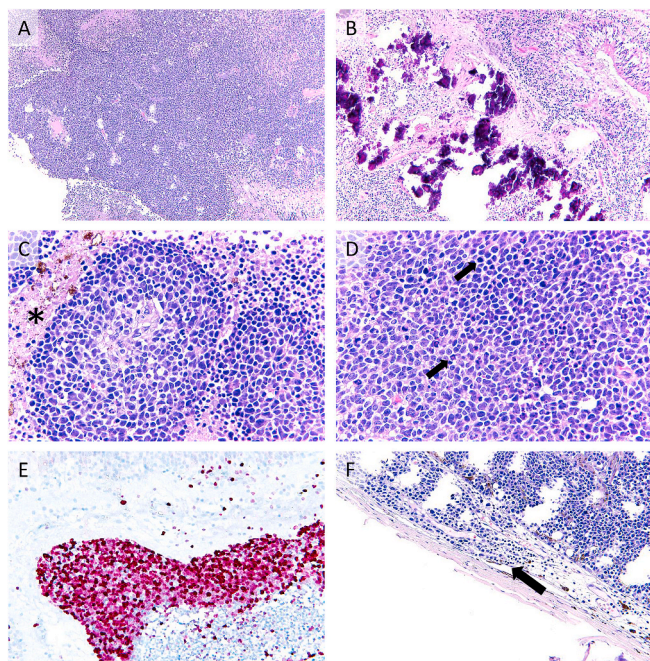


Fig. 2. Primary retinoblastoma of the right eye. (A) Tumor was composed of densely packed cells with sparse cytoplasm and condensed chromatin. Tumor tissue consisted predominantly of viable tumor cells (>80%). Small areas of regressive calcification after prior radio-chemotherapy (B). Higher magnification of viable tumor (C) with necrosis (*), karyorrhexis and apoptotic bodies (D, arrow). The viable tumor cells showed high Ki67 staining (>95%) (E). Tumor cells lying inside small vessels of the choroid due to choroidal invasion (F, arrow).

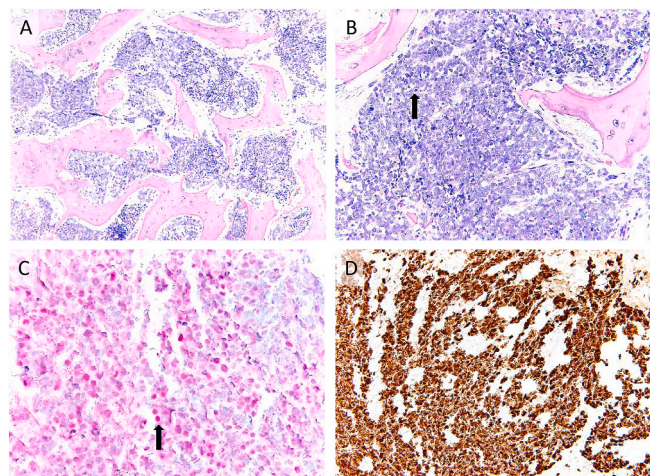


Fig. 3. Bone metastasis. (A) Nests of tumor cell infiltrate the bone marrow with arrosion of the bone trabecula. In higher magnification tumor cells exhibit the same morphological criteria as usually found in primary small blue round cell tumor, thus mimicking classical histology of Ewing's sarcoma (B; arrow: apoptosis). Immunohistochemistry of tumor cells demonstrated nuclear positivity with CRX (C, arrow). Proliferation was high with 100% index in Ki67 (D).

4. Discussion

Distant metastases in retinoblastoma represent a rare event in disease course. In patients with heritable retinoblastoma subsequent primary malignancies, especially sarcomas in the radiation field or elsewhere in the body are common, especially after previous EBRT for retinoblastoma. These tumors—if present in a heritable RB patient after

successful treatment of the primary tumor—represent major differential diagnoses of small blue round cell tumors.

The large and diverse group of small blue round cell tumors comprises different, but morphologically similar entities among pediatric malignancies. Tumors with small blue round cell morphology include leukemias, lymphomas, Ewing's sarcoma, alveolar rhabdomyosarcoma, desmoplastic small round cell tumor (DSRCT), Ewing's sarcoma-like tumors, undifferentiated round cell sarcomas or neuroblastoma with very different prognosis and varying treatments. Therefore, exact sub-classification is at utmost importance. However, small blue round cell tumors can be diagnosed and correctly classified by immunohistochemistry and/or methods of molecular pathology.

In our case, we excluded acute lymphatic leukemia by negative TdT staining, Ewing's sarcoma by negative CD99, FLI1 and negative *EWSR1*-FISH as well as other sarcoma subtypes by RNA-based sequencing. The latter technique is of particular relevance to exclude non-Ewing's sarcoma subtypes with small blue round cell morphology, which require a specific sarcoma-based treatment. The list of these sarcomas has been expanded enormously in the past few years and keeps still growing. These entities include the newly described sarcoma subtypes with *BCOR* or *CIC-DUX4* rearrangements, poorly differentiated synovial sarcomas (with various subtypes of *SS18* rearrangements) or myoepithelial tumors with *EWSR1/FUS-POU5F1* fusions among many others. These neoplasias are primarily defined by specific gene rearrangements, which correlate with clinical phenotypes and require specific treatment. Therefore, molecular fusion panels can significantly contribute to diagnostics of retinoblastoma patients if a subsequent primary malignancy is suspected. In our case, this technique helped to exclude any small blue round cell tumor as SPM. In contrast, we could demonstrate a cone rod homeobox (*CRX*)-expression in the majority of tumor cells, which in turn helped to confirm the diagnosis of distant metastasis of the formerly known RB. This finding underlines the diagnostic value of *CRX* expression for the diagnosis of RB.

CRX acts as a transcription factor in photoreceptor cells. It was identified as a strong marker for pineal lineage and photoreceptor differentiation. Thus, immunohistochemical staining with *CRX* is highly specific for discriminating retinoblastoma from other small blue round cell tumors.¹² In previous studies *CRX* was merely found in a small number of medulloblastomas,¹³ but these tumors present usually with a different morphology and do not represent a common differential diagnosis to retinoblastoma.

In the past, common diagnostic pathways required PCR testing to detect *RB1* mutations to prove a distant metastasis of retinoblastoma.⁴ Our case points out, that *CRX* immunohistochemistry is an alternative, fast, well established, valid and cost effective method to discriminate metastatic retinoblastoma from other small blue round cell tumors. Ancillary molecular testing can further contribute to exclude rare differential diagnoses and to classify SPM.

Retinoblastoma patients commonly receive globe-preserving therapy regimens and achieve survival rates of more than 95%. Therefore, an increase of primary subsequent malignancies or metastases may be expected, especially years after initial treatment. This observation should be considered for duration and frequency of follow-up, and new strategies of surveillance can be discussed. Up-to-date diagnostic procedures including immunohistochemistry and molecular tests need to be applied to reliably diagnose RB metastases and SPM subtypes.

Moreover, distant metastases of retinoblastomas including bone lesions should be included in the differential diagnosis of small blue round cell tumors in RB patients.

Patient consent

Written consent to publish case details is obtained from the patient's parents.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Declaration of competing interest

All authors have no financial disclosures.

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