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CASE REPORT

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Brain involvement in multicentric Epstein–Barr virus-associated smooth muscle tumours in a child after kidney transplantation

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Abstract Epstein–Barr virus (EBV)-associated smooth muscle tumours (SMT) have been reported in young patients with induced immunosuppression associated with organ transplantation, acquired immunodeficiency syndrome or congenital immunodeficiencies. EBV-associated SMT are frequently multicentric or multifocal and often occur in unusual locations. We are reporting a case of EBV-associated multicentric SMT that occurred after kidney transplantation in a 2-year-old boy with a history of oligomeganephrony. Headaches and left VIth cranial nerve paralysis led to the discovery of a brain tumour 3 years after transplantation. There were multiple pulmonary, hepatic and splenic nodules and enlarged mesenteric lymph nodes. Histological examination revealed multicentric SMT of uncertain malignant potential. Further investigations using in situ hybridisation demonstrated EBV early RNAs in the nucleus of most tumour cells. The immunosuppressive therapy was reduced, and the child was treated with chemotherapy, but died 2 months later, due to neurological complications.

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Introduction

Leiomyosarcomas are very rare in the paediatric age group [13]. In children with acquired immunodeficiency syndrome (AIDS) or induced immunosuppression following transplantation, the incidence of apparently benign or malignant spindle-cell (usually smooth-muscle) tumours is higher than expected for this age group [3, 9, 20]. Epstein–Barr virus (EBV)-associated smooth muscle tumours (SMT) are uncommon, distinctive mesenchymal tumours found in immunocompromised patients, including children, with AIDS [16], induced immunosuppression following transplantation [15], severe congenital immunodeficiency [12, 17, 28] or ataxia–telangiectasia [22]. The intracranial location of EBV-associated SMT in immunocompromised patients is extremely rare [12].

In this report, we are presenting a case of EBV-associated multicentric SMT that occurred after kidney transplantation in a child. Tumours were located in the brain, lungs, liver, spleen and mesenteric lymph nodes.

Clinical history

At birth, the boy was premature, with bilateral renal hypoplasia related to oligomeganephrony and renal insufficiency necessitating haemodialysis from the age of 15 months. Kidney transplantation was performed at 2 years of age. The recipient was EBV-seronegative and the donor seropositive. EBV primary infection was detected within 6 months of the renal transplant. Post-transplant immunosuppressive therapy included anti-lymphocyte globulin (days 1–10 post-transplantation); methylprednisolone (2 mg/kg per day on days 1–10, then progressively reduced to 0.35 mg/kg per day at 6 months) and cyclosporin (from day 8, 5 mg/kg). Azathioprine was given initially, but the patient was switched to mycophenolate mofetil (600 mg/m^2) at day 5 because of severe leukopenia. Long-term treatment included cyclosporin (7 mg/kg per day), prednisone (0.35 mg/kg per day) and mycophenolate mofetil (400 mg/m^2). The patient was admitted to hospital with headaches and left VIth cranial nerve paralysis 3 years after

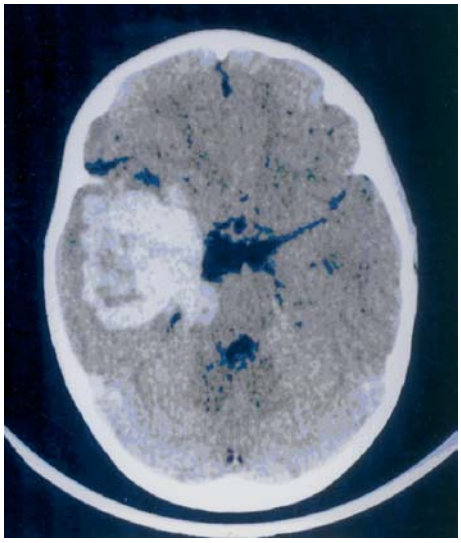

Fig. 1 Cerebral computed tomography scan showing a right temporal mass that was displacing the brain stem

transplantation. Physical examination revealed hepatomegaly and splenomegaly. A cerebral computed tomography (CT) scan demonstrated a right temporal mass, measuring 5 cm, that was displacing the brain stem (Fig. 1). A thoracic–abdominal CT scan revealed multiple pulmonary, hepatic and splenic nodules as well as enlarged mesenteric lymph nodes. Semiquantitative polymerase chain reaction demonstrated a high viral load in the blood (1000–2000 Eqv genome/10^5 cells). A fine-needle biopsy was taken of a hepatic nodule and a splenic nodule (3×2×2 cm) was resected. Following the diagnosis of EBV-associated SMT, the immunosuppressive therapy was reduced to prednisone, and the child was treated with chemotherapy (temozolomide 8 mg/kg per day on 5 days every month). He died after 2 months with seizures and coma. No autopsy was performed.

Materials and methods

Formalin-fixed tissue was sectioned (4 μm) and stained with haematoxylin–eosin–saffron. The immunohistochemical procedure used a panel of antibodies recognising relevant markers, such as vimentin, smooth muscle actin, desmin, CD31, CD34, S100 protein, Ki-67 and EBV latent membrane protein (LMP-1) (Dako, Glostrup, Denmark). Positive staining was visualised using peroxidase conjugates (EnVision+ system, Dako). EBV-encoded early RNAs (EBERs 1 and 2) were detected using in situ hybridisation with biotin-conjugated oligonucleotides (Kreatech, Amsterdam, The Netherlands).

Results

Macroscopically, the splenic nodule was encapsulated, firm and white. Microscopic examinations of biopsies taken from the liver and spleen demonstrated interlacing bundles of spindle cells with moderate cellularity in combination with some loosely textured myxoid areas of low cellularity. Tumour cells displayed eosinophilic cytoplasm and vesicular nuclei. The nuclei were elongated to cigar-shaped and slightly variable in size (Fig. 2). Mitotic figures were very scarce, and there was no haemorrhage or necrosis. Immunohistochemical staining and analysis demonstrated diffuse staining for vimentin and smooth muscle actin. Nuclear staining for Ki-67 was demonstrated in 10% of tumour cells. The results for desmin, CD31, CD34, S100 protein and LMP-1 were negative. The nucleus of most tumour cells expressed EBERs (Fig. 3).

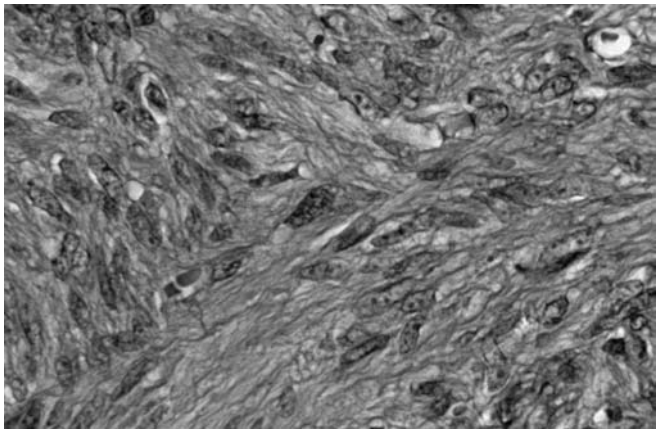

Fig. 2 This micrograph of a splenic nodule demonstrates interlacing bundles of spindle cells with slight nuclear atypia. Haematoxylin–eosin–saffron (original magnification ×600)

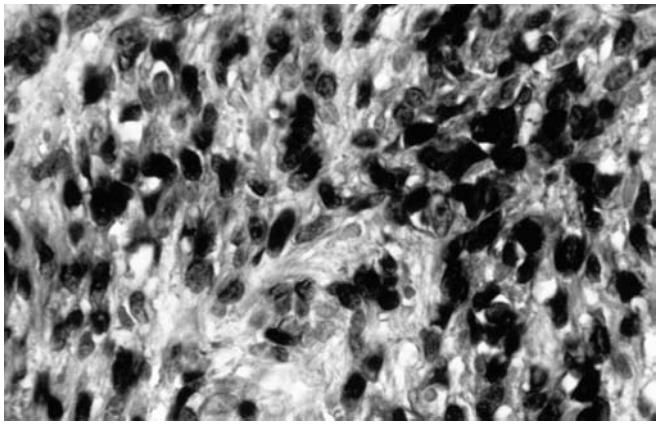

Fig. 3 This microphotograph shows in situ hybridisation in a splenic nodule demonstrating nuclear expression of EBERs by most tumour cells (original magnification ×600)

Discussion

Patients who have undergone organ transplantation require lifelong immunosuppressive therapy. The incidence of various proliferative disorders, including SMT and susceptibility to EBV are increased in immunosuppressed patients. EBV is a DNA herpes virus that is able to immortalise infected cells. It is commonly found in adults, primarily infecting B-lymphocytes and capable of persisting indefinitely in a latent form. Infected B-cells may undergo chromosomal rearrangement and become

Table 1 Clinical and pathological features of Epstein–Barr virus (EBV)-associated smooth muscle tumour (SMT) following organ transplantation. *transpl* transplantation, *UMP* uncertain malignant potential, *LMS* leiomyosarcoma, *m* multifocal, *s* single, *SR* surgical resection of tumor(s), *Red. IT* reduced immunosuppressive therapy, *AVT* antiviral therapy, *CT* chemotherapy

Reference	Sex	Age at transplant	Transplant	Delay transplant—SMT	Location of SMT	Pathology of SMT	Treatment for SMT	Follow-up
[15]	Female	18 months	Liver	3 years	Liver (donor), m	UMP	SR	Alive, no SMT (3 years)
[15]	Female	15 months	Liver	5.5 years	Liver, lungs, heart, stomach, small bowel, colon, m, retroperitoneum (10 cm), s	UMP	Red. IT, AVT, CT	Dead (candidosis), SMT
[15]	Female	20 months	Liver, small bowel	12 months	Colon (recipient), m	UMP	Red. IT, AVT	Dead, SMT (4 m)
[21]	Male	Child	Liver, pancreas, stomach, bowel		Bowel	UMP		Dead (sepsis)
[26]	Male	7 years	Liver	2 years	Liver (donor), s, lungs, m	Poorly differentiated LMS	CT	Dead, SMT (6 months)
[26]	Female	7 years	Liver	5 years	Peritoneum (10 cm) (recipient), s	Well-differentiated LMS	SR	Alive, no SMT (2 years)
[6]	Male	1 year	Heart	4 years	Liver (15 cm), s	UMP	SR, AVT	Alive (10 months)
[11]	Female	2 year	Heart	2 years	Liver, spleen, m	UMP	–	Dead (sepsis, rejection), SMT (1 years)
[14]	Female	49 years	Kidney	4 years	Liver (recipient), spleen, m	Well-differentiated LMS	Red. IT, AT, CT, SR	Dead, SMT (16 months)
[24]	Female	15 years	Kidney	6 years	Liver, m	UMP	–	Dead (cerebral hemorrhage)
[1]	Male	44 years	Heart	4 years	Heart, s	UMP	–	Dead (ruptured aortic aneurysm), SMT
[25]	Male	11 years	Heart, lung	3.5 years	Lung (donor), liver (recipient), m	LMS	Red. IT, AVT	Dead (sepsis) (1 month)
[29]	Male	26 years	Kidney	2 years	Lungs, liver, spleen, lymph nodes, thigh, m	LMS	–	Dead (1 year)
[8]	Male	2 years	Liver	5 years	Mesentery, s	UMP	SR, red. IT	Dead (retranspl.)
[23]	Male	23 years	Heart	3 years	Liver, m, paravertebral, m, vein (ankle), s	Low-grade LMS	SR, red. IT, AVT	Alive, no SMT (3 years)
[27]	Male	34 years	Kidney	4 years	Bones, liver, lungs, m	UMP	Red. IT	Alive, SMT (8 years)
[2]	Female	3 years	Liver	2 years	Liver, lymph nodes, mesentery, m	LMS	Red. IT, CT	Alive, SMT (12 years)
[5]	Female	18 days	Heart	5 years	Epidural intracranial, s, endobronchial, s	SMT	SR	Alive, SMT (3.5 years)
This report	Male	2 years	Kidney	3 years	Brain, s, lungs, liver, spleen, lymph nodes, m	UMP	Red. IT, CT	Dead (brain tumor) (2 months)

hyperproliferative. T-lymphocytes normally constitute the major pool of proliferating cells associated with the host's response to EBV infection [19]. Latent EBV infection is associated with a number of lymphoid, epithelial and mesenchymal tumours. CD21, the B-cell receptor for EBV, is found on the cell surface of SMT in human immunodeficiency virus (HIV)-positive (strong immunostaining) and HIV-negative (weak immunostaining) children [16]. EBERs 1 and 2 are demonstrated using in situ hybridisation in latently infected cells. Large amounts of EBV DNA and RNA have been demonstrated in SMT in immunocompromised patients [15, 16]. Monoclonal or biclonal EBV strains have been identified in SMT found both in patients following organ transplantation [14, 15] and in children with AIDS [10, 16]. Such EBV monoclonality suggests a primary role of the virus in oncogenesis of this tumour [4]. An EBV-associated liver tumour that had occurred after kidney transplantation in a 10-year-old patient presented a phenotypical spectrum, ranging from SMT to inflammatory pseudotumour, with genomic rearrangement of the ALK loci and co-localisation of the viral DNA and the ALK sequences [7]. EBV-

associated SMT following organ transplantation may occur concurrently or sequentially with post-transplant lymphoproliferative disorders (PTLD), which often regress after reduction of immunosuppression. EBV-associated SMT and PTLD occur after similar tumour-free periods post-transplantation, exhibit EBV type-III latency, involve either donor or recipient tissues, are of clonal or multiclonal origin and display a wide spectrum of histological grade and clinical behaviour [19].

EBV-associated SMT following organ transplantation have been reported in 14 children (including the subject of this report) [2, 5, 6, 8, 11, 15, 18, 21, 24, 25, 26, 30] and 5 adults [1, 14, 23, 27, 29]: 10 males, 9 females, aged 18 days to 49 years at transplantation (mean 12.9 years and median 5.1 years). A variety of organs have been transplanted in the subjects of these studies, most often the liver (eight patients), kidney, or heart. The delay between the transplantation and the occurrence of SMT ranged from 1 year to 6 years (mean 3.6 years and median 3.8 years), with a single SMT measuring up to 15 cm [6] or, more often, multifocal or multicentric lesions in multiple organs or tissues. The most common locations of SMT were the liver (12 patients), originating from the donor or recipient, lung (from the donor in one patient), heart and colon (native in one patient) (Table 1). SMT was found in the intracranial, epidural area in a 5-year-old girl following heart transplantation. Immunosuppression was reduced, and the epidural mass was stable 3.5 years after biopsy [5]. The child reported in this study represents the only patient with brain tumour. In 12 of the patients described in the literature, the diagnosis was SMT of uncertain malignant potential. A further seven patients were diagnosed with leiomyosarcoma (well-differentiated in three patients, poorly differentiated in one patient). The malignant potential of these tumours is difficult to assess, and the diagnosis of SMT of uncertain malignant potential might be preferred instead of leiomyosarcoma or leiomyoma. The biological significance of criteria, such as tumour size, cellularity, atypia and mitotic counts probably differs according to the location of such tumours, with some apparently benign tumours proving to be lethal. The tumours did prove to be fatal in most patients, with death most often being a result of sepsis (four patients) or haemorrhage. Five of the seven patients who survived (at a 10-month to 12-year follow-up) have been treated by surgical resection (Table 1). In the case reported here, the brain tumour was lethal, despite the reduction of immunosuppression and the use of chemotherapy with good diffusion through the blood–brain barrier.

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