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De novo cancers in paediatric renal transplant recipients: a multicentre analysis within the North Italy Transplant programme (NITp), Italy

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Abstract

The purpose of this study was to determine the frequency and the outcome of *de novo* malignancies in a cohort of renal transplant paediatric patients. The records of 493 kidney transplants, carried out in 454 paediatric recipients at the three paediatric transplant centres of the North Italy Transplant programme (NITp, Italy) were reviewed. 10 cases of malignancies (2.2%) comprising both PTLD (post-transplant lymphoproliferative disorders) (6 cases, 1.3%) and non-PTLD malignancies (4 cases, 0.88%) were reported. Non-PTLD included one urothelial carcinoma and one Wilms' tumour of the recipient's left native kidney, one abdominal dysgerminoma and one optic nerve glioma of the left eye. The PTLD consisted of localised or disseminated Epstein–Barr virus (EBV)—associated B-lymphocyte monoclonal (5 cases) and polyclonal (1 case) proliferations. All patients suffering from PTLD had been EBV-negative at the time of transplantion, but developed EBV primary infection after transplantion. All PTLD patient donors were EBV-positive. In addition, all but 1 patient received, before and/or after transplantation, a range of immunosuppressive drugs in addition to the baseline prophylactic immunosuppressive regimen. Moreover, 3 patients suffered from syndromes associated with a genetic predisposition to cancer. Finally, the malignancies reported here were associated with 20% graft failure and 20% mortality rates.

Keywords: Renal transplantation; Paediatric graft recipients; Post-transplant tumours; Immunosuppressive therapy; Epstein–Barr virus; Post-transplant lymphoproliferative disorders; Denys–Drash syndrome; Frasier syndrome

1. Introduction

Organ transplant recipients are at high risk of developing tumours, the reported average incidence being approximately 6% [1]. Although paediatric solid-organ recipients have been found to have a significantly lower risk of malignancy in general and of skin cancer in particular than adult recipients, an increased incidence of certain neoplasms after organ transplantation is observed in paediatric patients [2–4]. Indeed, post-transplant lymphoproliferative disorders (PTLD) which include a wide range of B-cell lymphoproliferations, ranging from plasmacytic hyperplasia to very aggressive disseminated immunoblastic lymphomas, account for

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approximately 50% of neoplasms in paediatric allograft recipients, but for only 14% in their adult counterparts [2–6]. The other tumours in paediatric recipients are represented by skin and lip cancers (20%) and by miscellaneous tumours (30%) including carcinoma of the vulva and the anus, non-Kaposi sarcomas, hepatic neoplasms, thyroid carcinomas and others [2, 3]. Although the occurrence of neoplastic events is consistently observed in immunosuppressed transplant recipients, due to the loss of immunosurveillance whatever the immunosuppressive regimen used, for almost a decade an increased incidence of life-threatening PTLD has been observed in association with the use of cyclosporin A, OKT3 and ATG/ALG immunosuppressive agents [7, 8]. Therefore, the recognition of major risk factors for carcinogenesis in graft recipients seems increasingly relevant in order to determine appropriate clinical management and improve graft outcome [9–12].

In the light of the above considerations, we were prompted to analyse the cumulative incidence and the clinical spectrum of neoplastic disorders occurring at the three paediatric renal transplant centres (Genoa, Milan and Padua) of the North Italy Transplant programme (NITp), which is the organisation that co-ordinates cadaveric organ sharing and allocation in six regions located in northern Italy.

2. Patients and methods

From January 1987 to September 1998, a total of 493 paediatric renal transplants (454 first transplants and 39 second transplants) were performed at the three paediatric transplant centres of the NITp. There were 253 males and 201 females, with a mean age of 13.7 ± 5.2 years.

Kidneys came from cadaver donors in 456 cases, whereas the remaining 37 were from living related donors (LRD), represented by parents in all cases but 1 (sister). All patients received a kidney allograft with a negative standard T cell cross-match and with additional negative flow cytometry T and B cross-matches in the case of LRD transplants. All grafts were harvested in a similar manner and stored in Euro-Collins solution or University of Wisconsin solution. Post-transplant immunosuppression (baseline regimen) consisted of a standard double therapy including cyclosporine A (CSA) and prednisone; azathioprine or, more recently, micophenolate mofetil (MMF) were added to the above regimen in selected cases within certain categories such as LRD transplants, patients needing a lower CSA dosage for related nephrotoxicity, second transplant and poorly human leucocyte antigen (HLA)-matched patients. Acute rejection episodes, diagnosed histologically and/or clinically, were treated with steroid pulses and, in the case of steroid resistance, with a course of antilymphocyte globulin (ALG/ATG) or OKT3 murine monoclonal antibody. All patients on the waiting list were screened before transplantion for Epstein–Barr virus (EBV) as well as other viral infections including cytomegalovirus (CMV), according to standard serological methods. Briefly, an enzyme-linked immunoadsorbent assay (ELISA) technique was used to determine IgG and IgM antibody titres against CMV and EBV-associated antigens such as EBNA (Epstein–Barr virus nuclear antigens) and VCA (viral capsid antigens). The same screening was performed on blood specimens from donors at the time of transplantion or retrospectively on frozen donor serum samples at the time of this analysis. After transplantion, in addition to the above serological methods, the following techniques were also used.

2.1. CMV infection

An immunocytological assay was used for the detection of CMV antigens (pp65KD) in circulating peripheral blood leucocytes (PBL). By the use of a mixture of monoclonal antibodies and a second step fluorescence antibody as a detection system, CMV lower matrix protein was detected in cytocentrifuged blood leucocytes, and the results expressed quantitatively by reporting the number of CMV antigen positive cells per 2×10^5 PBL. The presence of five or more positive cells per 2×10^5 PBL was taken as indicative of CMV infection [13].

2.2. EBV infection

Recently, EBV viral loads were serially measured by quantitative competitive PCR (polymerase chain reaction) of peripheral blood from EBV-negative patients showing serological seroconversion after transplantion, as described in [14].

The presence of EBV-DNA sequences in cell suspensions or tissues from PTLD localisations was detected by using appropriate primers targeting the U2 region of the virus that encodes the EBNA 2 protein, which is expressed in latently infected cells [10].

3. Results

Tumours occurred in 10 patients (2.2%) who underwent a first cadaveric graft. None occurred in patients receiving grafts from a living relative or from a second cadaver. There were 6 cases of EBV-associated post-transplant B lymphoproliferative disorders (cases 1–6; 60% of the total tumour number; 1.32% of all patients. These were represented by: two polymorphic lymphomas localised to grafted kidneys (cases 1 and 3), one plasmacytic hyperplasia localised to tonsils (case 6), one

Table 1
Kidney graft paediatric tumour patients' characteristics

Case number	Sex	Age (yrs)	PreTx EBV status recipient	EBV status donor	Tumour type	Time of appearance post Tx	Immunosuppression post Tx beyond baseline	Outcome	Cancer therapy	Primary disease
1	M	9	Negative[a]	Positive	EBV-associated intrarenal polymorphic B cell lymphoma	45 days	Steroid boluses, OKT3 for intrarenal lymphoma simulating an acute rejection	Alive	Graft removal	Joubert syndrome (autosomal recessive disorder with cerebellar hypoplasia, hypotonia and multicystic kidney disease)
2	M	9	Negative[a]	Positive	EBV-associated disseminated immunoblastic B cell lymphoma including cerebral localisation	66 days	Steroid boluses, ALG plus ATG for acute rejection	Dead	Decreased immunosuppression	FGS[b]
3	F	16	Negative[a]	Positive	EBV-associated intrarenal polymorphic B cell lymphoma	47 days	Steroid boluses, ALG plus ATG for acute rejection	Alive	Graft removal	Renal dysplasia
4	M	6	Negative[a]	Positive	Disseminated Burkitt's lymphoma	6 months	None	Alive	Discontinuation of immunosuppression, chemotherapy (vincristine, cyclophosphamide, doxorubicin, methotrexate (Mtx), intrathecal Mtx and cytarabine)	Denys–Drash syndrome
5	M	18	Negative[a]	Positive	EBV-associated disseminated immunoblastic B cell lymphoma	5 months	Steroid boluses, ALG for acute rejection	Dead	Interferon α	Bilateral renal hypo-dysplasia
6	M	2	Negative[a]	Positive	EBV-associated tonsilar plasmacytic hyperplasia	21 months	Steroid boluses for acute rejection	Alive	Surgery, CSA tapering	Congenital nephropathy secondary to ACE inhibitors administration during pregnancy
7	M	14	Positive	Unknown	Urothelial carcinoma of left native kidney	25 months	Steroid and cyclophosphamide boluses for early post Tx FGS recurrence	Alive	Graft removal for FGS recurrence, nephrectomy of left native kidney	FGS[b]
8	F	2	Negative	Negative	Wilms' tumour of left native kidney	13 months	Steroid boluses for acute rejection	Alive	Nephrectomy of left native kidney, radiotherapy, chemotherapy (actinomycin D, vincristine)	Denys–Drash syndrome
9	F	10	Positive	Unknown	Abdominal dysgerminoma	25 months	Steroid boluses for acute rejection	Alive	Chemotherapy (cisplatinum, etoposide, bleomycin) substitutive hormonal treatment	Frasier syndrome associated with FGS[b,c]
10	F	5	Positive	Unknown	Optic nerve glioma of left eye	24 months	Steroid boluses for acute rejection	Alive	Radiotherapy	Bilateral renal hypoplasia

[a] In this patient there was laboratory evidence of a post Tx primary EBV infection as documented by EBV antibody seroconversion and/or presence of EBV-DNA sequences in tumour specimens.

[b] This patient received, before transplantation, immunosuppressive drugs for treatment of FGS, as detailed in the text.

[c] The diagnosis of Frasier syndrome was performed, after transplant, following abdominal dysgerminoma detection.

M, male; F, female; Tx, transplant; EBV, Epstein–Barr virus; CSA, cyclosporine A; ACE, angiotensin correcting enzyme; FGS, focal glomerulosclerosis.

disseminated Burkitt's lymphoma (case 4) and two disseminated immunoblastic lymphomas (cases 2 and 5), one of which comprised cerebral localisations. The remaining 4 cases (cases 7–10; 40% of total tumour number; 0.88% of all patients) were represented by other tumour types, such as urothelial carcinoma (case 7) and Wilms' tumour (case 8) of the left native kidney, abdominal dysgerminoma (case 9) and optic nerve glioma of the left eye (case 10) (Table 1). With the exception of case 4, all tumour patients received, before and/or after transplantation, an array of immunosuppressive drugs, including powerful immunosuppressive agents such as chlorambucil, cyclophosphamide, steroid boluses, ATG/ALG and OKT3, in addition to the baseline prophylactic immunosuppressive regimen (Table 1).

The 3 patients affected by focal glomerulosclerosis (FGS) received both pretransplant (to treat primary nephropathy) and post-transplant additional immunosuppression (to treat acute rejection episodes in 2 cases and early FGS recurrence in 1 case) (Table 1).

FGS patients were treated in the pretransplant period as follows: patient 2 received 0.15 mg/kg/day chlorambucil for 2 months and oral prednisone for 5 years; patients 7 and 9 received 2 mg/kg/day cyclophosphamide for 2 months and oral prednisone for 3 and 6 years, respectively. Oral prednisone dosage in these patients ranged between 0.25 and 2 mg/kg/day and the treatment was not given on a continuous basis. The remaining 6 patients received additional immunosuppression only in the post-transplant period for acute rejection treatment, and in one case (case 1) for an intrarenal polymorphic B cell lymphoma simulating an acute rejection episode. As the main reason for additional immunosuppression was rejection treatment, it was interesting to compare the global cancer incidence in rejecting and non-rejecting patient populations. As 250 patients in the whole series (55.1%) were treated for clinical or biopsy proven acute rejection, and 7 of these developed a neoplasm, the calculated relative risk (RR) of *de novo* cancer development (CD) was 1.93 in rejecting patients as compared with non-rejecting ones. In this latter group there were 3 neoplastic patients. The RRCD was not significant (confidence interval at 95% probability ranged between 0.49 and 7.56).

The RRCD was also calculated for patients receiving immunosuppressive drugs before transplantion (steroids and alkylating agents) for the treatment of primary renal disease such as idiopathic and secondary glomerulonephritis (102 patients, 22.5% of the whole series) in comparison with patients who did not receive any pretransplant treatment. There were 3 neoplastic patients in the former group and 7 in the latter. In this case the RRCD was 1.49 and again it was not significant (confidence interval at 95% probability ranged between 0.38 and 5.88).

In addition, 3 patients were found to be affected by primary renal diseases as components of complex syndromes such as Denys–Drash (diffuse mesangial glomerulosclerosis: cases 4 and 8) and Frasier syndrome (FGS: case 9). Both the above syndromes are characterised by a cancer predisposition as a consequence of genetic mutations occurring at different chromosomal sites [15–18]. The above syndromes had the following incidence in our whole series: 5 patients out of 454 (11%) were found to be affected by Denys–Drash syndrome and 2 of these (40%) developed a *de novo* cancer; 2 patients out of 454 (0.4%) suffered from Frasier syndrome and 1 was affected by abdominal dysgerminoma (data not shown).

In only 2 patients, both affected by monoclonal highly aggressive disseminated immunoblastic B-cell lymphomas (cases 2 and 5), did the tumour determine a fatal outcome (Table 1), even though in both cases the grafts were still functioning. It is noteworthy that patient 6, who showed only a plasmacytic hyperplasia localised to tonsils, had to be admitted to the intensive care unit because of an obstructive dyspnoea due to bilateral tonsilar hyperplasia. This patient underwent bilateral tonsillectomy; histological examination revealed diffuse architectural effacement with polyclonal proliferation (staining for both κ and λ) of small lymphocytes, plasma cells and occasionally large lymphoid cells or immunoblasts. Three other grafts failed, two because of intrarenal polymorphic B cell lymphomas and one because of the recurrence of primary FGS associated with urothelial carcinoma of the left native kidney (cases 1, 3 and 7, respectively). The global incidence of graft loss and patient death, due to tumours, proved to be high (40%). Decreased immunosuppression, surgery, chemotherapy and α-interferon for PTLD malignancies and surgery, radiotherapy, chemotherapy and hormones for non-PTLD malignancies were utilised as single or variably combined treatments (see Table 1 for details).

4. Discussion

In this study a particular effort was made to retrospectively assess cancer incidence and outcome in a series of 454 paediatric kidney graft recipients, in order to identify the main risk factors for carcinogenesis.

As reported by other investigators, we confirmed the increased incidence of EBV-associated B cell PTLD. The reasons for this finding may be manifold but are mainly related to the larger amount of lymphoid tissue in children, and to the fact that primary EBV infections are more common in children than in adults. Indeed, in our series all PTLD patients experienced a primary EBV infection, as documented by their EBV-negative serological status before transplantion and by the subsequent demonstration of post-transplant seroconversion

and/or PCR amplification of EBV-DNA in tumour tissues. It is worth mentioning here that it was possible to demonstrate that all PTLD patients received a graft from an EBV-positive donor. This is in accordance with the current knowledge of the pathogenetic mechanisms triggered by EBV infection: primary chronic EBV infection and, less frequently, secondary EBV-reactivation constitute the initial events leading to B-cell clone immortalisation. Subsequently, a severe loss of T-cell surveillance, secondary to immunosuppressive therapy, allows further expansion of EBV infection and proliferation of multiple EBV-infected and immortalised B-cell clones (polyclonal proliferation). In an intermediate phase of this process, some clones may display a proliferative advantage, so that a few (oligoclonal) or one of them (monoclonal) eventually predominate. Possible subsequent transformation into highly malignant B cell lymphomas involves further genetic events consisting of the mutation of known oncogenes and/or tumour suppressor genes [19, 20]. With the exception of case 6, which involved polyclonal intratonsilar plasmacytic hyperplasia at an early stage of the above process, all PTLD cases involved the development of monoclonal B lymphomas.

Unfortunately, no research could be carried out in this study on the identification in our PTLD cases of any oncogene and tumour suppressor gene alterations, such as the *N-ras* codon 61 point mutation, *Bcl-1*, *Bcl-2* and *c-myc* gene rearrangements or *TP53* gene mutations, which are known to be associated with disseminated immunoblastic B cell lymphomas [19, 20].

It is well known that immunosuppressive therapy is responsible for major alterations in the immunosurveillance ability of the host which normally plays a major role in hampering cancer occurrence [21]. Furthermore, immunosuppressive agents can also display a direct carcinogenic effect. With the exception of case 4, all our tumour patients received additional immunosuppression.

However, from the analysis of our patients receiving additional immunosuppression either for treatment of primary renal diseases or for rejection, it was not possible to conclude that additional immunosuppression alone could represent a significant risk factor for cancer development.

Rather, the incidence of neoplasms after renal transplantation in our paediatric cohort seems to result from a complex interplay between two factors, which are primary EBV infection and exposure to additional immunosuppression. These primary conditions [22] may be associated with other risk elements constituted by renal disorders related to complex syndromes bearing a genetic predisposition to cancer, such as in Denys–Drash and Frasier-affected patients (cases 4, 8 and 9 respectively; Table 1) [18, 23–25].

From the incidence data in our whole series it seems that the genetic predisposition to cancer associated with these syndromes plays a significant role in cancer development. As for malignancies that occurred in Denys–Drash and Frasier patients the following issues are worth noting.

4.1. Denys–Drash syndrome

4.1.1 Denys–Drash syndrome may include, in addition to diffuse mesangial sclerosis, Wilms' tumour, male pseudohermaphroditism and gonadal dysgenesis [23]; the risk of Wilms' tumour development in these patients is strictly related to the presence of a germline mutation in the Wilms' tumour suppressor gene *WT1*, which is located on chromosome 11p13 [17, 18];

4.1.2 Serial renal ultrasounds are recommended in this patient category from the time of diagnosis to the time of transplantation, when bilateral nephrectomy should be performed [23]. In accordance with the above protocol, native kidneys were removed in patient 4 at the time of transplantation, in order to prevent Wilms' tumour development. As detailed above, this patient developed a disseminated Burkitt's lymphoma after transplantation. As previously reported [26, 27], a germline missense mutation affecting one of the zinc finger domains of the *WT1* gene was observed in the constitutional DNA of this patient, but no alterations of the constitutionally wild-type *WT1* allele and no expression of the gene were observed in his neoplastic cells, thus indicating that *WT1* was unlikely to be involved in the onset of Burkitt's lymphoma in our case. However, since the patient did not receive any additional immunosuppressive treatment, it is difficult to rule out completely the role of other genetic factors, indirectly associated with *WT1* gene mutation, in the development of Burkitt's lymphoma. Indeed, PTLD developed in all the other patients of our series in the presence of both primary EBV infection and additional immunosuppressive treatment;

4.1.3 Patient 8 was not nephrectomised at the time of transplantion. He developed Wilms' tumour 13 months after transplantion and underwent left nephrectomy, radiotherapy and chemotherapy. His clinical conditions are at present good and the transplanted kidney displays a normal function. As a proportion of Denys–Drash paediatric patients do not develop Wilms' tumour during follow-up [25], a possible role of immunosuppression in accelerating the occurrence of Wilms' tumour in this patient cannot be totally excluded. We are currently analysing this patient for the presence of *WT1* gene mutation.

4.2. Frasier syndome

4.2.1 Frasier syndrome is characterised by the association of chronic renal failure, XY gonadal dysgenesis and gonadoblastoma/dysgerminoma. In approximately 50% of cases, focal glomerulosclerosis is the pathological lesion leading to renal failure. The final diagnosis of Frasier syndrome was performed in our patient following the detection of a large abdominal mass, which was histologically revealed to be a dysgerminoma. After initial chemotherapy with four cycles of cisplatinum, etoposide and bleomycin, the abdominal dysgerminoma was surgically removed. During laparatomy, gonadal dysgenesis was also diagnosed. The patient karyotype was 46XY in the presence of a female phenotype.

4.2.2 Gonadal dysgenesis, as well as the genetic predisposition to cancer leading to gonadoblastoma/dysgerminoma, has been associated in a proportion of Frasier syndrome patients (15–20%) with mutation/deletion events affecting the developmental SRY gene (sex-determining region on chromosome Y) [18, 25]. According to the above studies the abdominal dysgerminoma is very likely to be correlated, in our patient, to the genetic background of Frasier syndrome. However, as prophylactic gonadectomy before renal transplantion has been recently proposed to avoid an increased risk of gonadal tumours following immunosuppression, we believe that a possible role of immunosuppression in gonadal cancer development should still be considered.

Indeed, these latter two cases seem to support the hypothesis that, even in cancer predisposing syndromes, immunosuppressive treatment may still play some role in cancer development, as already discussed for its synergistic role with EBV primary infection.

In addition to the above statements regarding syndromes with a genetic predisposition to cancer, a practical approach, based on the identification of the principal carcinogenesis factors for PTLD, may be to adopt strategies aimed at risk reduction.

These include: (i) selection of donors and recipients according to EBV serological status (i.e. avoiding the use of EBV-positive donors for EBV-negative recipients); (ii) the use of immunosuppressive protocols that do not include agents such as ATG/ALG and/or OKT3 in selected recipients, such as EBV-negative patients receiving a transplant from an EBV-positive donor. Indeed, we have already adopted this policy;

4.2.3 Pre-emptive antiviral therapy with gancyclovir/acyclovir, which has been used increasingly in recipients at high risk for PTLD, including paediatric patients [28, 29];

4.2.4 Periodic screening for circulating EBV DNA in peripheral blood lymphocytes (PBL) in order to lower the dosage of immunosuppressive agents in patients showing a high number of EBV-DNA genome copies. Very recently, EBV viral loads >200 genome copies/10^5 PBL have been suggested to constitute an increased risk for PTLD development [30]. In the same patient categories, an additional strategy could involve a policy of organ attribution with a high HLA compatibility, in order to minimise the incidence of severe acute rejection requiring the use of drugs such as ATG/ALG and/or OKT3 in addition to steroid boluses.

Finally, as PTLD are the principal cause of death and graft loss, new therapeutic approaches for such disorders should be encouraged. Amongst these, adoptive immunotherapy with *in vitro* expanded EBV-specific recipient cytotoxic T lymphocytes has recently shown promising results [31, 32] and may represent a feasible therapeutic option in selected high-risk PTLD patients.

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