Strategies for Prevention of Infections in Pediatric Oncology Patients and Hematopoietic Stem Cell Transplant Recipients

By

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CONTENTS

Tables and Figures

Abbreviations

ALD	Adrenoleukodystrophy
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
CMV	Cytomegalovirus
Con A	Concanavalin A
CVAD	Central venous access device
DNA	Deoxyribonucleic acid
DTP	Diphtheria-Tetanus-Poliovirus
EBV	Epstein-Barr virus
HL	Hodgkin lymphoma
HHV-6	Human herpes virus-6
HHV-7	Human herpes virus 7
HHV-8	Human herpes virus 8
HSV	Herpes simplex virus
HZ	Herpes zoster
HSCT	Hematopoietic stem cell transplantation
LPR	Lymphoproliferative response
MMF	Mismatched family donor
MSD	Matched sibling donor
MRD	Minimal residual disease
MUD	Matched unrelated donor
NHL	Non-Hodgkin lymphoma
NK	Natural killer cells
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHA	Phytohemagglutinin
PTLD	Post-transplant Lymphoproliferative Disorder
PWM	Pokeweed mitogen
UCB	Umbilical cord blood transplantation
VZV	Varicella zoster virus

Preface

This series of original studies presented in this thesis were completed over a period of seven years. As a pediatrician who is responsible for taking care of children with malignancies, I am particularly interested in prevention of infectious diseases in immunocompromised children. Children with malignancies who are on systemic chemotherapy and/or stem cell transplantation are immunocompromised. Infection is one of the potentially life-threatening complications. Parents of long term survivors always seek opinions from their oncologists about recommendations on strategies of prevention of infectious diseases since their children's immunities had been severely compromised by various treatment modalities and primary diseases as well. At the time when I started these series of studies, there were no formal recommendations or guidelines in preventing vaccine-preventable infectious diseases especially the strategies of revaccination in pediatric oncology patients. In order to address this particular question in term of exploring different strategies in prevention of infections in pediatric oncology patients and stem cell transplant recipients, various original studies described in this thesis were therefore performed.

These studies included patients with hematological malignancies, solid tumors, and transplant recipients. They were conducted in Lady Pao Children's Cancer Centre, Department of Pediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong. The availability of excellent clinical and research facilities in the Children's Cancer Centre and the Chinese University of Hong Kong provided an excellent environment for performing clinical studies to investigate the various strategies for prevention of infection in pediatric oncology patients and transplant recipients.

The studies in the chapters 6, 7, 8, 9, 10, 11 and 12 had been published in peer-reviewed international journals and being accepted for presentations in scientific meetings of worldwide-reputable organizations in pediatric oncology, stem cell transplantation and pediatric infectious diseases.

In all the studies, I was responsible for the generation of research ideas, planning of research logistics, organization of logistics, most of the data collections, data analysis and writing up the results and submission of manuscripts to peer-reviewed international journals for publication. Prof Patrick Man Pang Yuen, founder of the Bone Marrow Transplant Unit and Lady Pao Children's Cancer Center, and Dr Chi Kong Li, Chief of Division of Hematology / Oncology / Bone Marrow Transplant Unit of Department of Pediatrics had provided continuous and tremendous support and advice throughout my study period. Prof Ting Fan Leung and his research team (Mr Raymond PO Wong, Ms Brenda CY Li) of the Department of Pediatrics provided the technical support of various immunological investigations. Drs Ming Kong Shing, Vincent Lee, Wing Kwan Leung, Ms Hing Wah Lau, Ms Jeanny Cheung and her nursing team had contributed significantly in clinical management of patients. Prof Paul Kay Sheung Chan and his research team of Department of Microbiology, The Chinese University of Hong Kong is responsible for providing valuable microbiological and virologic support and advice. Ms Stella Chan and Ms Anita Lee had provided excellent secretarial support to my work. Prof Tai Fai Fok, Dean of Faculty of Medicine and Professor in Pediatrics and Prof Pak Cheung Ng, Chairman of Department of Pediatrics, The Chinese University of Hong Kong have given endless support, guidance and encouragement to my work during the past years.

The following studies were supported by research grants: (i) Humoral Immune Response After Post-Chemotherapy Booster Diphtheria–Tetanus–Pertussis Vaccine in Pediatric Oncology Patients by the Hong Kong Pediatric Bone Marrow Transplant Fund Research Grant; (ii) Lymphoproliferative Response to Herpes Simplex Virus Type 1, Cytomegalovirus, Epstein-Barr Virus, Varicella Zoster Virus, Human Herpes Virus 6, 7 and 8 antigens stimulation in Pediatric Allogeneic Stem Cell Transplant Recipients was supported by the Children's Cancer Foundation Peter Nash Pediatric Oncology Research Grant. I have no conflict of interest to declare.

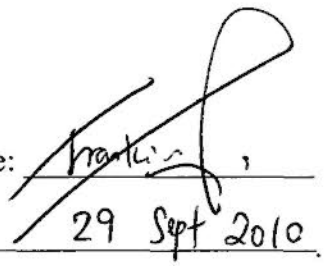
Informed consents were obtained form all participating parents and/or patients. The intervention studies had granted the ethical approval from the Joint New Territories East Cluster-Chinese University of Hong Kong (NTEC-CUHK) Clinical Research Ethics Committee.

Many other colleagues in the various studies have given me valuable support. They will be acknowledged at the end of this thesis.

Declaration

I hereby declare that the thesis entitled "Strategies for Prevention of Infections in Pediatric Oncology Patients and Hematopoietic Stem Cell Transplant Recipients" has been carried out in the Lady Pao Children's Cancer Centre, Department of Pediatrics, The Chinese University of Hong Kong. The work is original and has not been submitted in part or full by me for any degree or diploma at any other university.

I further declare that the materials obtained from other sources have been explicitly acknowledged in the thesis. I also acknowledge that I am aware of the University policy and regulations on honesty in academic work.

Signature: 

Date: 29 Sept 2010

Section 1

Background and Overview

Chapter 1 An Overview of Spectrum of Diseases in Pediatric Oncology in Hong Kong

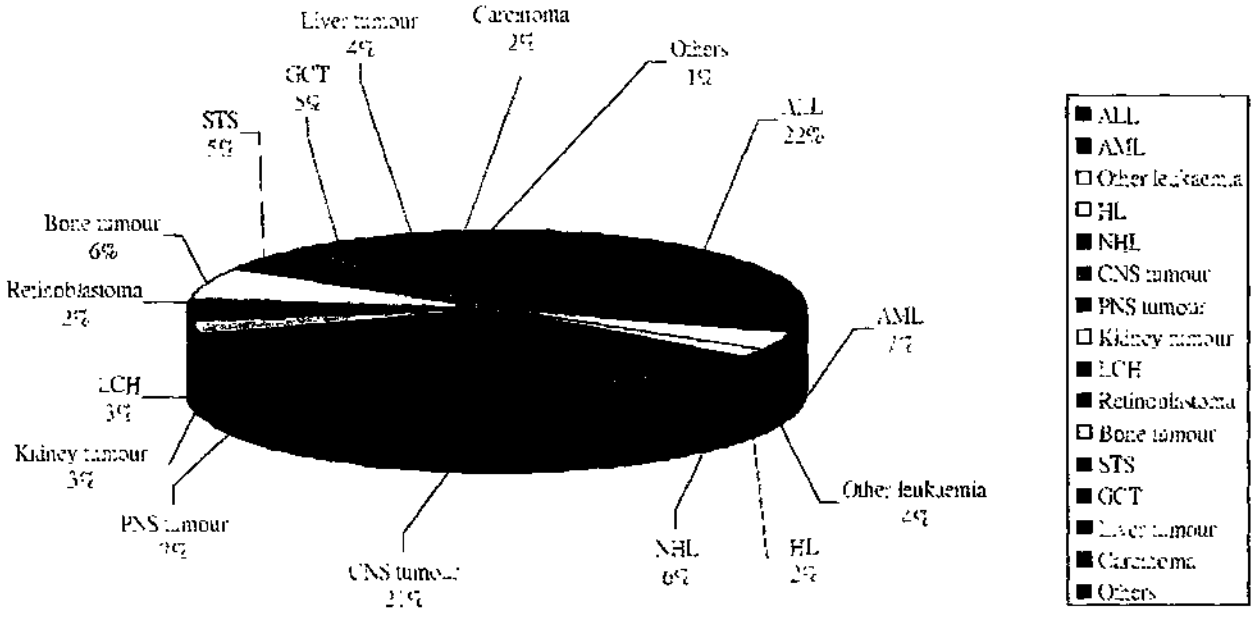
1.1 Introduction

In Hong Kong, from year 2001-2005, the incidence of childhood malignancies was 134.2 cases per million children in population aged 0 to 19 years old (1). Unpublished data from Hong Kong Pediatric Hematology and Oncology Study Group (HKPHOSG) revealed that 1164 new cases of childhood cancer diagnosed from year 2001 to 2007. Acute leukemia which include acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML); central nervous system (CNS) tumors and lymphomas which include non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) are the three commonest childhood malignancies. The frequency and distribution are summarized in Figure 1 and Table 1 (2).

Pediatric malignancies rank the second major causes of death in Hong Kong children (5-14 years old) which account for 4.3 deaths per 100,000 population whereas injury and poisoning rank the first (4.4 deaths per 100,000) (3). With the improvement in chemotherapy protocols and various treatment modalities in childhood malignancies, cancer mortality has declined substantially in the past two decades. There are statistically significant declines in mortality for each of the five-year age groups (<5, 5-9, 10-14, and 15-19 years old) for cancers combined. The declines by age group range from 2.0 to 3.2% per year. The overall decline in mortality is up to 40% between 1975 and 1995 (4).

In a recent published population-based study to assess the all-cause and cause-specific (recurrence / progression of primary disease, external cause, and non-recurrence / non-external cause) late mortality during four consecutive time periods from 1974 through 2000, among 26,643 5-year survivors of childhood cancer, Armstrong *et al* showed that all-cause late mortality improved during more recent eras, dropping from 7.1% (95% CI, 6.4% to 7.8%) among children diagnosed during 1974 to 1980 to 3.9% (95% CI, 3.3% to 4.4%) among children diagnosed during 1995 to 2000 ($p < 0.001$). The main reason was due to reduction in mortality from recurrence or progression of primary diseases, while there was no significant reduction in mortality attributable to other health-related conditions (including treatment-related health conditions) (5).

Figure 1. Distribution of new cases of childhood cancer from 2001 – 2007 in Hong Kong (Unpublished data, 2008 Annual workshop HKPHOSG)



Abbreviations: ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, CNS tumor: central nervous system tumor, PNS tumor: peripheral nervous system tumor, LCH: Langerhans cell histiocytosis, STS: soft tissue sarcoma, GCT: germ cell tumor

Table 1. Frequency distribution of new cases of childhood cancer in Hong Kong from 2001 – 2007 (Unpublished Data, 2008 Annual workshop HKPHOSG)

	2001	2002	2003	2004	2005	2006	2007	Total count
ALL	46	37	36	41	39	40	31	270
AML	11	9	10	10	11	17	16	84
Other leukaemia	2	7	6	4	6	6	11	42
HL	5	5	0	3	4	3	2	22
NHL	13	11	13	6	8	10	15	76
CNS tumour	31	41	26	40	30	37	33	238
PNS tumour	8	8	12	7	12	9	12	68
Kidney tumour	5	6	2	4	2	3	8	30
LCH	6	4	5	3	5	3	5	31
Rennoblastoma	8	7	3	3	4	5	2	32
Bone tumour	8	15	17	9	11	10	15	85
STS	9	12	6	11	16	6	2	62
GCT	8	7	6	11	4	5	16	57
Liver tumour	5	4	7	6	4	5	6	37
Carcinoma	2	1	1	3	3	5	4	19
Others	3	2	2	3	0	0	1	11
Total count	170	176	152	164	159	164	179	1164

Abbreviations: ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, CNS tumor: central nervous system tumor, PNS tumor: peripheral nervous system tumor, LCH: Langerhans cell histiocytosis, STS: soft tissue sarcoma, GCT: germ cell tumor

1.2 Overview of acute leukemia

Acute leukemia is the commonest form of pediatric malignancy. Acute lymphoblastic leukemia (ALL) is the commonest form of childhood leukemia and acute myeloid leukemia (AML) ranks the second. In Hong Kong, about 40 and 12 new cases of ALL and AML are diagnosed each year (2). Concerning ALL, the peak incidence occurs from 2-5 years old. Male has a slightly higher prevalence than female. Chemotherapy is the mainstay treatment of which consists of: remission induction, consolidation, delayed intensification and maintenance therapy together with CNS-directed therapies. The duration of treatment lasts for about two years. The intensity of treatment depends on a risk-stratification approach based on patients, disease characteristics, which include cancer genetic characteristics and subsequent treatment response which include steroid responsiveness and level of minimal residual disease (MRD) by flow cytometry and clonal-specific polymerase chain reaction (PCR) study during different stages of treatment.

Li *et al* conducted a population-based multi-centre study for childhood ALL in Hong Kong from 1993 to 1997. One hundred and forty-five newly diagnosed ALL patients were treated by the HKALL 93 protocol. Patients were stratified into three risk groups according to age, presenting white cell count (WBC), immunophenotyping and cytogenetic study in standard risk (SR), intermediate risk (IR) and high risk (HR) groups. The induction remission rate was 97.2% with 2% induction death. Two patients died during first complete remission. The 5-year overall and event-free survival of the whole group was 81.3 and 62.6% respectively. According to risk groups, the event-free survival was 79, 61% and 49% for SR, IR and HR patients respectively, while the overall survival was 96, 73 and 68% for SR, IR and HR patients respectively (6). From 2003 to 2008, we adopted the IC-BFM-2002 protocol, the 4-year event-free survival rate for SR, IR and

HR was increased to 79.3%, 83.6% and 63.2% whereas the overall survival was 86.3%, 93.4% and 66.6% respectively (unpublished data). Our results were comparable to major published western series.

Schrappe *et al* and Pui *et al* demonstrated that with the modification of treatment protocols according to risk-stratification approach based on various parameters namely, white cell counts, age, cytogenetics, and recently the clonal-specific PCR-based detection of minimal residual disease (MRD), they achieved the success in getting more children in remission after induction therapy with fewer long-term sequelae and more rational use of allogeneic HSCT to those very high risk patients. The survival of childhood ALL has increased significantly from a 6-month median survival to approaching 90% overall 5-year survival rate with only 1.5% non-relapse mortality rate (2, 7, 8).

Acute myeloid leukemia (AML) has traditionally considered as an oncological emergency. Timely and promptly initiation of chemotherapy is the most important factor to minimize disease-related morbidity and mortality. In an ideal setting with young patients with favorable cytogenetic abnormalities, standard anthracycline- and cytarabine-based induction chemotherapy can result in complete remission rate approaches 85% with long-term disease free survival rate up to 60% or greater (9).

1.3 Overview of central nervous system tumors

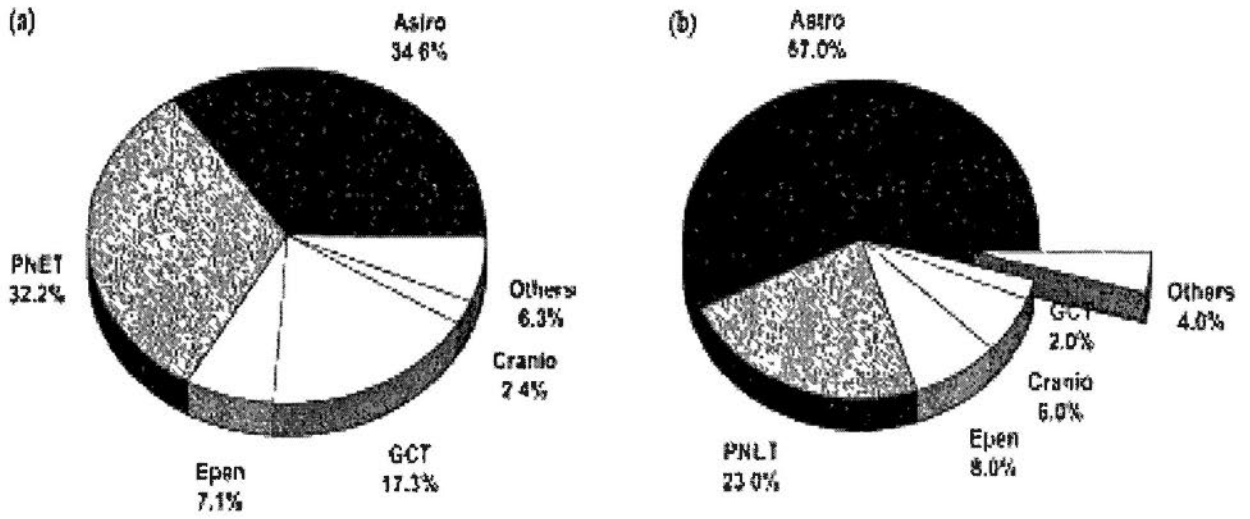
This is the second commonest cancer in children and the leading cause of death of solid tumors in children less than 15 years old. It is also the third leading cause of death from cancer in adolescents and adults aged 15-34 years old in United States (10).

In Hong Kong, 25 to 40 children aged less than 15 years are diagnosed to have various types of brain tumors each year. Based on the data from Hong Kong Pediatric Hematology & Oncology Study Group (HKPHOSG) and Hong Kong Cancer Registry (HKCR), 131 children were diagnosed to have brain tumors in Hong Kong from January 1999 to December 2003 (11, 12). The annual incidence of brain tumors for Chinese children in Hong Kong is 22.9 cases per million children. A much higher incidence of intracranial germ cell tumors is observed in Hong Kong when compared with western data (13, 14). The commonest histological subtype of brain tumors in children is astrocytoma. Primitive neuroectodermal tumor (PNET) including medulloblastoma ranks the second. The relative frequency of each subtype in our patients' cohort is shown in Figure 2. Forty to fifty percents of the childhood brain tumors are originated from infratentorial territory whereas medulloblastoma is the commonest form of tumor arising from that region.

Poor prognostic factors of medulloblastoma include presence of metastatic site at presentation, large cell/anaplastic phenotype, chromosome 17p13.3 loss, and high-frequency *MYC* amplification. Outcome of children with high risk features is significantly poorer than low-risk group who has none of these tumor characteristics (15) ($p=0.0002$).

Other relatively common childhood infratentorial brain tumors include juvenile pilocytic astrocytoma, ependymoma and brain stem glioma. More than 95% of brain stem gliomas are high-grade astrocytoma and locate mainly in the pontine region. Germ cell tumors mainly arise from the mid-line structures of the brain in particularly over the pineal region. Whereas ependymoma characteristically arises from the para-ventricular areas along the whole central nervous system (12). These characteristics are similar to what have been described in the Western literatures (13, 14).

Figure 2. (a) Comparing incidences of childhood brain tumors in Hong Kong (HKPHOSG & HKCR, January 1999-December 2003, n=131), with (b) Western series (13, 14)



Abbreviations: Astro: astrocytoma (including all grades and brain stem), PNET: primitive neuroectodermal tumors (including supratentorial and medulloblastoma), Epen: ependymoma, Cranio: craniopharyngioma, GCT: germ cell tumors

1.4 Overview of Lymphoma

Lymphoma is the third commonest childhood malignancies which accounts for approximately 8-10% of paediatric oncology patients (16). World Health Organization (WHO) classified lymphoma according to cell type, phenotype, molecular and cytogenetic characteristics. There are 5 main groups: (i) B cells; (ii) T cells and (iii) Natural killer cells lymphoma (iv) Hodgkin lymphoma and (v) Immunodeficiency-associated lymphoproliferative disorders (17).

In pediatric population, the common types include: (i) diffuse large B cell lymphoma; (ii) Burkitt lymphoma; (iii) lymphoblastic lymphoma (primarily precursor T-cell lymphoma and less frequently, precursor B-cell lymphoma); and (iv) anaplastic lymphoma (T-cell or null cell lymphoma) (18, 19). These are fast-growing tumors and may also disseminate widely, especially to bone marrow and central nervous system. Abdomen is the most common primary site (30%-45%), and most of the cases are Burkitt lymphoma. Mediastinal tumors (25%-35%) are typically T-cell lymphoblastic lymphoma. The third most common site is at head and neck regions (10%-20%). A diagnosis can be rapidly obtained by tissue biopsy or bone marrow for histology, immunohistochemistry, cytogenetic and molecular studies. Until 1970s, childhood NHL had a poor prognosis and the majority of children died within weeks of diagnosis because of progression of primary disease, or dissemination to bone marrow or central nervous system (CNS). Significant improvements in survival have been achieved in the past 20 years mainly due to advances in chemotherapy. In Hong Kong, from the year 1995 to 2007, 133 new cases of NHL were diagnosed. The overall 5-year event-free survival of Burkitt lymphoma was 87.7%, lymphoblastic lymphoma was 79.7% and large cell lymphoma was 60.7% (20). In our hospital, 44 cases were diagnosed from 1995 to 2007 (33 males; 11 females). The median age of presentation was 9.2 years old. Twenty-six cases were B-cell NHL

included Burkitt and Burkitt-like lymphoma and diffuse large B-cell lymphoma, 14 cases were lymphoblastic lymphoma; and 4 cases were anaplastic large cell lymphoma. Abdomen was the most common presenting site (14 cases in abdomen; 11 cases in mediastinum; 4 cases in head and neck; 7 cases in cervical lymph nodes; 4 cases had multiple sites involvement; 3 had involvement at other anatomical sites which included 1 skin, 1 bone and 1 pericardium). The cases are predominately stage III and stage IV diseases (18 cases and 13 cases respectively). Chemotherapy regimen differs according to the histological subtype. Burkitt and large B-cell NHL are treated with intensive, pulsed chemotherapy whereas lymphoblastic lymphoma is treated with prolonged chemotherapy, and currently most centers adopt treatment protocol for acute lymphoblastic leukemia. CNS-directed therapy is essential and is based on intrathecal chemotherapy rather than radiotherapy. There is little role for surgery in the management of NHL (21).

Sun *et al* studied the treatment outcome of Chinese children and adolescents with lymphoblastic lymphoma from 1998 – 2006. This study was designed to evaluate the efficacy and toxicity of a modified acute lymphoblastic leukemia (ALL)-Berlin-Frankfurt-Münster (BFM)-90-based protocol in Chinese children and adolescents with lymphoblastic lymphoma. The study period was from March 1998 to November 2006, 60 untreated patients with lymphoblastic lymphoma (age <18 years) were enrolled. All patients were treated with the modified ALL-BFM-90 protocol. The median age of the patients was 10 years (range 2.5-18 years old). Forty-eight (80%) patients had T-cell lymphoblastic lymphoma, and 59 (98.3%) patients were in advanced stage of disease. At the end of induction remission, 3 patients died of treatment-related toxicity. In the remaining 57 patients, complete remission (CR) or CR undetermined (CRu) had occurred in 47 patients (82.5%), who were designated as the moderate-risk group and partial remission (PR) had occurred in 10 patients (17.5%), who were

designated the high-risk group. All patients experienced grade 3-4 hematological toxicity. At a median follow-up of 35 months, event-free survival was 78.8% for all patients. Among the moderate-risk group, event free survival was 88.3% (90.9% for stage III, 87.7% for stage IV, 100% for those with B-cell LBL, 84.8% for those with T-cell LBL, and 82.9% for stage IV patients). The event-free survival in the high-risk group was 60%. They showed that modified ALL-BFM-90 protocol was an effective regimen against lymphoblastic lymphoma and greatly improved the survival rate of Chinese children and adolescents with lymphoblastic lymphoma compared with the ALL protocols that were used previously (22).

1.5 Conclusion

In this chapter, an overview of various pediatric oncology conditions is presented, chemotherapy plays an important role in treatment of the four commonest pediatric cancers. The survival improves significantly from 10% to almost 90% over the last 50 years due to improvement in various treatment protocols.

Chapter 2 An Overview of Different Treatment Modalities in Pediatric Oncology and Impact on Destruction of Immune System

2.1 Introduction

Chemotherapy, radiation therapy (RT) and surgery are the main treatment modalities in pediatric oncology. It is too straightforward to think that only chemotherapy +/- RT will induce immunosuppressive effect on the host. In fact, the interaction between cancer and host's immune system is far more complex. It is now clear that cancer patients, particularly patients with acute leukemia and lymphoma, display different degrees of immunosuppression even at the time of presentation (23). As with the increase in treatment dose intensity of various anti-cancer regimens, the immunosuppressive effects of these regimens also increased. With the advance in treatment protocols, cooperative multi-center and interdisciplinary approaches, the survival rate of childhood cancers is now up to 75-80% (24). In this chapter, different treatment modalities in pediatric oncology and the impact on destruction of immune system are described.

2.2 Primary diseases lead to immunodeficiency

Patients with acute leukemia are already shown to have signs of immunosuppression at the time of presentation. Walker *et al* studied the immune function of 20 patients with AML. *In vitro* phytohemagglutinin (PHA) transformation of washed lymphocytes obtained from these subjects was assessed by measuring the rate of DNA synthesis after 70 hours of incubation. The time point of assessment included pre-treatment, post-induction and post-consolidation samples. They showed that 42% of pretreatment sera already showed inhibitory response. Reduced *in vitro* immunological reactions were also shown in patients of induction failure due to presence of blasts in samples. The inhibitory activity could be overcome by high PHA concentration. Serum inhibitory factors may also pose significant immunosuppressive effect *in vivo* (25). Patients with

untreated Hodgkin lymphoma frequently have impaired lymphocyte proliferation to a variety of antigens (26). Patients with Burkitt lymphoma, have been reported to show variable levels of lymphocyte depletion which is related to the stage of disease (27). Therefore, children with various hematological malignancies are already immunocompromised before starting immunosuppressive therapies.

2.3 Chemotherapy

The use of chemotherapy can be dated back in the early 20th century in World War II that a group of military personals incidentally exposed to mustard gas and were later found to have leucopenia. In 1940s, Goodman *et al* reported the use of this agents in terminal stage lymphoma patients, it showed temporary but significant improvement (28). Thereafter, many other agents with anti-neoplastic properties were developed.

In general, most chemotherapeutic agents target at dividing cells by impairing mitosis or DNA synthesis at various stages of cell cycle. In general, fast-growing tumors are more susceptible to these agents than slow-growing tumors. However, normal human fast-dividing cells namely hematopoietic cells, hair cells and mucosal epithelial cells can also be affected by these agents and cause debilitating side effects namely bone marrow aplasia, alopecia and mucositis.

2.3.1 Cell cycle

Cell cycle is a series of events that takes place and leads to division and replication of cells. The cell cycle consists of five major phases: (i) resting phase (G_0); (ii) G_1 phase; (iii) S phase; (iv) G_2 phase and (v) mitosis (M phase).

Resting (G_0) phase refers to cells in quiescent state, that is, non-proliferating cells. G_1 phase is the first phase of interphase. It represents the stage of growth and is marked by the synthesis of various enzymes that are required for DNA replication. S phase is the phase which DNA synthesis occurs. By the end of S phase, all the chromosomes have been replicated. G_2 phase is the phase before mitosis occurs. Significant protein synthesis occurs which mainly involves microtubules which are required during the process of mitosis. In the M (mitosis) phase, it is further subdivided into prophase, prometaphase, metaphase, anaphase, telophase and then to cytokinesis in which the cells are actually dividing.

2.3.2 Major groups of chemotherapy

The major groups of chemotherapeutic agents with examples are as follow:

(i) Alkylating agents

They are the oldest and most commonly used agents. They form interstrand and intrastrand DNA crosslinkages and lead to cell death. They are cell-cycle non-specific. The alkylation of DNA has been linked to the mutagenic properties of these agents and may be associated with secondary malignancies (29). Cyclophosphamide, thiotepa, busulphan and melphalan are examples.

(ii) Antimetabolites:

They inhibit DNA and RNA synthesis by competitively inhibit key enzymes that are crucial to the synthesis of purine or pyrimidine nucleotides and they are cell-cycle specific at S-phase. Mercaptopurine and methotrexate are examples.

(iii) Antitumor antibiotics:

They work through DNA intercalation and inhibit the progression of topoizomerase II. They are cell-cycle non-specific. Examples include actinomycin D, dactinomycin and bleomycin

(iv) Tubulin binding agents

They are derived from natural occurring plants. They are mitotic inhibitors and act by prevention of formation or stabilization of microtubules. They are cell-cycle non-specific agents. Plant alkaloids include paclitaxel and vinca alkaloids include vincristine and vinblastine

(v) Topoisomerase inhibitors:

Topoisomerase facilitates the relaxation and unwinding of DNA by permitting the formation of a controlled break in double-stranded DNA and permits another double strand to pass through the break. Normally the topoisomerase-mediated double-strand break is resealed but these agents act by stabilizing the topoisomerase II/DNA cleavable complex and prevent resealing of double-strand break. The persistence of strand breaks can result in apoptosis and block DNA replication. They are cell-cycle specific agents at S or early G_2-phase (29). Examples include topotecan, irinotecan and etoposide.

(vi) Enzymes

L-asparaginase is an example of this class. Malignant cells are unable to synthesize asparagine from aspartic acid since they lack L-asparagine synthetase. Therefore, cancer cells are dependent on extracellular source of asparagine. L-asparaginase depletes this source by converting L-asparagine to aspartic acid and ammonia and deprives the malignant cells from this essential nutrient.

(vii) Glucocorticoids

Glucocorticoids bind to glucocorticoid receptor and induce apopotosis by inhibition of IL-2 production, down-regulation of c-myc and repression of transcription factors such as AP1. Prednisolone and dexamethasone are standard agents used in the treatment of leukemia and lymphoma (29).

There are newer agents that do not directly act by interfering DNA synthesis and mitosis. They target at specific tumor surface antigens and induce clearance of tumor cells via host's immune response or act at specific molecular abnormalities of cancer cells and block subsequent signaling pathway. Examples of monoclonal antibodies include (i) Rituximab (Rituxan, Genentech, Inc.) is a chimeric anti-CD 20 monoclonal antibody which directs against human B-lymphocyte; (ii) Gemtuzumab ozogamicin (Mylotarg, Wyeth) is a humanized anti-CD33 antibody conjugated with a cytotoxic antitumor antibiotic, calicheamicin which acts on surface of myeloid leukemia cells; (iii) Imatinib mesylate (Glivec, Novartis Pharma) is a tyrosine kinase inhibitor which targets at the molecular abnormality in chronic myeloid leukemia. The advantage of these targeted agents is to minimize the effects of non-tumor cells and aim at reducing the impact on these healthy cells and reduce the systemic side effects of these agents.

2.4 Radiation therapy

Radiation therapy has been used in cancer therapy for more than a century. The concept was invented by Wilhelm Conrad Röntgen in 1895. This field began to grow rapidly since 1900s after the great achievement grounded by Marie Sklodowska Curie who discovered radioactive elements radium and polonium. Medical linear accelerators have been used as source of radiation since late 1940s.

Photon, electron, or ion beams directly ionizes the atoms which make up the DNA chain of tumor cells and thus cause damage in DNA. Indirect ionization induces ionization of water by photons and electrons presented in radiation source, forming free hydroxyl radicals and causes damage in DNA chain. In general, normal human cells have excellent repair mechanisms but cancer cells have diminished ability to repair these damages and hence continue to produce cells with these damages. These cells with the damaged DNA will either die or reproduce slowly.

There are three main forms of radiation therapy which include (i) external beam radiotherapy (conventional and stereostatic); (ii) systemic radioisotopes (unsealed source radiotherapy) and (iii) brachytherapy (sealed source radiotherapy). In pediatric malignancies, the first two forms are the mainstay of radiation therapy.

Conventional external beam radiotherapy is delivered via two-dimensional beams using linear accelerator to target tissue. The technique is well-established and reliable. The main limitation is the radiation tolerance in surrounding healthy tissues. Stereotactic radiotherapy focuses high doses radiation at target tissue and thus minimizes damage to adjacent healthy tissues.

Systemic radioisotope is a form of targeted therapy. Targeting can be achieved by attaching the radioisotope to another molecule or antibody to guide it to target tissue. Infusion of metaiodobenzylguanidine (MIBG) for neuroblastoma is one of the examples.

The side effect profile depends on the dose and field of irradiation. It causes damage to skin, oral, pharyngeal and bowel mucosas and causes breakdown of skin or mucositis. Irradiation of spine causes bone marrow suppression due to presence of plenty of bone marrow tissue within the medullary cavity of vertebral bones. Irradiation to thymic region, namely in total body irradiation (TBI) or in Hodgkin lymphoma, induces tissue damage to epithelial cells of the thymus and impairs its function in T-cell maturation. Fractionation of radiation dosage helps to diminish these dose-related side effects. In long term, secondary malignancy, mainly sarcoma, at radiation field is always a rare but serious long term complication of radiation therapy.

In our unpublished series, 1425 new cases with median age 5.9 years (range 0.1-21.1 years old) treated in our oncology unit in 24-year study period were reviewed. The incidence of secondary malignancies was about 1%. The median time to develop secondary malignancies was 6.5 years after diagnosis of primary tumors. Cranial irradiation was a major risk factor. Patients developed secondary central nervous system tumor and osteosarcoma had the poorest prognosis.

2.5 Impact of chemotherapy and radiation therapy on immune system

Pediatric oncology patients are high-risk group of acquiring opportunistic and serious infections. The reasons are as follow: (i) breaking down of natural defective barriers, namely mucositis after chemotherapy and / or radiation therapy; (ii) presence of forcign bodies, namely indwelling central venous catheters or ventriculoperitoneal shunts (iii) prolonged period of neurtropenia due to sequence of primary disease and marrow aplasia after receiving chemotherapy and (iv) impaired cellular and humeral immunities after chemotherapy and/or radiation therapy (30).

2.6 Immunosuppressive effects of chemotherapy

Virtually all chemotherapeutic agents can cause different degree of suppression of immune system. Since they act on rapid-dividing cells and therefore, bone marrow is one of the most severely affected sites. Pancytopenia develops at 5-7 days, and reaches nadir at about 10-14 days after commencement of chemotherapy. Neutropenia is one of the most significant predisposing factors in causing various opportunistic infections. Kosmidis *et al* prospectively studied the immune functions of 72 children with ALL from diagnosis to 12 months after termination of treatment. They showed that CD19-expressing B-lymphocyte number was very depressed (median absolute count 24 x 10^9/L) with low immunoglobulin levels at the end of intensive chemotherapy. CD4 / CD8 ratio was persistently less than 1 at the end of intensive chemotherapy. CD4 T-lymphocyte dropped significantly after starting treatment and continued to be at low level uptil 12 months after stopping all treatment. The above data showed that both the humoral and cellular immunities were significantly suppressed during and after commencement of intensive chemotherapy (31).

Therapy regimens which include chemotherapy such as cyclophosphamide, purine nucleoside analogs or corticosteroids particular have severe suppressive effects on lymphocyte function. For example, treatment regimens for acute lymphoblastic leukemia (ALL) are targeted against lymphoid cells and can have significant adverse influence on lymphocyte function, while chemotherapy for the treatment for low risk Wilm's tumor namely actinomycin D and vincristine are not particular immunosuppressive.

Alkylating agents, especially high dose cyclophosphamide, are profoundly immunosuppressive (32). Purine nucleoside analog, namely fludarabine monophosphate, is another class of agents with a predilection for lymphocyte depletion (33). These agents have a remarkable capacity of depletion of both dividing and resting lymphocytes and result in severe opportunistic infections.

Moreover, studies have shown that despite immune recovery can be demonstrated after completion of chemotherapy, the "acquired" immunity through active vaccinations which require immune "memory" may not be fully recovered especially in young children, leaving them vulnerable to vaccine-preventable infectious diseases which are potentially life-threatening (34).

2.7 Effect of radiotherapy on immune system

The data on the effects of radiotherapy on immune system is relatively scarce when compared with chemotherapy. Irradiation to spleen may cause functional hyposplenia and asplenia and increases probability to infection by polysaccharide encapsulated bacteria. Irradiation to thymic region in children with Hodgkin disease, which is immunologically important for children, may have negative impact on subsequent immune recovery (35). Irradiation to spine, such as in patients with medulloblastoma can develop prolonged neutropenia due to extensive irradiation to normal marrow tissues in the spinal vertebrae.

2.8 Effect of chemotherapy and radiation therapy on mucosa

Mucositis induced by chemotherapeutic agents is an important, dose-dependent side effect of anti-cancer therapy. The breakdown of mucosa not only causes pain and restriction of oral intake, it also acts as port of entry of endogenous oral and/or gut flora causing secondary blood-stream infections. About 40% of patients develop mucositis at various stages of their treatment. Up to 50% of them require treatment modification and support by parenteral analgesia (36, 37). Mucositis is a complex process which occurs in four phases (i) vascular phase; (ii) epithelial phase; (iii) bacteriological phase and (iv) healing phase. Not all anti-cancer agents are equally active in this process, antimetabolites, namely methotrexate, cytarabine and 5-fluorouracil which affect DNA synthesis (S-phase cell cycle), are the commonest agent. The period of ulcerative phase usually coincides with the period of neutropenia after chemotherapy. Bacterial colonization of mucosal ulceration commonly leads to local infection and systemic translocation of bacteria into bloodstream. Therefore, Gram negative organisms and alpha-hemolytic streptococci are the main infective agents causing bacteremia in this group of patients (37, 38).

2.9 Immunosuppressive effect of viral infection

Even in immune-competent individuals, cytomegalovirus (CMV) infection is associated with impairment of T-cell function. Giebel S *et al* evaluated the influence of CMV infection in allogeneic hematopoietic stem cell transplantation recipients, treated pre-emptively with ganciclovir, in T-cell function. Mitogen-stimulated T-cell proliferative activity, together with cell surface markers, was tested in 49 patients on days 30, 45, 60, and 90 after allogeneic hematopoietic stem cell transplanation and, additionally, in cases of positive CMV pp65-antigenemia. T-cell proliferative activity was significantly decreased on days when CMV antigenemia was positive as compared to days without antigenemia. The number of pp65-positive cells negatively correlated with proliferative response. They demonstrated that even clinically asymptomatic, CMV infection posed negative impact on T-cell proliferation capacity in allogeneic hematopoietic stem cell transplant recipients. Pre-emptive therapy with ganciclovir might modify this immunosuppressive effect (39).

2.10 Central venous assess devices and infection

Central venous assess devices (CVAD) have been used for at least 20 years in treatment of childhood cancers. On one hand, they provide a straightforward, safe and reliable route for administering cytotoxic agents, antimicrobial agents, analgesics, blood products, hyper-osmolar parental nutrition and allow painless blood collection. On the other hand, long term implementation of foreign materials into vascular system increases risk of catheter-related blood-stream infections. Microbiological colonization of CVAD usually occurs within the first 24 hours of catheter implantation. The colonization may involve the catheter lumen and extra-luminal surface. Bacteria are capable of binding to foreign materials and cross-link with extracellular glycopolysaccharides, fibrin, fibronectin and form extracelluar biofilm matrix and prevent adequate immune response against adhering bacteria and hinder the eradication of microorganisms despite treatment with appropriate antimicrobial agents (39, 40). Most common pathogens are Gram-positive bacteria, particularly coagulase-negative staphylococci. In order to deliver antimicrobials to bacteria effectively, disruption of biofilm by urokinase or ethanol may have a role (41, 42).

With the better understanding of the pathophysiology of sepsis, aggressive use of appropriate broad spectrum empirical antibiotics and improvement in intensive care support, the mortality of septic shock decreased from more than 50% in 1980s to about 16% in a recent study. However, it still causes significant morbidities and mortalities in pediatric oncology patients (43, 44).

2.11 Infections associated with monoclonal antibodies

Treatment with rituximab (Rituxan, Genentech, Inc.), a chimeric anti-CD 20 monoclonal antibody directed against human B-lymphocyte, can cause profound and prolonged complement-mediated B-lymphocyte depletion and hypogammaglobulinemia. In the background of immunosuppression, reactivation of various viruses (CMV, enterovirus, HBV, HCV, Parvovirus B19, Polyomavirus, VZV) have been reported after receiving rituximab. This causes additional morbidity or even mortality in pediatric oncology patients (45-47). Tumor necrosis factor – α (TNF-α) blocking agents, namely infliximab (Remicade, Schering-Plough) and etarnercept (Enbrel, Wyeth), are now one of the effective agents in treatment of acute or chronic graft-versus-host disease which is one of the serious complications of pediatric allogeneic hematopoietic stem cell transplantation. With the importance of TNF-α in host response in formation of granuloma and maintenance, anti-TNF-α therapy is linked with increased susceptibility to various infections, which include *Mycobacterium tuberculosis*. Reactivation of latent tuberculosis is increased in patients treated with TNF-α blockers (48, 49).

2.12 Conclusion

In this chapter, the impact of primary diseases and various treatment modalities on the host's immune system are discussed. This makes the host vulnerable to various potential life-threatening infectious diseases. These impacts may not be fully reversed after completion of treatment of primary diseases. This makes the formulation of various preventive strategies for preventing of infection after completion of treatment a justifiable and reasonable act.

Chapter 3 Current Understanding of Immune Reconstitution in Pediatric Oncology Patients Received Chemotherapy and Hematopoietic Stem Cell Transplant Recipients

3.1 Introduction

In this chapter, the current understanding of immune reconstitution in children who have received immunosuppressive therapy is discussed. We know that after receiving chemotherapy, the nadir of bone marrow suppression is about 10-14 days. After the nadir, neutrophils are the first one to return to normal (3-4 weeks) and then follow by natural killer cells (NK cell), while B and T lymphocytes take longer time to recover. This process of recovery varies with children's age. However, residual deficits may persist until 12 months after completion of treatment protocol or even longer. Moreover, the recovery of "immune memory" may not be fully completed especially in young children (34). This will be further elaborated.

3.2 Immune reconstitution of cell-mediated immunity

Alanko S *et al* studied the recovery of cell-mediated immunity after cessation of chemotherapy in children with acute lymphoblastic leukemia (ALL) in 1990s. The total T lymphocyte count normalized at 1 to 3 months after stopping chemotherapy. Whereas for T-cell subset, the regeneration of naïve T lymphocytes (CD4, CD45RA+) seemed to be faster in children aged 3 to 6 years old. However in older children (7 to 18 years old), they were normalized only after 6 months of stopping chemotherapy (50). Regeneration of memory lymphocytes (CD4, CD45RO+) were more effective in adults than children. Therefore, despite the recovery of "number" of lymphocytes, the effective response to previous vaccinated antigens which require immune "memory" may not be fully recovered in young children. The recovery time of children with different primary diseases is different and this is partly due to the difference in immunosuppressive effect

of primary disease and treatment protocol. Alanko S *et al* showed that children with Hodgkin lymphoma or Burkitt lymphoma had a slower recovery of total lymphocyte counts and T-lymphocyte counts (50, 51).

With the increase in treatment intensity of pediatric cancer, the degree of immunosuppression increased. Kosmidis *et al* studied the immune recovery of 72 children with ALL longitudinally up to 12 months after stopping all chemotherapy (including maintenance therapy) in 2000s. They showed that CD4+ T-lymphocyte number dropped significantly at 6 months after stopping intensive chemotherapy and persisted up to 12 months. CD45RO+ T-lymphocyte subset continued to decline up to 18 months after stopping intensive chemotherapy but the numbers were significantly higher in children older than 7 years old than young children. CD 16/56+ NK cell count was persistently low until 12 months after cessation of all therapy. Again, NK cell count was higher in older children (>7 years) when compared with young children. The CD4/CD8 ratio was less than 1 at end of intensive chemotherapy but persistently > 1 at later evaluation points (31).

Immunosuppression persist despite children are receiving maintenance chemotherapy. El-Chennawi FA *et al* evaluated the immune reconstitution in children with ALL during the maintenance chemotherapy. Thirty-six children with ALL (24 females and 12 males) in the maintenance phase of therapy and 12 healthy age and sex-matched control were recruited in this study. Laboratory investigations included complete blood count, serum creatinine, liver function tests and evaluation of the immune system by measuring CD3+, CD4+, CD8+, and CD16/56+ (cellular immunity) by flow cytometry at the first and the third month of maintenance therapy were performed. The results of the study documented presence and persistence of leucopenia and lymphopenia during maintenance therapy with decreased medians of CD3+, CD4+ and CD8+ from the

first to the third month of therapy. CD16/56+ and CD4/CD8 ratio showed increasing median from the first to the third month of therapy. In summary, T-cell reconstitution showed delayed in recovery of both T helper and T suppressor cells even in the phase of maintenance chemotherapy in children with ALL (52).

3.2 Immune reconstitution of humoral immunity

Kosmidis *et al* showed that CD19+ B lymphocyte was suppressed at the end of intensive chemotherapy (median absolute count 0.24 x 10^9/L) and returned to normal value by 6 months after stopping chemotherapy. Immunoglobulin levels were dropped significantly by the end of intensive chemotherapy. IgG and IgA were increased back to normal by 6-12 months after cessation of chemotherapy. IgM remained low and unchanged throughout the study period (31).

El-Chennawi FA *et al* also evaluated humoral immune reconstitution in children with ALL during the maintenance phase of therapy and to correlate between the presence of severe infections and the abnormalities in immune system during reconstitution. Thirty-six children with ALL (24 females and 12 males) in the maintenance phase of therapy with 12 healthy age and sex-matched control were recruited in this study. The results of the study documented presence and persistence of leucopenia and lymphopenia during maintenance therapy with decreased medians of CD3+, CD4+ and CD8+ from the first to the third month of therapy. The other markers CD19+, IgA, IgM, IgG and CD4 / CD8 ratio showed increasing median from the first to the third month of therapy. They also demonstrated a significant correlation between infection and CD19+ and serum IgM at the first month and between infection and CD19+, IgM and CD4/CD8 ratio at the third month of therapy. They concluded that persistent immunosuppression was presented in children with ALL during maintenance therapy. Reconstitution of B lymphocytes

occurred earlier than T cell reconstitution (52).

Summarizing the results of various studies, both B and T lymphocytes are suppressed during treatment period. B-lymphocytes start to restore to normal numbers about 1 month after stopping therapy. For T-lymphocyte series, CD4+ lymphocytes recover more slowly than CD8+. Total B and T-lymphocytes usually recover fully in term of quantity by 6 months after stopping chemotherapy, although in some extreme cases, can be up to 12 months (50, 51, 53). The recovery of immunoglobulin level can take up to 12 months after completion of treatment, especially IgG_2 (54). Moreover, young patients are shown to be more likely to suffer immune suppression after receiving chemotherapy (55, 56).

3.3 Immune reconstitution after hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation was conceived more than 50 years ago as treatment for fatal irradiation injury. It is now mainly used for treatment of high risk hematological malignancies and many other disorders including immunodeficiency syndromes, hemoglobinopathies, autoimmune disorders, myelodysplastic syndrome, bone marrow failure syndromes and inborn errors of metabolism (57).

After conditioning, due to the immune suppressive effects of intensive chemotherapy and tissue injury associated with conditioning regimen, and with the administration of immunosuppresents to prevent graft rejection and graft-versus-host disease, recipients become severely immunocompromised and are prone to various bacterial, viral and invasive fungal infections.

After donor stem cells infusion, monocytes are the first cells to engraft, follow by granulocytes, platelets and natural killer cells. These cells restore the innate immunity of host. The recovery of lymphocytes comes by two pathways. The first batch of T-lymphocyte recovery is progeny of donor T cells co-transfused with the donor graft that expands after entering the host (58). This occurs in the first year of transplantation. The clonal expansion of a limited number of donor T lymphocytes may only contain a few antigen specificities. They are mainly memory and effector (CD45RA+RO+) T cells (59-61).

The second pathway is the thymic emigrants. This depends on a functioning thymus. This produces mainly naïve T cells which are CD45+RO-. This appears approximately 6 months after transplantation. Patients with low thymic output, namely adult patients or received irradiation to thymus after total body irradiation, will have a major delay of T-cell output by this pathway. Therefore, in adults or thymectomized patients, the naïve T lymphocytes reconstitute late in transplant period (usually more than 6 months) and the partial restoration of this pool may require up to 12 to 24 months and may only appear in individuals younger than 40-50 years (62, 63).

B lymphocyte is low in number during the first 2 months after transplantation. The number of B lymphocyte increases gradually in the first 1-2 years and memory B cells remain scarce in number. Serum immunoglobulin isotypes recover in the sequence of IgM, IgG_1/IgG_3, IgG_2/IgG_4 and IgA. Hence, B cell reconstitution after transplant resembles a recapitulation of ontogeny that is likely to occur more slowly in young children because follicular dendritic cells and CD4+ T cells in germinal centers needed for isotype switching are scarce (64-66).

The degree and extent of immune reconstitution depends on a number of factors, namely (i) the degree of immunosuppression in conditioning regimen; (ii) use of anti-thymocyte immunoglobulin; (iii) irradiation to thymus; (iv) graft manipulation by *ex vivo* or *in vivo* T cells depletion and (v) presence of acute and / or chronic graft-versus-host disease (GVHD). Therefore, post-transplant infections remain a major cause of morbidity and mortality in this group of patients.

3.4 Conclusion

Over the last 50 years, with the improvement in treatment protocols and increases in dose intensity of various chemotherapy regimens, the survival rates of various childhood cancers have risen drastically from 10% to almost 80% (67). Children treated with intensive chemotherapy suffer from more severe and prolonged depression in both humoral and cellular immunities. The immune recovery continues but there is still persistent partial immune deficiency in term of low IgG_2, IgM, NK cell count and CD45RO+ T-lymphocyte subsets up to 12 months after stopping all chemotherapy. Older children perform better than young children. This may have significant implication in term of formulating various preventive strategies in protecting children, especially young children from various infectious diseases during and after stopping chemotherapy.

Section 2

Strategies for Prevention of Infectious Diseases in Pediatric Oncology Patients

Chapter 4 Literature Review

4.1 Introduction

Opportunistic infection is an important cause of morbidity and mortality in pediatric oncology patients. It is not only life-threatening, but also causes prolonged hospitalization, compromises subsequent chemotherapy delivery, affects quality of life, and increases healthcare utilization. The presentations can be either: (i) unusually severe clinical course due to common pathogens; (ii) atypical presentations of relatively benign pathogens or (iii) occurrence of opportunistic infections. Prolonged exposure to antimicrobial, antifungal and antiviral agents may induce drug-resistant strains that pose difficulties in treatment of subsequent infective episodes.

In this chapter, the important aspects of infection in immunocompromised host are highlighted and the present strategies for prevention of infection in pediatric oncology patients are summarized.

4.2 Impact of infectious diseases in pediatric oncology patients

Sung *et al* showed that 30% - 60% of children experienced at least one episode of microbiologically-proven infection throughout the treatment period of malignancies (68, 69). Although the overall survival rate of children with cancer is now up to 70% - 80% in recent years due to significant advances in treatment protocols and supportive care, infection remains a major cause of therapy-associated morbidity and death (70). Moreover, the prevalence of infections varies depending on the type of chemotherapy, with the highest risk of infections associated with intensive induction and consolidation courses of chemotherapy (68, 69).

With the increase in severity of immunsuppression resulting from (i) underlying diseases, namely leukemia, and (ii) intensive chemotherapy, pediatric oncology patients often suffer from prolonged period of severe neutropenia secondary to marrow hypoplasia and are unable to mount adequate cellular and humoral immune response to protect them from various infectious agents. This can result in severe clinical courses even relatively benign pathogens are encountered. Prolonged symptomatic and asymptomatic shedding of virus are commonly observed in immunocompromised patients. This can last for weeks or months and contributes significant risk of nosocomial transmission of disease in oncology units. Cheng *et al* reported a case of 15-month-old boy with stage 3 neuroblastoma who had prolonged shedding of respiratory syncytial virus (RSV) for 7 months during his course of intensive chemotherapy treatment, which was the longest period being reported in literatures (71). This poses significant implication in hospital infection control and planning of in-patient isolation care facilities for pediatric oncology patients since at least 30% of all rooms need to be allocated for isolation of patients infected or colonized with communicable pathogens (72, 73).

In Hong Kong, the incidence rates of invasive pneumococcal disease (IPD) caused by *Streptococcus pneumoniae* were 18.8 and 15.6 per 100,000 in children $\leqslant 2$ and $\leqslant$ 5 years old respectively (74). Miesel *et al* conducted a 6.5-year national-wide surveillance for IPD in Germany. They showed that children with acute lymphoblastic leukemia (ALL) carried 10-fold higher risk for IPD when compared with general pediatric population and the risk was actually similar to recipients of hematopoietic stem cell transplantation (HSCT). Over 90% of IPD cases presented as bacteremia. The mortality rate was about 10%. The highest risk of IPD occurred during the first 2 years after initial diagnosis and 50% of cases occurred in ALL children on maintenance chemotherapy. The highest risk was observed in children aged 5-9 years old who had

50-fold increase in IPD risk when compared with general pediatric population (75).

Protective strategies of IPD include chemoprophylaxis and active immunization. Administration of trimethoprim-sulphamethoxazole at prophylactic dose for *Pneumocystis jirovecii* prophylaxis during treatment period does not provide protection from IPD (75). With the emergence of penicillin-resistant *Streptococcus pneumoniae* in Hong Kong, up to 65% and 37% of isolated strains of *Streptococcus pneumoniae* from nasopharyngeal aspirate are not susceptible to penicillin and second generation cephalosporin respectively (76). Moreover, in our patient population with extensive exposure to various broad spectrum antibiotics, the percentage of penicillin-non-susceptible species is expected to be much higher than the general population although the data in this area is lacking. Thus chemoprophylaxis is unlikely to be an effective protective method for IPD in pediatric oncology patients. Alternative strategies for prevention of infectious diseases need to be explored.

Apart from a complicated clinical course, they can also encounter atypical presentations of common infectious agents. Epstein-Barr virus (EBV) was first discovered in 1964 by Epstein, Achong and Barr (77). It is a very "successful' virus that causes infection in over 90% of humans and persists for the lifetime as latent state in humans. The manifestation of infection range from asymptomatic or have non-specific symptoms on the benign side to fatal EBV-related lymphoproliferative disorder and it is also implicated in a number of malignancies, namely Burkitt lymphoma and nasopharyngeal carcinoma. Management of EBV-related lymphoproliferative disorder is sometimes problematic due to difficulties and controversies with laboratory surveillance, diagnosis, prevention and treatment. Allen *et al* led a group of infectious diseases experts in American and Canada to formulate consensus and recommendations in these aspects (78). Our group illustrates the case presentations and clinical courses of post-transplant

patients complicated with EBV-related post-transplant lymphoproliferative disorder (PTLD) in Chapter 10. Human herpesvirus 6 (HHV-6) was first isolated in peripheral blood of patients with AIDS and other lymphoproliferative disorders in 1986 (79). Primary HHV-6B is now established as the cause of exanthema subitum (roseola infantum) which is a common infectious diseases in infancy and has a life-long latency period (80). The clinical course is a self-limiting disease with benign clinical outcome. However, in immunocompromised host, particular hematopoietic stem cell transplant recipients, reactivation of latent HHV-6 infection can result in encephalitis in which the outcome is very grave (81). Our group performed an extensive review in this aspect and evaluated the role of antiviral prophylaxis in HHV-6 infection. The detail is discussed in Chapter 11.

Concerning opportunistic infection in immunocompromised patients, Pneumocystis pneumonia (PCP) is caused by *Pneumocystis jirovecii*, a yeast-like fungus. This pathogen is specific to humans. Pneumocystis pneumonia in humans was initially recognized in Central Europe after the World War II in premature and malnourished infants. In the 1960-1970s, additional cases with significant mortality were described in adults and children with hematological malignancies. Before the initiation of routine prophylaxis for PCP with trimethoprim/sulfamethoxazole (TMP/SMX), the attack rate was up to 43%. In 1977, Hughes *et al* published results of the first study to document successful prophylaxis with daily TMP/SMX in pediatric oncology patients (82). The success of daily and intermittent prophylactic dosing of TMP/SMX has subsequently been recognized by other authors (82, 83). Current recommendations for PCP prophylaxis in immunocompromised patients are based on either daily dosing or dosing three consecutive days per week (83).

4.3 Preventive strategies in pediatric oncology patients

There are a number of well-established guidelines for preventing opportunistic infections in oncology patients and patients received hematopoietic stem cell transplantation (82). Our group also formulated various recommendations and guidelines in term of isolation of children with respiratory and enteric infections (71, 83).

Vaccination is now a well-established method to protect children from a number of infectious diseases. The term vaccine derives from Edward Jenner in 1796. Edward Jenner was an English scientist who discovered the fact that vaccination with cowpox would prevent smallpox after his observation of milkmaids who had contact with cowpox did not get smallpox. His works was continued by Louis Pasteur and others in 19th century and Louis Pasteur discovered the first vaccine for rabies. There were major breakthroughs in vaccine technology in twentieth centuries with a number of successful vaccines developed over this period and caused a dramatic decrease in the incidences of various infectious diseases.

However in immunocompromised children, they may not have completed their primary vaccination series before starting treatment or the recovery of immune system, particularly the "immune memory", can be incomplete, as discussed in previous chapters, they are vulnerable to various infectious diseases despite months after completion of chemotherapy. Zignol *et al* studied serum antibody levels for poliovirus, tetanus, hepatitis B, rubella, mumps, and measles in 192 children who were in remission after completion of chemotherapy. Absence of protective serum antibody titer for hepatitis B, measles, mumps, rubella, tetanus, and poliovirus were detected in 46%, 25%, 26%, 24%, 14%, and 7% of patients, respectively. The loss of antibodies against rubella, mumps, and tetanus was associated significantly with young age ($p=0.001$, $p=0.02$, and $p=0.001$, respectively), and loss of antibodies against measles was significantly associated with young age and

the female gender (p=0.0003 and p=0.008, respectively). These data showed that young children, even though after completion of chemotherapy, were vulnerable to various vaccine-preventable infectious diseases (56).

Patel *et al* evaluated the immunity to vaccines at a median of 7 months after completion of chemotherapy in ALL children. They demonstrated protective antibody concentration for all patients to tetanus, 86% to *Haemophilus influenzae* type B, 71% to measles, 12% to *Neisseria meningitidis* group C, and 11% to all poliomyelitis serotypes. In these studies, in general they showed a need to re-immunize patients after completion of chemotherapy (84).

However, as a pediatrician who is responsible for taking care children with malignancies, I always have a question whether post-chemotherapy immunization is one of the effective strategies to protect these children from vaccine-preventable infectious diseases. At the time of writing up this thesis, there were very little data from controlled trials to study the effectiveness of vaccination in this group of vulnerable patients (85).

Basically, there are two reasons to postulate booster or revaccination being necessary in children completed chemotherapy. Firstly, there may be interruption of vaccination program due to onset of cancer and need to start chemotherapy urgently. Secondly, the protective antibodies from previous vaccination may be lost after the treatment with chemotherapy. The latter has been well-proven from published literature (56).

Based on the various published evidences, there is a fairly satisfactory recovery of immune system at 6 months after stopping chemotherapy. Our group designed and performed a series of original studies to evaluate the effectiveness of various vaccines in pediatric oncology patients and stem cell transplant recipients. Moreover, our group also explored different strategies so as to prevent infections in this vulnerable group.

4.4 Conclusion

Although recommendations in term of treatment of infections in this vulnerable group are well established, opportunistic infections continue to pose significant impact in pediatric oncology patients and stem cell transplant recipients. A series of studies that will describe in the following chpaters is to forumate different strategies to protect our patients from various infectious diseases.

Chapter 5 Objectives and Planning of the Original Studies

5.1 Introduction

The objectives of this series of original studies are to explore different strategies in protecting pediatric oncology patients from infections (both vaccine-preventable and non-vaccine preventable infectious diseases) which are the major treatment-related morbidities and mortalities in modern era.

The immune system of pediatric oncology patients and stem cell transplant recipients is severely depressed and the depression can persist after the completion of intensive chemotherapy, various immune studies have shown that a significant percentage of children will lose protective antibodies to various vaccine-preventable infectious diseases. Post-chemotherapy booster vaccination is one of the possible immunomodulatory strategies that can be further explored to reconstitute the defective "memory".

Currently, there are clear guidelines for revaccination in pediatric stem cell transplant recipients (86). However, uptil now, data from vaccination study in pediatric oncology is scarce (85). There are still large numbers of questions remain unanswered: (i) Who are the potential candidates? (ii) Is there any difference in vaccination strategy between children with hematological malignancies and children with solid tumors? (iii) When is the optimal timing for revaccination? (iv) What is the strategy for booster vaccination?

The potential strategies include complete revaccination of primary series or only vaccinate unprotected patients after performing serology screening. Practically, there are a few laboratories that are able to perform all assays in one goal. Moreover, the "protective" levels are derived from studies in healthy children and may not be totally applicable in this selected population. Therefore, published literature did not support

routine screening of antibodies to vaccine preventable antigens or measuring immune response before booster vaccinations (87).

The vaccination strategy in this aspect is still a matter of debate due to paucity of clinical data and specific recommendations are still lacking with respect to the use of protein-conjugated vaccines during the maintenance phase of therapy and whether or not there is need for revaccination for patients with malignancies who are in remission, during therapy and/or after completion of treatment and whether the vaccines are immunogenic in immunocompromised state. However, there is little debate in avoiding live-attenuated vaccines during intensive phases of therapy, for example, induction, re-induction and consolidation therapies.

Concerning those non-vaccine preventable infectious diseases, alternative strategies namely routine monitoring of viral load and timely use of antiviral agents as prophylaxis may have a role. Finally, in transplant recipients, with the introduction of various myeloablative conditioning regimens, total body irradiation and the use of new immunosuppressents in controlling acute and / or chronic graft-versus-host diseases, the timing of recovery of immunity to various viral antigens may be different from decades ago. Moreover, the role of transferring donors' immunity to recipients so as to fasten the recovery of hosts' immunity can be further explored.

With these lines of thinking in mind, various original studies were planned and performed.

5.2 Planning of original studies

In the first place, we performed a 18-month longitudinal study to evaluate the natural course of immune recovery to major vaccine-preventable diseases include diphtheria, pertussis, tetanus, hepatitis B, measles, mumps and rubella in pediatric oncology patients which included both hematological malignancies and solid tumor patients from 6 months after stopping chemotherapy to 18 months after stopping chemotherapy. This study was one of the longest cohort studies that ever reported in English literatures to address this question. It can tell us the duration of vulnerable period that these children may be infected with these vaccine-preventable pathogens if no revaccination is given especially in the era of "intensive" treatment of childhood malignancies and provides us the information about the status of immune recovery at 18 months after stopping chemotherapy. The study detail is illustrated in Chapter 6

In Chapter 7, we describe our first randomized controlled trial to investigate the effect of booster diphtheria-tetanus-pertussis (DTP) vaccine in children who have completed treatment for hematological malignancies for six months. This study can answer whether DTP booster vaccine is immunogenic in this group of children and whether the immune response can be sustained throughout the vulnerable period, i.e. at least 18 months after stopping chemotherapy (88).

Immunosuppressive effects of different chemotherapy regimens are different and children suffered from solid tumors in general do not suffer from immunosuppressive effects of primary diseases. Therefore, the degree of immune recovery may be different in solid tumors when compared with children with hematological malignancies. We evaluated the effect of DTP booster vaccine separately in children with solid tumors who have stopped treatment for six months. The study can give us recommendation on whether booster DTP booster vaccine is necessary in children with solid tumors. The detail is illustrated in Chapter 8 (88).

In the spring of 2009, the emergence of a novel influenza virus (H1N1) strain caused a global pandemic outbreak. Immunocompromised patient is a well-known high risk group of severe influenza infection. The clinical presentation and outcome of this novel infection remained unknown at the time of writing up this thesis. We performed a territory-wide evaluation of the clinical characteristics of pandemic H1N1 infection in the pediatric oncology and hematopoietic stem cell transplant recipients in Hong Kong. This study involved all the five hospitals that are responsible for taking care of pediatric oncology patients and stem cell transplant recipients in Hong Kong. We found that the early start of antiviral therapy and prolonged course of antiviral treatment in patients with recurrent or persistent symptoms may relate to the benign clinical course of this novel infection in our cohort. The study detail is illustrated in Chapter 9.

Children who have undergone hematopoietic stem cell transplantation belong to the highest risk group among immunocompromised children to suffer from life-threatening infections. With the improvement in various measures in preventing infections in this highly vulnerable group mentioned in the previous chapter, a number of non-vaccine preventable diseases still cause significant morbidity and mortality in these children.

Human herpesvirus 6 (HHV-6) was firstly isolated in peripheral blood of patients with AIDS and other lymphoproliferative disorders in 1986. Primary infection occurs in early childhood and has a life-long latency period. Reactivation of HHV-6 commonly occurs within the first thirty days post-transplant and may cause significant transplant-related morbidity and mortality.

Epstein Barr Virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication following solid organ transplantation and HSCT using bone marrow or peripheral blood stem cell sources, but rarely reported in umbilical cord blood transplant setting. The impact of Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6) infections in children who have undergone allogeneic hematopoietic stem cell transplantation were evaluated. The role of routine monitoring of viral load in plasma and the early use of antiviral agents as prophylaxis in preventing lethal complications are discussed in Chapter 10 and 11.

Although there are well-validated guidelines for revaccination strategies in children who have undergone hematopoietic stem cell transplantation. Non-vaccine preventable infectious diseases still have significant impacts in these children's morbidity and even mortality as illustrated in Chapters 10 and 11. Moreover, with the introduction of various myeloablative conditioning regimens, technique of total body irradiation and the use of new immunosuppressents in controlling acute and/or chronic graft-versus-host diseases, the timing of recovery of immunities to various viral antigens may be different from decades ago. Moreover, the role of transferring donors' immunities to recipients so as to fasten the recovery of hosts' immunities remains unknown. Our group studied the profile of immune reconstitution of various herpes viruses (HSV-1, CMV, EBV, VZV, HHV-6, HHV-7, HHV-8) by lymphoproliferative response (LPR) which is a reflection of recovery of cell-mediated immunity (CMI) in transplant setting and the potential role of adoptive transfer of cell-mediated immunity from donor to receipt in prevention of these non-vaccine preventable infectious disease in children who underwent hematopoietic stem cell transplantation. The result is illustrated in Chapter 12.

In the final part of the thesis, the results of my series of studies are summarized. The new knowledge that was generated from our series of original studies and actual measures that were and are going to apply in clinical settings are listed.

5.3 Conclusion

The results of this series of original studies can provide clinical data on (i) whether post-chemotherapy vaccination is necessary in pediatric oncology patients; (ii) whether post-chemotherapy vaccination is effective in regenerating protective antibodies to various vaccine-preventable infectious diseases; (iii) whether there is a difference in recommendation in children with hematological malignancies and solid tumors as there is fundamental difference in the degree of immunosuppression due to different primary diseases and chemotherapy regimens; (iv) the role of routine plasma viral load monitoring and the early use of antiviral prophylaxis in prevention of non-vaccine preventable infectious diseases; (v) the timing of completion of immune reconstitution to various non-vaccine preventable infectious agent in children with hematopoietic stem cell transplantation and whether adoptive transfer of cell-mediated immunity is an effective mode of prevention of these infectious diseases.

Chapter 6 Recovery of Humoral and Cellular Immunities to Vaccine-Preventable Infectious Diseases in Pediatric Oncology Patients

6.1 Introduction

With the improvement of various treatment protocols of pediatric malignancies, the overall and event-free survival rates in this vulnerable group improve significantly.(7, 89) However, both primary diseases and their treatments will inevitably cause immunosuppression. In this modern era, various studies have been performed to evaluate the immune recovery after intensive chemotherapy (31, 90). A number of them showed persistent immune deficit at the end of study period and left the period of complete or satisfactory recovery of immune system unanswered.

In view of the exact duration of immune suppression after chemotherapy remains unanswered, our group performed this longitudinal study to study the recovery of immune system in children with hematological malignancies and solid tumors up to 18 months after stopping chemotherapy which is one of the longest ever reported in English literatures. Our group also studied the recovery of antibodies to various vaccine antigens at that period and thus estimated the percentage and duration of subjects that were still vulnerable to these vaccine-preventable infections.

6.2 Subjects and Methods

Study subjects were diagnosed and received treatment in a tertiary referral center for pediatric cancers in Hong Kong. The Clinical Research Ethics Committee of our University approved this clinical trial. Patients and / or their parents gave informed written consent prior to study according to the principles of the Declaration of Helsinki.

Patients aged 1-18 years who had been completed treatment for pediatric hematological malignancies and solid tumors for 6 months were recruited consecutively for evaluation of recovery of humoral and cellular immunities against seven major vaccine-preventable infectious diseases (hepatitis B, diphtheria, tetanus, pertussis, measles, mumps and rubella). Hematological malignancies included children with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and other leukemias. They were all treated according to their disease-specific treatment protocols.

Exclusion criteria included (1) patients with past history of those listed vaccine-preventable diseases; (2) evidence of immunodeficiency before diagnosis of malignancies; (3) patients developed relapse of primary disease or secondary malignancies during study period; (4) patients were still receiving systemic steroid for their primary diseases or other conditions with dosage greater or equivalent to prednisolone 2 mg/kg/day for more than 14 days; (5) hematopoietic stem cell transplant recipients.

Immunization status of patients was obtained from the history and vaccination records. Past medical history was reviewed to exclude those with history of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella infections before evaluation. They were monitored for development of these vaccine-preventable infectious diseases and other severe infections during the study period. Criteria of severe infections were defined as (i) culture-proven bacteremia; (ii) culture or histological confirmed or probable invasive fungal infections; (iii) severe infective episodes which required critical care and / or respiratory support.

6.2.1 Serological studies

Serum concentrations of antibodies to diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B were monitored serially started from 6 months (visit 1), 8 months (visit 2), 12 months (visit 3) and 18 months (visit 4) after completion of all chemotherapies including maintenance chemotherapy.

Serum samples from the above four visits were stored at -30°C until analysis in one batch. Commercially available kits were used for the determination of antibody titers. Serum levels of specific antibodies to diphtheria, tetanus and pertussis were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany). The protective antibody levels were defined as 4 IU/mL for diphtheria, 2 IU/mL for tetanus and 24 IU/mL for pertussis respectively.(88) Titer to Hepatitis B surface antibody (anti-HBs) was measured by using an enzyme-linked immunosorbent assay. (BioSupply, United Kingdom) Antibody level ≥ 10 mIU/ml was considered as protective. Measles and mumps IgG antibodies were tested by a modification of the NOVUM measles/mumps virus IgG ELISA test kits (Novum Diagnostica, Germany) and rubella by Imx Rubella IgG2.0 antibody assay (Abbott Laboratories, Chicago, IL, USA). Antibody levels exceeding a cutoff value of 10 IU/ml were interpreted as seropositive (91).

6.2.2 Assessment of lymphocyte subsets and measurement of serum immunoglobulin levels

Lymphocyte subset and serum immunoglobulin level were checked at six months (visit 1) and eighteen months (visit 4) after stopping chemotherapy. Subsets of circulating lymphocytes were quantified by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA) using fluorescence-labeled monoclonal antibodies CD3+, CD4+ (helper T lymphocyte) and CD8+ (cytotoxic T lymphocyte), CD19+ (mature B lymphocyte) and CD16/56+ (natural killer cells). Serum IgG, IgA and IgM concentrations were measured by nephelometry (Binding Site, Birmingham, UK).

6.2.3 Statistical Analysis

Data were expressed as median and standard deviation unless otherwise stated. Demographic data between different subgroups were analyzed using Student *t* test for independent parametric variables and χ^2 for categorical variables between different disease or patient groups. Wilcoxon signed ranks test was used to analyze between values at visit 1 (6 months after stopping chemotherapy) and visit 4 (18 months after stopping chemotherapy). Statistical significance was defined as p-value <0.05. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA).

6.3 Results

Twenty-eight patients with hematological and solid tumors were recruited (18 males and 10 females); median age was 7.0 ± 3.8 years old (range 2.6 - 16.2 years old). All patients had completed primary series of childhood immunization schedules before malignancy developed. Primary diagnosis included fourteen cases of hematological malignancies and fourteen cases of solid tumors. Hematological malignancies included acute lymphoblastic leukemia (ALL), n=12; acute myeloid leukemia (AML), n=2. Solid tumors group included central nervous system (CNS) tumor, n=3; Hodgkin lymphoma, n=3; osteosarcoma, n=2; and 1 case of germ cell tumor, rhabdomyosarcoma, neuroblastoma, nasopharyngeal carcinoma, clear cell sarcoma, and Ewing's sarcoma respectively. The median duration of chemotherapy was 22.4 ± 7.7 months in hematological groups which included cumulative dose of prednisolone 1680 mg/m^2 and dexamethasone 210 mg – 740 mg/m^2 during intensive phase of treatment of ALL and 10.0 ± 4.0 months in solid tumors groups respectively. No radiation was given to the mediastinal or thymic regions as part of their primary therapies.

6.3.1 Recovery of humoral and cellular immunities

At six months after stopping chemotherapy (visit 1), the median absolute neutrophils, CD3+ cells (1.43 ± 0.52 x 10^9/L), CD4/CD8 ratio, CD16/56+ cells and immunoglobulin levels were within normal limit, but CD4+ cells (0.39 x 10^9/L), CD8+ cells (0.39 x 10^9/L) and CD19+ cells (0.37 x 10^9/L) were low when compared with age-specific normal lymphocyte subset values.(92) Throughout the eighteen-month study period, there was an overall statistical significant increase in various lymphocyte subsets and immunoglobulin levels. The median CD3+ lymphocyte raised from 1.43 x 10^9/L ± 0.52 at visit 1 to 1.76 x 10^9/L ± 0.68 (p=0.01) at visit 4; CD4+ raised from 0.39 x 10^9/L ± 0.17 to 0.54 x 10^9/L ± 0.28 (p=0.01); CD8+ raised from 0.39 x 10^9/L ± 0.20 to 0.47x10^9/L ± 0.20 (p=0.04); CD19+ increased from 0.37 x 10^9/L ± 0.17 to 0.45 x 10^9/L ± 0.20 (p=0.03). CD4/CD8 ratio was > 1.0 throughout the study period (visit 1: 1.09 ± 0.44; visit 4: 1.14 ± 0.33). IgG increased from 9.52 ± 2.43 g/L to 10.28 ± 2.73 g/L (p=0.02); IgA raised from 1.27 ± 0.59 g/L to 1.57 ± 0.6 g/L (p=0.01) and IgM changes from 0.92 ± 0.41 g/L to 1.22 ± 0.66 g/L (p=0.01). Overall, all immune parameters were nearly normal at the end of study period (18 months after stopping all chemotherapy) except median CD4+ and CD8+ cell counts still lower than age-specific normal values.(92)

No subjects developed infections due to these vaccine-preventable pathogens during the study period. Neither of them fulfilled the criteria of severe infection. The detail is shown in Table 2.

6.3.2 Antibodies to vaccine preventable infectious diseases

Concerning the recovery of protective antibody levels to seven major vaccine-preventable infectious diseases, only 82% of subjects had protective anti-diphtheria antibody levels, the percentages to hepatitis B, measles, mumps and rubella were 29.6%, 70.4%, 55.6% and 70.4% respectively at six months (visit 1) after stopping chemotherapy. The seropositive rate of pertussis and tetanus were 96.5% at that time point.

Throughout the study period, there was no significant increase in various antibody levels. Up till eighteen months (visit 4) after stopping chemotherapy, there were still 11%, 15%, 60%, 30%, 49% and 30% of subjects remained susceptible to diphtheria, tetanus, hepatitis B, measles, mumps and rubella infections respectively. The detail is shown in Table 3.

Table 2. Recovery of humoral and cellular immunities in pediatric oncology patients (n=28)

	6 months after stopping chemotherapy (visit 1)	18 months after stopping chemotherapy (visit 4)	p^*
Absolute neutrophil count (10^9/L)	4.13 ± 1.81	4.47 ± 1.99	0.38
CD3+ (10^9/L) 2.39 (1.40 - 3.70)^	1.43 ± 0.52^^	1.76 ± 0.68	0.01**
CD3+/CD4+ (10^9/L) 1.38 (0.70 - 2.20)	0.39 ± 0.17	0.54 ± 0.28	0.01**
CD3+/CD8+ (10^9/L) 0.84 (0.49 - 1.30)	0.39 ± 0.20	0.47 ± 0.20	0.04**
CD4+ : CD8+ ratio	1.09 ± 0.44	1.14 ± 0.33	0.43
CD19+ (10^9/L) 0.75 (0.39 - 1.40)^	0.37 ± 0.17	0.45 ± 0.20	0.03**
CD16/56+ (10^9/L) 0.30 (0.13 - 0.72)	0.15 ± 0.08	0.14 ± 0.06	0.43
IgG (g/L) (5.49 – 15.84)	9.52 ± 2.43	10.28 ± 2.73	0.02**
IgA (g/L) (0.61 – 3.48)	1.27 ± 0.59	1.57 ± 0.60	0.01**
IgM (g/L) (0.23 - 2.59)	0.92 ± 0.41	1.22 ± 0.66	0.01**

*Analyzed by Wilcoxon signed ranks test between values at visit 1 (6 months after stopping chemotherapy) and visit 4 (18 months after stopping all chemotherapy); ** Statistical significance: $p<0.05$; ^ normal median value (10 percentile -90 percentile); ^^ expressed as median ± standard deviation

Table 3. Seropositive rate of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella in pediatric oncology patients

Vaccine-preventable infectious diseases	Visit 1	Visit 2	Visit 3	Visit 4
Diphtheria (Total)	82.1%	78.6%	75.0%	89.3%
Diphtheria (Hematology)	78.6%	71.4%	71.4%	78.6%
Diphtheria (Solid Tumor)	85.7%	85.7%	78.6%	100%
Tetanus (Total)	96.4%	96.4%	92.9%	85.2%
Tetanus (Hematology)	92.3%	96.4%	92.9%	78.6%
Tetanus (Solid Tumor)	100%	96.4%	92.9%	92.3%
Pertussis (Total)	96.5%	96.2%	100%	100%
Pertussis (Hematology)	92.9%	100%	100%	100%
Pertussis (Solid Tumor)	100%	92.3%	100%	100%
Hepatitis B (Total)	29.6%	25.9%	32.1%	40.7%
Hepatitis B (Hematology)	15.4%	14.3%	35.7%	42.9%
Hepatitis B (Solid Tumor)	42.9%	38.5%	28.6%	38.5%
Measles (Total)	70.4%	-	71.4%	70.4%
Measles (Hematology)	76.9%	-	71.4%	71.4%
Measles (Solid Tumor)	54.3%	-	71.4%	69.2%
Mumps (Total)	55.6 %	-	60.7%	51.9%
Mumps (Hematology)	53.9%	-	57.1%	42.9%
Mumps (Solid Tumor)	57.1%	-	64.3%	61.5%
Rubella (Total)	70.4%	-	64.3%	70.4%
Rubella (Hematology)	69.2%	-	64.3%	69.2%
Rubella (Solid Tumor)	71.4%	-	64.3%	71.4%

6.4 Discussion

Various studies have shown that immune impairment can persist up to twelve months after stopping intensive chemotherapy. The degree of immune reconstitution depends on the treatment intensity. The most heavily treated patients, namely survivors of high-risk ALL and AML are anticipated to have persistent abnormalities in cellular and humoral immunities for at least six months after stopping treatment. Despite normalization of T- and B-cell functions *in vitro*, they respond poorly to immunization with T-cell dependent antigens indicating residual *in vivo* immune deficit.(93, 94) Our study prospectively evaluate the natural recovery of humoral and cellular immunities and the recovery of antibodies to various vaccine antigens in pediatric oncology patients longitudinally from 6 months to 18 months after stopping chemotherapy which is one of the longest periods of evaluation in reported English literatures.

In our cohort, although the T-helper (CD4+), cytotoxic T (CD8+) cells and B lymphocytes (CD19+) remained at low level at six months after stopping chemotherapy, the median absolute neutrophil, lymphocyte counts and CD4 / CD8 ratio remained at a relative normal range. This might be the reason for the absence of severe infections, namely bacteremia, invasive fungal infections or septic episodes which required critical care and / or respiratory support, despite the extent of the depression of cellular and humoral immunities.

We demonstrate there was satisfactory recovery of humoral and cellular immunity throughout the study period and the immune parameters of both groups were nearly normal at the end of study. However, the CD4+ and CD8+ cells remained low at the end of study period indicating residual immune deficit that could be persisted up to 18 months after stopping chemotherapy.

T-lymphocytes are composed of a heterogenous group of short- and long-lived cells. Under normal circumstances, the "long-lived" cells typically contain the "naïve" subset which are quiescent and remain in a noncycling state for months or even years while awaiting antigen exposure. "Short-lived" cells, generally contain the effectors and memory subsets, undergo variable levels of cell cycling in response to antigen and result in ongoing modulation of their contribution to overall T-cell repertoire. When the T-cells are acutely depleted after chemotherapy, restoration of heterogenous populations of T-cells and re-establishment of T-cell immunocompetence is a slow, continuous and frequently incomplete process (95, 96). Therefore, despite the persistence of memory T-cells, loss of immune "memory" is still noted (34, 97). In our study, loss of immune "memory" is demonstrated by persistent loss of previous antibodies to various vaccine antigens up to eighteen months after stopping chemotherapy although other parameters indicating a good recovery to humoral and cellular immunities *in vivo*.

Although vaccine-preventable infectious diseases become uncommon or almost being erradicated in developed worlds with the advancement in vaccine technology, they are always one of the major threats in immunocompromised patients. Kaplan *et al* showed that severe complications occurred in about 80% of immunocompromised patients who acquired measles infection. The case fatality rate of measles infection was about 70% for oncology patients and about 40% for HIV-infected patients. More importantly, classical maculopapular rash could be absent in 30% of cases which posed significant implications in patient management and infection control policy in oncology centers (98).

Our study measured serially the recovery of antibodies and estimated the duration of vulnerable periods to seven major vaccine-preventable infectious diseases that children would be exposed to. Our results clearly showed that there was no significant increase in percentage of seropositivity to various vaccine antigens although immune studies demonstrated a good recovery of humoral and cellular immunities *in vivo*. A significant number of subjects (range 15% to 60%) still had excessive risk of acquiring potentially life-threatening vaccine-preventable infectious diseases up to eighteen months after stopping chemotherapy. Our data is compatible with other published series (56, 99).

In 2009, there were 26, 163 and 45 cases of measles, mumps and rubella infections were diagnosed respectively and reported to Centre for Health Protection (100). Therefore, this will certainly become a significant public health problem if no further intervention is implemented as the survival rate of pediatric oncology patients improves significantly with the improvement in various cancer treatment protocols as stated in previous chapters.

Herd effect is defined as the reduction of infection or disease in the unimmunized segment as a result of immunizing a proportion of the population (101). The estimated herd immunity thresholds for vaccine preventable infectious diseases are as follow: diphtheria: 85%, pertussis: 92-94%, poliomyelitis: 80-86%, measles: 83-94%, mumps: 75-86%, rubella: 80-85% (96, 102). Therefore, our cohort was below the herd immunity thresholds for diphtheria, measles, mumps and rubella during the whole 18-month study period. This can evolve to a significant public health problem if no interventions are implemented as we anticipate an increase in number of long-term survivors of pediatric oncology patients in the future.

Booster vaccinations started after stopping chemotherapy may have a role to decrease the proportion of unprotected subjects in the community. Patel *et al* showed that children who had completed treatment of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia developed satisfactory responses to diphtheria, tetanus, acellular pertussis, *Haemophilus influenza* type b, meningococcus C, poliomyelitis, measles, mumps and rubella vaccines which were given at median six months after stopping chemotherapy. The response sustained up to 12 months after vaccinations (84). Ek *et al* also showed that children who were intensively treated with chemotherapy, namely children with high risk ALL, showed insufficiency immune response to revaccination and the suboptimal response was correlated with the numbers of memory B cells and antibody secreting cells (55).

Therefore, although according to our study results and other published data, six months after stopping chemotherapy, including maintenance therapy, seems to be a reasonable time to restart booster vaccinations (88). Currently, our unit adopts the following policies in our pediatric oncology patients: (i) revaccinate with three doses of diphtheria-tetanus-acellular pertussis vaccines started at six months after stopping all chemotherapy, each dose is two-month apart. We do not routinely perform antibody testing of diphtheria, tetanus and pertussis because this is not part of the routine service of our virology laboratory; (ii) evaluate the serostatus of measles, mumps, rubella and hepatitis B at 6 months after stopping all chemotherapy and revaccinate with 1 dose of measles-mump-rubella and 3 doses of hepatitis B vaccines for those seronegative patients. (iii) patients can return to school three to six months after stopping chemotherapy.

We should admit that there is still not enough data to suggest the detailed strategy of revaccination, namely (i) what is the optimal number of doses of revaccination? (ii) Should we adopt a universal approach or only revaccinate those unprotected subjects after immune check-up? (iii) Whether we need to have a different revaccination schedules for standard and high risk patients?

One limitation of our study is lacking of complete set of pretreatment serology data to strongly support the low seropositivity rate to various vaccine antigens was solely due to immunosuppressive effect of chemotherapy. We did have pre-treatment data for hepatitis B and measles to support our argument. For hepatitis B, overall 68% were seropositive before starting chemotherapy (53.9% in hematology malignancies group and 84.4% in solid tumors group respectively) and after completion of treatment, the overall seropositivity dropped to about 30% and stayed at 40% up to 18 months after stopping chemotherapeutic agents. For measles, 85.7% of subjects were protective but the level

was 70% after treatment completed. Despite of this, our study results are comparable with other published literatures which evaluate antibody levels from the time of diagnosis of malignancies (99). Immunosuppressive effect of chemotherapeutic agents plays an important role in this aspect. Moreover, due to the limitation of the design of the study and relative small sample size in different age group, it is difficult to evaluate the age effect on immune reconstituation. A prospective multi-centers study which involves a larger study population can answer this question. Finally, in order to have a more comprehensive evaluation of the recovery of the immune system, functional assessment of cell-mediated immunity should be performed. These can be further elaborated and answered in future studies.

6.5 Conclusion

Our study shows that although there was near complete immune recovery in our subjects, a significant proportion of subjects remained susceptible to various vaccine-preventable infectious diseases up to 18 months after stopping chemotherapy which can evolve to significant public health problem if no interventions are implemented as we anticipate an increase in number of long-term survivors of pediatric oncology patients in the future.

Chapter 7 Humoral Immune Response After Post-Chemotherapy Booster Diphtheria-Tetanus-Pertussis Vaccine in Pediatric Patients with Hematological Malignancies.

7.1 Introduction

In the last chapter, we have discussed that although there was near complete immune recovery in pediatric oncology patients, a significant proportion of subjects remained susceptible to various vaccine-preventable infectious diseases up to eighteen months after stopping chemotherapy.

As vaccination plays a key role in preventing infectious diseases, effective vaccines have curtailed dramatically or almost eliminated diphtheria, measles, mumps, poliomyelitis, rubella and tetanus in developed countries. Consistent high level of vaccine coverage accompanies with intensive surveillance and effective public health disease control measures provide basis for effective prevention of various vaccine-preventable infectious diseases and possibly eradication of these infectious diseases (103). From the year 2004 – 2008, the vaccine coverage rates of diphtheria, tetanus, measles, mumps, rubella and hepatitis B vaccines in Hong Kong were persistently higher than 98% (104). The results were encouraging.

In children with hematological malignancy, chemotherapy is the mainstay of treatment modality. Vaccination of these children presents challenges due to efficacy and safety concerns (85). There are well-established and validated recommendations for revaccination in hematopoietic stem cell transplant recipients (86). However, to date, there are very few guidelines in recommending routine booster vaccination in children who have received intensive chemotherapies. Most of them are established based on data from limited published studies and expert opinions (105-107).

With the advance in treatment protocols and increase in dose intensity of chemotherapies which result in marked improvement in overall and event-free survivals of pediatric oncology patients in the past decades, these strategies also induce marked impairment in humoral and cellular immunities which can persist up to 18 months after stopping chemotherapy. This has been discussed in details in the last chapter (108). Various cohort studies which include ours also demonstrate persistent and significant loss of protective serum antibodies against poliomyelitis, tetanus, hepatitis B, measles, mumps and rubella in pediatric oncology patients who had received intensive chemotherapy up to eighteen months (31, 56, 108).

Restoration of normal number of B-lymphocytes and achievement of normal serum immunoglobulin levels by six months after stopping chemotherapy in most children that have completed treatment of cancers provide a rational basis of starting revaccination program in these patients since a prolonged period of susceptibility to various vaccine-preventable infectious diseases is not of the best interest of our patients (50, 51, 53).

In this chapter, we describe our first randomized control trial to evaluate the antibody response of diphtheria, pertussis and tetanus after completion of treatment in children with hematological malignancies after three doses of booster diphtheria, tetanus and pertussis (DTP) vaccines so as to answer some of the unanswered questions.

7.2 Subjects and Methods

7.2.1 Patients and Samples

From June 1, 2003 to October 31, 2004, twenty nine consecutive patients aged 1-18 years who had been treated successfully for pediatric hematological malignancies were recruited for evaluation of humoral immunity against seven major vaccine-preventable diseases (hepatitis B, diphtheria, tetanus, pertussis, measles, mumps and rubella) and their responses to three doses of booster DTP vaccine. They were all diagnosed and received treatment in our oncology unit which was a tertiary pediatric oncology referral centre in Hong Kong. All patients were in clinical, morphological, cytogenetic and molecular remissions. The Clinical Research Ethics Committee of our University approved this clinical trial, and patients and / or their parents gave informed written consent prior to study.

Exclusion criteria included (1) patients with past history of those listed vaccine-preventable diseases; (2) evidence of immunodeficiency before diagnosis of malignancy; (3) history of allergic or severe reactions to DTP vaccine or its components and (4) relapse of primary disease or development of secondary malignancies during the study period. Eight patients did not consent for the study, two patients were excluded because of history of severe febrile reaction to previous DTP vaccines and the other two were due to relapse of primary disease during study period.

The subjects included patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Patients in each group were randomized into vaccine or control group. Immunization status of patients was obtained from the history and vaccination records. Past medical history was reviewed to exclude those with history of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella infections before enrollment.

7.2.2 Randomization

Eligible patients were randomly assigned at 1:1 ratio into treatment and control groups in this randomized clinical trial. An independent research staff was responsible for generation of the randomization sequence by a computer program.

7.2.3 Surveillance of post-vaccination humoral and cellular immunities

Serum concentrations of antibodies to diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B were monitored serially started from six months after completion of intensive chemotherapy (visit 1). In the vaccination group, three doses of booster DTP vaccine were given at eight months (visit 2), ten months (visit 3) and twelve months (visit 4) after completion of chemotherapy. Each 0.5 ml dose of DTP vaccine (Aventis Pasteur, Lyon, France) contained ≥ 30 IU purified diphtheria toxoid, ≥ 60 IU purified tetanus toxoid and ≥ 4 IU heat-inactivated *Bordetella perutssis*. These vaccines were stored at 4°C until use. No intervention was performed for the control group. Blood for diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella antibodies were checked at visit 1, visit 2, visit 4 and visit 5. Table 4 illustrates the study timeline.

Table 4. Study flowchart for children with hematological malignancies receiving booster DTP vaccines

	Time Following Completion of Chemotherapy				
	6 months (Visit 1)	8 months (Visit 2)	10 months (Visit 3)	12 months (Visit 4)	18 months (Visit 5)
Immunological Surveillance					
Complete blood count with differentials	+	+	-	+	+
Lymphocyte subsets by flow cytometry	+	-	-	-	+
Serum IgG, IgM and IgA levels	+	-	-	+	+
Antibodies to diphtheria, tetanus and pertussis	+	+	-	+	+
Antibody to hepatitis B virus surface antigen (Anti-HBs)	+	+	-	+	+
Antibodies to mumps, measles, rubella	+	-	-	+	+
Booster vaccination					
DTP vaccine		+	+	+	

7.2.4 Serological studies

Serum samples obtained from the above four visits were stored at -30°C until analysis in one batch. Commercially available kits were used for the determination of antibody titers. Serum levels of specific antibodies to diphtheria, tetanus and pertussis were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany). The protective antibody levels were defined as 4 IU/mL for diphtheria, 2 IU/mL for tetanus and 24 IU/mL for pertussis respectively.(88) Titer to Hepatitis B surface antibody (anti-HBs) was measured by using an enzyme-linked immunosorbent assay. (BioSupply, United Kingdom) Antibody level ≥ 10 mIU/ml was considered as protective. Measles and mumps IgG antibodies were tested by a modification of the NOVUM measles/mumps virus IgG ELISA test kits (Novum Diagnostica, Germany) and rubella by Imx Rubella IgG2.0 antibody assay (Abbott Laboratories, Chicago, IL, USA). Antibody levels exceeding a cutoff value of 10 IU/ml were interpreted as seropositive (91).

7.2.5 Assessment of cellular and humoral immunities

Subsets of circulating lymphocytes were quantified by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA) using fluorescence-labeled monoclonal antibodies CD3+ (total T-lymphocyte), CD4+ (helper T-lymphocyte) and CD8+ (suppressor T-lymphocytes), CD19+(mature B-lymphocytes) and CD16+/56+ (natural killer cells). Serum IgG, IgA and IgM concentrations were measured by nephelometry (Binding Site, Birmingham, UK).

7.2.6 Statistical Analysis

The primary outcomes were the differences in serum concentrations of specific antibodies to diphtheria, tetanus and pertussis between vaccine and control groups at the end of the twelve months study period. Secondary outcomes included the differences in serum concentrations of total IgG and anti-HBs, measles, mumps and rubella antibodies, as well as circulating lymphocyte sub-populations, between the two groups, and longitudinal changes in serum diphtheria, tetanus and pertussis antibody levels in the patients and controls at baseline (visit 1) and 12 months (visit 5) following vaccination.

Data were expressed as mean and standard deviation unless otherwise stated. Serum concentrations of specific antibodies were $\log_{10}$-transformed to achieve normal distribution of data before analysis. Demographic data between different subgroups were analyzed using Student *t* test for independent parametric variables and χ^2 for categorical variables between different disease or patient groups. Statistical analysis was performed only for subjects who had received at least two doses of DTP vaccines. Patients who relapsed during follow-up were excluded from analysis. Following DTP vaccination, the longitudinal changes in serum antibody levels between study visits were analyzed using paired *t* test. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was considered to be statistically significant.

7.3 Results

7.3.1 Study populations

During the study period, twenty nine patients with acute leukemia (24 ALL; 5 AML) were recruited. Fourteen were randomized to the vaccination group and 15 to the control group. All patients were followed up until the end of study. Forty-two booster vaccines were given. No significant vaccine-related adverse reactions were noted. The baseline characteristics of subjects in vaccination and control groups were comparable (Table 5).

Table 5. Baseline characteristics of hematological malignancies patients

Parameters	Vaccine group (n=15)	Control group (n=14)	p-value
Mean age	8.39 ± 3.67	8.41 ± 3.82	0.99
Male, n (%)	9 (60.0%)	10 (71%)	0.76
ALL	12	12	
AML	3	2	
Mean duration of chemotherapy, months	20.5 ± 8.79	22.4 ± 7.74	0.53
Total lymphocyte count, 10^9/L	2.35 ± 0.84	2.64 ± 0.98	0.39
Total T-lymphocyte counts (CD3+), 10^9/L	1.35 ± 0.51	1.54 ± 0.59	0.37
Mature B-lymphocyte (CD19+), 10^9/L	0.72 ± 0.33	0.42 ± 0.21	0.01*
Natural killer cells (CD16+ / CD56+), 10^9/L	0.13 ± 0.05	0.18 + 0.09	0.07
Helper T-lymphocyte (CD3+ / CD4+), 10^9/L	0.37 ± 0.17	0.39 ± 0.17	0.78
Suppressor T-lymphocyte (CD3+ / CD8+), 10^9/L	0.36±0.16	0.44±0.23	0.25
CD4 : CD8 ratio	1.12 ± 0.95	0.95 ± 0.31	0.22
Baseline IgG, g/L	8.59 ± 2.05	9.40 ± 1.62	0.25
Baseline IgA, g/L	1.23 ± 0.54	1.21 ± 0.63	0.93
Baseline IgM, g/L	0.88 ± 0.38	0.88 ± 0.45	0.77

Results expressed as mean ± standard deviation unless otherwise stated.

* Statistical significance ($p<0.05$)

7.3.2 Baseline antibody titers

At six months after stopping chemotherapy (baseline), 29.5% patients had diphtheria antibody titer below protective level, 10.5% and 6.9% of patients were seronegative for tetanus and pertussis respectively. For measles, mumps and rubella, 34.9%, 46.4% and 38.8% of patients did not have protective levels of these specific antibodies respectively after completion of chemotherapy. Concerning hepatitis B, our unpublished data showed that overall 68% were seropositive before starting chemotherapy, and the overall seropositivity dropped to about 30% and stayed at 40% up to 18 months after stopping chemotherapeutic agents. In hematology group, 53.9 % of patients were seronegative against hepatitis B surface antigen before starting chemotherapy and the percentage raised up to about 85% after completion of treatment (Table 3).

Table 6. Changes in seropositivity rates for vaccine-preventable diseases at visit 1 (six months after stopping of chemotherapy) and at visit 5 (eighteen months after stopping of chemotherapy) for children with hematological malignancies

	Vaccine group			Control group		
Disease	Visit 1	Visit 5	p*	Visit 1	Visit 5	p*
Diphtheria	62.5%	100%	0.03**	78.6%	78.6%	0.96
Tetanus	86.7%	100%	0.04**	92.3%	78.6%	0.23
Pertussis	93.3%	100%	0.74	92.9%	100.0%	0.89
Measles	53.3%	33.3%	0.02**	76.9%	71.4%	0.98
Mumps	53.3%	53.3%	1.00	53.9%	42.9%	0.73
Rubella	53.3%	60.0%	0.92	69.2%	69.2%	1.00
Hepatitis B	20.0%	20.0%	0.89	15.4%	42.9%	0.04**

* Analyzed by McNemar test for paired binomial variables.

** Statistical significant ($p<0.05$)

7.3.3 Humoral immunity to booster DTP vaccinations

After three doses of booster DTP vaccine, 100% of patients demonstrated seropositivity against diphtheria, tetanus and pertussis. The responses were sustained till twelve months after first dose of DTP vaccine, i.e. eighteen months after stopping chemotherapy (Table 6). The geometric mean of antibody titers against diphtheria, tetanus and pertussis were significantly higher than baseline and the response were sustained till twelve months after vaccinations (Table 7). Booster DTP vaccinations were efficacious in increasing the respective specific antibodies. During the twelve months follow up, the seropositivity rates were similar among unvaccinated patients. The seropositive rate of mumps, measles, rubella and hepatitis B in both groups remained unchanged throughout the study period. A significant proportion of subjects remained susceptible to these vaccine-preventable infectious diseases throughout the study period.

Table 7. Serial change of antibody titers (geometric mean) of children with hematological malignancies

Visit	Visit 1			Visit 2			Visit 5		
	Vaccine Group	Control group	p*	Vaccine group	Control group	p*	Vaccine group	Control group	p*
Diphtheria (> 4.0 U/ml)	9.8	9.5	0.96	286.1	8.2	<0.01	148.8	8.0	<0.01
Tetanus (>2.0 IU/ml)	15.4	23.9	0.42	448.5	19.9	<0.01	296.6	13.8	<0.01
Pertussis (>24 U/ml)	482.8	717.5	0.09	954.3	693.5	<0.01	10541.9	1292.5	<0.01

Results expressed as $\log_{10}$-transformed concentrations of specific antibodies.

*Analyzed by paired *t* test for comparisons of antibody levels post-vaccination (Visits 2 and 5) with that at baseline (Visit 1).

7.4 Discussion

The safe and effective use of vaccines has always been a major challenge and concern in immunocompromised patients. The key concerns are (i) the safety and (ii) the ability of patients to mount and sustain protective immune response to various vaccine antigens. There are well-established and validated recommendations for revaccination of patients who have undergone hematopoietic stem cell transplantation but such information is still missing for post-chemotherapy patients at the time of writing up this thesis (82, 86). Although a number of cohort studies were published to address the change of antibody titers and response to booster vaccine after chemotherapy, there are no validated recommendations for booster vaccination program for post-chemotherapy patients with hematological malignancies.

Thirty years ago, de Vaan GA *et al* conducted a study to investigate the serial changes in antibody titers to diphtheria, pertussis, tetanus and poliomyelitis (types I, II and III) after stopping chemotherapy in forty-nine children with acute lymphoblastic leukemia (ALL) and their response to revaccination with DT-Polio vaccine one year after cessation of anti-cancer treatment.(109) The antibody titers were lower than in healthy controls but still remained at protective levels in most patients. No spontaneous rise in antibody titer was shown before revaccination in the first year post-treatment, but a rise in antibody titers was demonstrated which was similar to healthy controls after revaccination.

With the increase in dose intensity of treatment of ALL and other hematological malignancies, the disease-free survival has improved significantly (7). In the 1990s, the five-year event-free survival rates for childhood ALL generally ranged from 70 to 83% in developed countries with an overall cure rate of approximately 80%. Emerging results suggest that a cure rate of nearly or more than 90 percent will be attained in the near future, particularly in standard risk ALL patients (7). However, immune reconstitution after treatment may be severely prolonged. In our study, chemotherapy induced a loss of protective serum antibody titers for diphtheria, tetanus, pertussis, measles, mumps, rubella and hepatitis B at six months after stopping chemotherapy respectively. Hepatitis B, measles, mumps and rubella antibody titers were mostly affected by the immunosuppressive effect of cytotoxic therapy when compared with diphtheria, tetanus and pertussis. These findings were consistent with other published data (93, 110-112).

The administration of booster DTP vaccines started at 6 months after stopping chemotherapy enabled 100% of patients in treatment group to recover protective antibody at a persistently high titers. The antibody titers were also sustained above the protective level throughout the one-year follow up period. This finding suggested that chemotherapy did not entirely abolish the specific humoral immune memory in patients with undetectable serum antibody titers after chemotherapy. The impaired capacity to mount an immune response was temporary and it tended to return to near normal level at six months after the end of chemotherapy. In the control group, although the process of immune reconstruction continued, there was no significant increase in percentage of patients that became seropositive and there was no significant change in the absolute antibodies titers throughout one year, indicating the recovery of immune "memory" as stated in previous chapter was not complete and

they were still susceptible to these vaccine-preventable diseases. There was a role for post-chemotherapy booster vaccination. A total of forty-two booster vaccinations were given. There were no significant or serious adverse events noted during the entire study period.

Since routine antibody testing for diphtheria, tetanus and pertussis is usually not part of the routine service of most of the virology laboratories. This is reasonable and logical to propose children with age less than two years old who have interrupted the primary vaccine schedule at the time of starting chemotherapy should restart the primary vaccine series six months after stopping chemotherapy and revaccinate according to vaccination schedule (113). For children who have completed the primary series before the diagnosis of malignancies, three doses of booster DTP vaccines can produce significant and sustained antibody responses in recipients. Antibody titers to hepatitis B, measles, mumps and rubella antibodies can be retested at 6 months after stopping chemotherapy and booster revaccination should be given to those seronegative patients.

One limitation of our study is lacking of actual functional assessment of cellular immunity in our study and control populations which is an important aspect of comprehensive assessment of recovery of cellular immunity. This can be addressed in future studies.

7.5 Conclusion

In conclusion, our study demonstrate that intensive chemotherapy led to loss of protective serum antibody titers for different types of vaccine-preventable infectious diseases which could be persistent till eighteen months after stopping chemotherapy. Booster vaccinations started at six-month after stopping chemotherapy in children with hematological malignancies that could be safely administered, and effectively restored a sustained effect in humoral immunity against various types of vaccine-preventable infectious diseases.

Chapter 8 Humoral Immune Response After Post-Chemotherapy Booster Diphtheria-Tetanus-Pertussis Vaccine in Pediatric Patients with Solid Tumors.

8.1 Introduction

As there are fundamental differences in primary diseases and treatment protocols between hematological and solid tumor malignancies, children with solid tumors may behave differently in immune recovery and subsequent immune response to booster vaccination. Therefore, in this chapter, we study the response of children with solid tumors who have completed their treatment protocols for six months to three doses of DTP booster vaccine.

In children with solid tumors, apart from surgery and/or radiotherapy for local disease control, systemic chemotherapy is the other mainstay of treatment modality aiming at clearance of minimal residual diseases or distant micrometastasis. As with patients with hematological malignancies, vaccination of these children presents challenges due to efficacy and safety concerns (85). With the limitation of data in this aspect, we conducted this study to evaluate the antibody levels of diphtheria, pertussis, tetanus, hepatitis B, measles, mumps and rubella after completion of treatment in children with solid tumors and to investigate their humoral immune responses to booster diphtheria, tetanus and pertussis (DTP) vaccinations in pediatric patients with solid tumors.

8.2 Subjects and Methods

8.2.1 Patients and Samples

From the period June 1, 2003 to October 31, 2004, 27 consecutive patients aged 1-18 years who had been treated successfully with different solid tumors were recruited for evaluation of humoral immunity against seven major vaccine-preventable diseases (hepatitis B, diphtheria, tetanus, pertussis, measles, mumps and rubella) and their responses to booster DTP vaccine. They were all diagnosed and received treatment in an our oncology centre of a university teaching hospital in Hong Kong, which is also a tertiary referral center for pediatric cancers in Hong Kong. All patients were in clinical and radiological remissions. The Clinical Research Ethics Committee of our University approved this clinical trial, and patients and / or their parents gave informed written consent prior to study.

Exclusion criteria included (1) patients with past history of those listed vaccine-preventable diseases; (2) evidence of immunodeficiency before diagnosis of malignancy; (3) history of allergic or severe reactions to DTP vaccine or its components and (4) relapse of primary disease or secondary malignancies during the study period. Three patients did not consent for the study, two patients were excluded because of history of severe febrile reaction to previous DTP vaccines and the other two were due to relapse of primary disease during study period.

The subjects included patients with different solid tumors. Patients in each group were randomized in blocks of 4 into the vaccine and control groups. Immunization status of patients was obtained from the history and vaccination records. Past medical history was reviewed to exclude those with history of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella infections before enrollment.

8.2.2 Randomization

Eligible patients were randomly assigned at 1:1 ratio into treatment and control groups in this randomized clinical trial. An independent research staff was responsible for generation of randomization sequence by a computer program.

8.2.3 Surveillance of post-vaccination humoral and immunities

Serum concentrations of antibodies to diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B were monitored serially started from six months after completion of intensive chemotherapy (visit 1). In the vaccination group, three doses of booster DTP vaccines were given at eight months (visit 2), ten months (visit 3) and tweleve months (visit 4) after completion of chemotherapy. Each 0.5 ml dose of DTP vaccine (Aventis Pasteur, Lyon, France) contained ≥ 30 IU purified diphtheria toxoid, ≥ 60 IU purified tetanus toxoid and ≥ 4 IU heat-inactivated *Bordetella perutssis*. These vaccines were stored at 4°C until use. No intervention was performed for the control group. Blood for diphtheria, tetanus, pertussis, hepatitis B, measles, mumps and rubella antibodies were checked at visit 1, visit 2, visit 4 and visit 5. Table 8 illustrates the study timeline.

Table 8. Study flowchart for children with solid tumors receiving DTP booster vaccines

	Time Following Completion of Chemotherapy				
	6 months (Visit 1)	8 months (Visit 2)	10 months (Visit 3)	12 months (Visit 4)	18 months (Visit 5)
Immunological Surveillance					
Complete blood count with differentials	+	+	-	+	+
Lymphocyte subsets by flow cytometry	+	-	-	+	+
Serum IgG, IgM and IgA levels	+	-	-	+	+
Antibodies to diphtheria, tetanus and pertussis	+	+	-	+	+
Antibody to hepatitis B virus surface antigen (Anti-HBs)	+	+	-	+	+
Antibodies to mumps, measles, rubella	+	-	-	+	+
Booster vaccination					
DTP vaccine		+	+	+	

8.2.4 Serological studies

Serum samples obtained from the above 4 visits were stored at -30°C until analysis in one batch. Commercially available kits were used for the determination of antibody titers. Serum levels of specific antibodies to diphtheria, tetanus and pertussis were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany). The protective antibody levels were defined as 4 IU/mL for diphtheria, 2 IU/mL for tetanus and 24 IU/mL for pertussis respectively.(88) Titer to Hepatitis B surface antibody (anti-HBs) was measured by using an enzyme-linked immunosorbent assay. (BioSupply, United Kingdom) Antibody level ≥ 10 mIU/ml was considered as protective. Measles and mumps IgG antibodies were tested by a modification of the NOVUM measles/mumps virus IgG ELISA test kits (Novum Diagnostica, Germany) and rubella by Imx Rubella IgG 2.0 antibody assay (Abbott Laboratories, Chicago, IL, USA). Antibody levels exceeding a cutoff value of 10 IU/ml were interpreted as seropositive (91).

8.2.5 Assessment of cellular and humoral immunities

Subsets of circulating lymphocytes were quantified by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA) using fluorescence-labeled monoclonal antibodies $CD3^+$, $CD4^+$ and $CD8^+$ (T lymphocytes), $CD19^+$ (mature B lymphocytes) and $CD16/56^+$ (natural killer cells). Serum IgG concentration was measured by nephelometry (Binding Site, Birmingham, UK).

8.2.6 Statistical Analysis

The primary outcomes were the differences in serum concentrations of specific antibodies to diphtheria, tetanus and pertussis between vaccine and control groups at the end of the 12-month study period. Secondary outcomes included the differences in serum concentrations of total IgG and anti-HBs, measles, mumps and rubella antibodies, as well as circulating lymphocyte subpopulations, between the two groups, and longitudinal changes in serum diphtheria, tetanus and pertussis antibody levels in the patients and controls at baseline (Visit 1) and 12 months (Visit 5) following vaccination.

Data were expressed as mean and standard deviation unless otherwise stated. Serum concentrations of specific antibodies were $\log_{10}$-transformed to achieve normal distribution of data before analysis. Demographic data between different subgroups were analyzed using Student *t* test for independent parametric variables and χ^2 for categorical variables between different disease or patient groups. Statistical analysis was performed only for subjects who had received at least two doses of DTP vaccines. Patients who relapsed during follow-up were excluded from analysis. Following DTP vaccination, the longitudinal changes in serum antibody levels between study visits were analyzed using paired *t* test. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was considered to be statistically significant.

8.3 Results

8.3.1 Study populations

During the study period, twenty seven patients with solid tumors were recruited. They included patients with different solid tumors (osteosarcoma, n=8; brain tumors, n=5; lymphoma, n=5; soft tissue sarcoma, n=2; others included Wilm's tumors, extracranial germ cell tumors, neuroblastoma, and nasopharygneal carcinoma, n=7). Thirteen were randomized to the vaccination group and fourteen to the control group. All patients were followed up until the end of study. Thirty-nine booster vaccines were given. No significant vaccine-related adverse reactions were noted. The baseline characteristics of subjects in vaccination and control groups were comparable (Table 9).

Table 9. Baseline characteristics of children with solid tumors

Parameters	Vaccine group (n=13)	Control group (n=14)	p-value
Mean age	9.25 ± 3.89	7.62 ± 3.79	0.28
Male, n (%)	11 (84.6%)	8 (57.1%)	0.12
Mean duration of chemotherapy, months	9.69 ± 3.12	9.71 ± 4.05	0.99
Total lymphocyte counts, 10^9/L	2.47 ± 1.30	2.17 ± 0.52	0.46
Mature B cells (CD19), 10^9/L	0.57 ± 0.39	0.59 ± 0.14	0.92
Total T-lymphocyte (CD3), 10^9/L	1.43 ± 0.82	1.31 ± 0.41	0.60
Natural killer cells (CD16/CD56), 10^9/L	0.12 ± 0.08	0.12 + 0.07	0.93
T-helper cells (CD3/CD4), 10^9/L	0.46 ± 0.34	0.40 ± 0.18	0.58
T-suppressor cells (CD3/CD8), 10^9/L	0.39 ± 0.26	0.33 ± 0.15	0.44
CD4 : CD8+ ratio	1.18 ± 0.40	1.27 ± 0.48	0.62
Baseline IgG, g/L	11.67 ± 1.70	9.66 ± 3.03	0.05*
Baseline IgA, g/L	2.14 ± 0.93	1.46 ± 0.50	0.38
Baseline IgM, g/L	1.12 ± 0.39	0.99 ± 0.37	0.03*

Results expressed as mean ± standard deviation unless otherwise stated.

* Statistical significant ($p<0.05$)

8.3.2 Baseline antibody titers

At baseline, 12.1% patients had diphtheria antibody titer below protective level, and 7.4% and 3.5% of patients were seronegative for tetanus and pertussis respectively. For measles, mumps and rubella, 30.5%, 29.2% and 33.5% of patients did not have protective levels of these specific antibodies respectively. Concerning hepatits B, our unpublished data showed that overall 68% were seropositive before starting chemotherapy (53.9% in hematology malignancies group and 84.4% in solid tumors group respectively) and after completion of treatment, the overall seropositivity dropped to about 30% and stayed at 40% up to eighteen months after stopping chemotherapeutic agents. Concerning the data in solid tumors group, only 15.6% of patients were seronegative against hepatitis B surface antigen before starting chemotherapy and the percentage raised up to about 57.0% after completion of treatment (Table 3).

Table 10. Changes in seropositivity rates for vaccine-preventable diseases at visit 1 (six months after stopping of chemotherapy) and at visit 5 (eighteen months after stopping of chemotherapy) in children with solid tumors

	Vaccine group			Control group		
Disease	Visit 1	Visit 5	p*	Visit 1	Visit 5	p*
Diphtheria	90.5%	100%	0.68	85.7%	100.0%	0.70
Tetanus	87.2%	100%	0.55	100.0%	92.3%	0.87
Pertussis	93.3%	100%	0.74	100.0%	100%	0.98
Measles	84.6%	76.9%	0.45	54.3%	69.2%	0.11
Mumps	84.6%	76.9%	0.45	57.1%	61.5%	0.34
Rubella	61.5%	53.8%	0.34	71.4%	71.4%	1.00
Hepatitis B	53.8%	46.1%	0.50	42.9%	38.5%	0.75

* Analyzed by McNemar test for paired binomial variables.

8.3.3 Humoral immunity to booster DTP vaccination

After three doses of DTP vaccine, 100% of patients demonstrated seropositivity against diphtheria, tetanus and pertussis. The responses were sustained till twelve months after first dose of DTP vaccine (Table 10). The geometric mean of antibody titers against diphtheria, tetanus and pertussis were significantly higher than baseline and the response were sustained till 12 months after vaccinations (Table 11). Booster DTP vaccinations were efficacious in increasing the respective specific antibodies. During the twelve months follow up, the seropositivity rates were similar among unvaccinated patients. There was no spontaneous rise in protective antibodies against mumps, measles, rubella and hepatitis B in both groups during the study period. A significant proportion of subjects remained susceptible to these vaccine-preventable infectious diseases throughout the study period.

Table 11. Serial antibody titers (geometric mean) of children with solid tumors

Visits	Visit 1			Visit 2			Visit 5		
	Vaccine group	Control group	p**	Vaccine group	Control group	p**	Vaccine group	Control Group	p**
Diphtheria (> 4.0 U/ml)	29.91	28.09	0.99	147.31	38.52	<0.01	114.43	36.02	<0.01
Tetanus (>2.0 IU/ml)	127.35	52.22	0.03	328.43	57.22	<0.01	268.14	52.42	<0.01
Pertussis (>24 U/ml)	1108.25	704.99	0.06	2213.30	552.16	<0.01	11843.51	1425.19	<0.01

* Results expressed as $\log_{10}$-transformed concentrations of specific antibodies.

** Analyzed by paired *t* test for comparisons of antibody levels post-vaccination (Visits 2, 4 or 5) with that at baseline (Visit 1). Same results were obtained on subgroup analyses for patients with hematological malignancies and solid tumors.

8.4 Discussion

In this study, chemotherapy for children with solid tumors were also shown to be able to induce a loss of protective serum antibody titers for diphtheria, tetanus, pertussis, measles, mumps, rubella and hepatitis B at six months after stopping chemotherapy. Hepatitis B, measles, mumps and rubella antibody titers were mostly affected by the immunosuppressive effect of cytotoxic therapy when compared with diphtheria, tetanus and pertussis antibody titers. These findings are consistent with other published data (31, 56).

The administration of booster DTP vaccines started at 6 months after stopping chemotherapy enabled 100% of patients in treatment group to recover protective antibody at a persistently high titers. The antibody titers could also be sustained above the protective level throughout the twelve-month follow up period. This finding suggested that chemotherapy did not entirely abolish the specific humoral immune memory in patients with undetectable serum antibody titers after chemotherapy. The impaired capacity to mount an immune response was temporary, and probably in a lesser extent than children with hematological malignancies, and it tended to return to normal at six months after the completion of chemotherapy. In the control group, although the process of immune reconstruction continued, there was no significant increase in percentage of patients became seropositive and there was no significant change in the absolute antibodies titers throughout one year indicating they were still vulnerable to these vaccine-preventable diseases, indicating there was a role for post-chemotherapy booster vaccination in this group of patients. A total of thirty-nine booster vaccinations were given. No significant or serious adverse events were noted during the entire study period.

Our studies described in Chapter 7 and 8 are the first series of randomized controlled studies published in English literatures evaluating immune responses of post-chemothearpy vaccination in pediatric oncology patients. Our results clearly illustrate that there are no spontaneous rise in antibodies level among various vaccine-preventable infectious diseases antigens if no post-chemotherapy booster vaccines are given. The rise in antibodies in diphtheria, tetanus and pertussis are solely due to the effect of vaccination.

Currently, with the available data including the data from our group, indicating there are no major differences in protection among patients with solid tumors and hematological malignancies, we can probably treat them as one group of patients in term of evaluating the strategies of booster immunization.

There are two main strategies that can be used for re-immunizing children who had completed chemotherapy. They are: (i) universal approach: re-immunization of all patients six months after completion of chemotherapy, regardless of their antibodies level; (ii) selected approach: immunization of those unprotected patients after assessment of antibodies to various vaccine antigens at six months after stopping chemotherapy. The adoption of each strategy needs a fine balance among (i) epidemiology and clinical consequences of acquiring these vaccine-preventable infectious diseases in local population; (ii) risk of vaccination; (iii) detailed cost and effectiveness analysis of the vaccine cost, cost of extra laboratory tests and cost of extra clinic visits for vaccination. However, before the detail logistics being worked out, as mentioned in my previous chapter (Chapter 7), young children, probably under 2 years old, especially who have not completed their primary schedule at the time of initiation of intensive chemotherapy, are even less protective against vaccine antigens than older counterparts should restart the revaccation program six months after

stopping all chemotherapies including maintenance chemotherapies.

Since routine antibody testing for diphtheria, tetanus and pertussis is usually not part of the routine service in most of the virology laboratories. This is reasonable and logical to propose children with age less than two years old who have interrupted the primary vaccine schedule at the time of starting chemotherapy should restart the primary vaccine series six months after stopping chemotherapy and revaccinate according to vaccination schedule (113). For children who have completed the primary series before the diagnosis of malignancies, three doses of booster DTP vaccines can produce significant and sustained antibody responses in recipients. Antibody titers to hepatitis B, measles, mumps and rubella antibodies can be retested at six months after stopping chemotherapy and booster revaccination should be given to those seronegative patients.

One limitation of our study is lacking of actual functional assessment of cellular immunity in our study and control populations which is an important aspect of comprehensive assessment of recovery of cellular immunity. This can be addressed in future studies.

8.5 Conclusion

In conclusion, our study demonstrates that chemotherapy for solid tumors leads to loss of protective serum antibody titers for different types of vaccine-preventable infectious diseases. Booster vaccinations started at six-month after stopping chemotherapy in children with different solid tumors can be safely administered and effectively restore a sustained effect in humoral immunity against various types of vaccine-preventable infectious diseases.

Chapter 9: Clinical Presentations and Outcome of Hospitalized Pediatric Oncology Patients with Laboratory-confirmed Pandemic H1N1 Influenza Infection in Hong Kong – Impact of Novel Virus and the Role of Novel Monovalent H1N1 Vaccine

9.1 Introduction

In April, 2009, a novel influenza virus (H1N1) strain which was characterized by a unique combination of gene segments that derived from two swine species from North American and Eurasian lineages, one avian and one human origin influenza viruses was identified (114). In June, 2009, World Health Organization (WHO) declared the status of influenza A (H1N1) pandemic had reached phase six indicating widespread community transmissions in at least two continents (17). The basic reproductive ratio (R_0), which is defined as the expected number of secondary infections arising from a single individual during his or her entire infectious period in a population of susceptible, of this novel influenza (H1N1) strain is similar to the spread of Asian pandemic influenza (H2N2) in 1957-1958 which is about 1.4-1.6 (17).

Although the reported case fatality rate of this influenza remains comparable to seasonal influenza in general population, the epidemiology is quite different from seasonal influenza infection, namely (i) the median age of reported confirmed cases is much younger than cases of seasonal influenza; and (ii) the attack rate is highest among children and adolescents (115). Prolonged viral excretion, lower respiratory tract infection and frequent development of anti-viral resistance during antiviral therapy are major additional concerns in immunocompromised pediatric population (116).

The overall clinical picture of pandemic H1N1 infection in one of the most vulnerable patient groups – pediatric oncology and hematopoietic stem cell transplant recipients (HSCT) is not fully understood. Traditionally, immunocompromised patients are well-known to suffer from serious complications of influenza infection. By knowing this fact, appropriate and timely treatment and preventive strategies can then be timely tailored made to this patient group. We, therefore, performed this territory-wide study to evaluate the symptomatology and clinical course of this infection in hospitalized pediatric oncology patients with laboratory-confirmed pandemic H1N1 infection in Hong Kong.

9.2 Subjects and Methods

9.2.1 Recruitment criteria

All local hospitals (2 university centres and 3 regional hospitals) which involve in managing pediatric oncology patients and hematopoietic stem cell transplant (HSCT) recipients in Hong Kong participate in this study.

The review period was started from the date which WHO declared the global pandemic of H1N1 infection, i.e. from 11th June, 2009 to 30th November, 2009. The following case definitions were used to select patients for review: (i) children aged ≦ 18 years old with oncologic conditions or hematopoietic stem cell transplant recipients who are receiving active treatment presenting with influenza-like illness (ILI) and laboratory-confirmed pandemic H1N1 influenza infection or (ii) children aged ≦ 18 years old with oncologic conditions or hematopoietic stem cell transplant recipients who have completed chemotherapy or discontinued immunosuppressants for less than twelve months, presenting with ILI and laboratory-confirmed pandemic H1N1 infection. The definition of ILI is fever (temperature ≧ 38°C) with either cough, sore throat or both for at least 24 hours. Laboratory-confirmed pandemic H1N1 infection was defined as the detection of H1N1-H1 gene by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) in nasopharyngeal aspirate (NPA) or nasopharyngeal swab or combined oropharyngeal and nasal swab samples.

9.2.2 Data collection

Medical chart review was performed by the physician-in-charge of each participating site. They used the same standardized form that included (i) baseline demographic data; (ii) presenting symptoms / signs and laboratory results at diagnosis; (iii) clinical course; (iv) treatment offered; (v) complications and (vi) outcome. All diagnostic tests and management were clinically driven and based on the recommendations of Hospital Authority of Hong Kong.

For time calculations, the first day of onset of fever was considered to be day 0.

9.3 Results

During the study period, sixteen patients (12 male: 4 female) fulfilled the case definition were recruited. The median age was 12.4 years (range 3.4 years – 14.6 years). The primary diseases included: (i) acute lymphoblastic leukemia (n=7); (ii) CNS tumors (n=4); (iii) acute myeloid leukemia (n=2); (iv) lymphoma, (n=1); (v) others (n=2). Nine of them were receiving chemotherapy according to their disease-specific protocols. Seven of them have received hematopoietic stem cell transplantation (HSCT) of whom six had chronic graft-versus-host disease (GVHD). Two patients (patient 10 and 15) developed moderate to severe chronic graft-versus-disease of the lung and one of them (patient 15) complicated with bronchiolitis obliterans and was on intensive immunosuppressive therapies.

The most common presenting symptoms of pandemic H1N1 infection were fever (100%), cough (75.0%), runny nose (56.3%) and sore throat (50.0%). One patient (patient 13) with history of epilepsy presented with generalized convulsion and fever. One patient (patient 8) presented with vomiting but none of them presented with diarrhea.

The median duration of fever was 4 days (range from 1 to 9 days). The median duration of hospitalization was 5 days (range from 3 to 12 days).

Concerning antiviral therapy, oseltamivir phosphate (Tamiflu®, Roche Laboratories Inc.) was started at median 1 day after fever onset (range from 1 to 6 days). Eight patients (50.0%) received one course of 5 to 7-day of oseltamvir phosphate (Tamiflu®, Roche Laboratories Inc.). Patient 3, 10 and 15 had recurrence of fever and worsening of respiratory symptoms after a 5-day course of oseltamivir phosphate (Tamiflu®, Roche Laboratories Inc.) and required an extra 5-day of high dose antiviral therapy.

The mean time of defervescence after stating antiviral therapy was 1.3 days (range 1-3 days) and 3.3 days (range 1-8 days) in oncology patients and HSCT recipients respectively.

Seven patients had repeated virologic studies, 4 turned negative by day 14 from diagnosis but 3 showed persistent positive PCR results up to day 24 of illness. No laboratory-confirmed secondary nosocomial spread of infection in health-care setting was recorded during the 5-month study period. The details are shown in tables 1 and 2.

Two of them (patient 3 and 15) developed tachypnea and required oxygen supplement via nasal cannula. They did not require intensive care or requirement of mechanical ventilation. All of our patients recovered fully and none of them complicated with residual morbidity.

Table 12. Patients' characteristics of pandemic H1N1 infection in pediatric oncology patients in Hong Kong

Patients	Sex / Age (years)	Primary disease	Treatment phase
1	F/3.4	Acute lymphoblastic leukemia (ALL)	Intensive chemotherapy
2	M/5.7	Cerebellar medulloblastoma	Intensive chemotherapy
3	M/13.7	Relapse ALL	Intensive chemotherapy
4	M/8.3	Relapse ALL	Maintenance chemotherapy
5	M/7.8	ALL	Maintenance chemotherapy
6	M/11.8	T-cell lymphoma	Maintenance chemotherapy
7	M/12.3	ALL	Maintenance chemotherapy
8	M/12.4	Oligodendroglioma	Intensive chemotherapy
9	M/13.2	CNS* germinoma	Intensive chemotherapy
10	F/7.1	Beta-thalassemia	Post-unrelated cord blood transplant (UCBT) 25 months with chronic GVHD** of skin and lung on cyclosporine A
11	F/7.2	T-cell lymphoma, MDS***	Post-unrelated CBT 23 months with chronic GVHD of skin on cyclosporine A
12	M/12.5	Ph$^{\#}$+ve ALL, choroid plexus tumor	Post-unrelated CBT 36 months with chronic GVHD
13	M/13.3	Adrenoleukodystrophy	Post-unrelated CBT 45 months with chronic skin GVHD on prednisolone
14	F/14.1	Relapsed acute myeloid leukemia (AML)	HLA-ID$^{\wedge}$ sibling post-bone marrow transplant (BMT) 9 months
15	M/14.2	Relapsed Ph+ve ALL	HLA-ID sibling PBSC$^{\#\#}$ transplant 20 months with extensive chronic GVHD of skin, lung (bronchiolitis obliterans), gut and mucosa on prednisolone, cyclosporine A, sirolimus and mycophenolate mofetil
16	M/14.6	Refractory AML	Post-UCBT 16 months with chronic skin GVHD on cyclosporine A

* Central nervous system; ** Gaft-versus-host disease; *** Myelodysplastic syndrome; # Philadelphia chromosome; ^ HLA-identical; ## Peripheral blood stem cell

Table 13. Clinical presentation and outcome of pandemic H1N1 infection in pediatric oncology patients in Hong Kong

Patients	Presenting symptoms	ANC*	LYM**	CXR# changes	Duration of fever (days)	Treatment	Antiviral therapy started on days of fever (days)	Duration of PCR remained positive (days)	Outcome
1	Fever, cough, runny nose, sore throat	5.7	1.8	No	6	Oseltamivir 30 mg BD for 5 days; Empirical antibiotics for 2 days	6	Remained positive on day 14 of illness	Discharge on day 7 of illness
2	Fever, cough	0.7	0.1	No	3	Oseltamivir 45 mg BD for 5 days; Empirical antibiotics for 7 days	3	10	Discharge on day 8 of illness
3	Fever, runny nose, cough, sore throat	0.1	0.3	Yes (bilateral hilar streakiness)	5	Oseltamivir 75 mg BD for 3 days then 150 mg BD for 7 days; Empirical antibiotics for 7 days	3	ND##	Required 2L/min O2 supplement. Discharge on day 9 of illness
4	Fever, runny nose, cough	1.5	0.5	Yes (bilateral hilar	1.2	Oseltamivir 60 mg BD for 5 days	1	ND	Discharge on day 3 of illness

				streakiness)					
5	Fever	3.1	1.1	No	3	Oseltamivir 60 mg BD for 5 days	1	ND	Discharge on day 4 of illness
6	Fever, cough, runny nose, sore throat	4.4	0.2	No	2	Oseltamivir 60 mg BD for 5 days	1	Remained positive on day 14 of illness	Discharge on day 4 of illness
7	Fever, cough, runny nose, sore throat	3.4	0.75	No	3	Oseltamivir 60 mg BD for 7 days; Empirical antibiotics for 5 days	2	ND	Discharge on day 6 of illness
8	Fever, cough, runny nose, sore throat, chills, rigor, vomiting	3.1	0.4	No	2	Oseltamivir 60 mg BD for 5 days	2	8	Discharge on day 4 of illness
9	Fever, sore throat, cough	0.2	0.4	No	4	Oseltamivir 75 mg BD for 5 days	2	7	Discharge on day 7 of illness
10	Fever, cough, myalgia, chills, rigor, malaise	4.4	1.2	No	4	2 courses of oseltamivir 60 mg BD for 5 days; Empirical antibiotics for 5 days	1	ND	Discharge on day 6 of illness
11	Fever, runny nose	11.0	1.4	No	1	Oseltamivir 60 mg BD for 10 days; Empirical	1	ND	Discharge on day 4 of illness

						antibiotics for 5 days			
12	Fever	4.3	2.2	No	9	Oseltamivir 75 mg BD for 10 days	1	ND	Discharge on day 10 of illness
13	Fever, cough, runny nose, tonic-clonic seizure	7.6	3.8	No	4	Oseltamivir 150 mg BD for 10 days	1	ND	Discharge on day 6 of illness
14	Fever, sore throat, malaise	4.0	3.1	No	4	Oseltamivir 75 mg BD for 10 days	1	ND	Discharge on day 5 of illness
15	Fever, cough, runny nose	2.3	0.9	No	2	2 courses of oseltamivir 75 mg BD for 5 days; Empirical antibiotics	1	Remained positive on day 14 of illness	Required 2L/min O2 supplement, discharge on day 12 of illness
16	Fever, cough, sore throat, chills	3.2	0.9	No	4	Oseltamivir 75 mg BD for 10 days	1	ND	Discharge on day 6 of illness

* Absolute neutrophil count (10^9/L); ** Absolute lymphocyte count (10^9/L); # Chest X-ray; ## Not done

9.4 Discussion

Influenza viruses are enveloped segmented RNA viruses. Influenza viruses are members of the family Orthomyxoviridae. They are divided into three types: A, B, and C. The majority of the human cases of influenza are caused by types A and B in annual winter epidemics. Influenza A viruses are further divided into subtypes based on the hemagglutinin (H) and neuraminidase genes (N). World Health Organization (WHO) nomenclature for classification of influenza strains is as follows: type (A, B, or C)/ geographic origin / year of isolation/subtype (hemagglutinin and neuraminidase), for example A / Sydney / 5 / 97 (H3N2). There are sixteen hemagglutinin subtypes and nine neuraminidase subtypes. Hemagglutinin 1, 2, and 3 and neuraminidase 1 and 2 typically circulate in humans. Variations in yearly influenza strains cause epidemics, and these are due to point mutations and progressive variation in protein sequence, called antigenic drift. The reassortment of gene segments that leads to complete change of the hemagglutinin or neuraminidase proteins is called antigenic shift and is a major source of pandemic strains of influenza.

Laboratory-confirmed cases of human infections with the novel influenza A (H1N1) virus were mostly occurred in children and young adults. A spectrum of disease presentation ranging from non-febrile, mild upper respiratory tract illness to severe or fatal pneumonia has been described (115). In our cohort of immunocompromised cancer children, the most commonly reported symptoms included fever, cough and runny nose. Gastrointestinal symptoms (nausea, vomiting and/or diarrhea) previously reported with pandemic H1N1 influenza were not common in our series (115).

To date, although the clinical presentation of patients who are hospitalized with 2009 H1N1 influenza are generally similar to those reported during peak periods of seasonal influenza, the epidemiology of mostly affected population is quite different. Jain S *et al* studied two hundreds and seventy two hospitalized patients with pandemic H1N1 infection in United States. During peak periods of seasonal influenza, hospitalizations are more common among persons sixty five years of age or older and those under the age of five years, For pandemic H1N1 infection, up to 45% of the hospitalizations involved persons under the age of eighteen years; more than one third of the patients were between the ages of eighteen and forty nine years, and only 5% were sixty five years of age or older. Seventy-three percent of the patients had at least one underlying medical condition which included asthma, diabetes, heart, lung, and neurologic diseases and pregnancy (117).

Launes *et al* recently reviewed ten consecutive patients with acute lymphoblastic leukemia (ALL) and pandemic H1N1 influenza infections treated in a single institution in Spain. The median age of the cohort was seven years (range 3–12 years). All patients were treated with standard first-line antiviral agents. There were no deaths reported in the series. Two patients treated under intensive chemotherapy developed lower respiratory tract infections and one required intensive care and ventilatory support. ALL patients treated with maintenance therapy had mild disease and none of them developed severe complications. They concluded that patients who were treated under intensive treatment protocols developed moderate to severe H1N1 disease. Based on their study results, patients are needed to ascertain risk factors for severe disease in pediatric ALL. Patients who are in good clinical condition and are not neutropenic and receive maintenance treatment can be safely managed as outpatients. Patients who are still receiving intensive chemotherapy should begin antiviral

treatment as soon as influenza is suspected and do not require to wait for the result of the RT-PCR (118).

Cao *et al* described the clinical features of four hundreds and twenty six cases of pandemic H1N1 infection in China. Although they showed that majority of cases ran a benign course, independent risk factors for prolonged real-time RT-PCR positivity included patients' age of less than fourteen years, male sex, and a delay from the onset of symptoms to treatment with oseltamivir of more than forty eight hours (119). Among the patients who required intensive care, 93% were patients younger than sixty five years old and 10% were pregnant women (120). All these data suggested that young patient is one of the highest risk groups of pandemic H1N1 infection. To *et al* also showed that younger age was associated with prolonged shedding in the respiratory tract and higher viral load in the stool (121).

In contrast to the findings of Launes *et al*, our patient series who were traditionally believed to be one of the highest risk groups of severe influenza infection, ran a relative uncomplicated clinical course and all of them recovered uneventfully from this novel infection. This was probably due to the early start of antiviral therapy (median 1 day after onset of fever) which halted the propagation of virus and decreased the viral load in the immunocompromised hosts. However, three patients (patient 3, 10 and 15) required additional course of high dose antiviral therapy due to recurrence of fever and respiratory symptoms. We also observed that the duration to achieve defervescence was longer in HSCT recipients and prolonged duration or recurrence of respiratory symptoms were more commonly observed in HSCT patients, therefore, we routinely prescribed a ten-day course of antiviral therapy in these patients in the latter half of the study period. Jain S *et al* also demonstrated that the early use of antiviral drugs was beneficial in hospitalized patients (117). To *et al*

recently demonstrated that patients who died from acute respiratory distress syndrome (ARDS) had a slower decline in nasopharyngeal viral load and higher plasma levels of proinflammatory cytokines and chemokines than in patients who survived without (ARDS) or mild disease groups (122).

Despite the benign clinical course, the phenomenon of persistent symptoms and laboratory evidence of prolonged viral excretion after a standard course of antiviral poses a continuous threat in term of infection control policy in pediatric oncology and hematopoietic stem cell transplant unit (116). Cheng *et al* reported a fifteen-month old patient with stage 3 neuroblastoma with persistent respiratory syncytial virus (RSV) shedding for seven months after primary infection. Regular surveillance of the shedding of virus should be performed and confirmation of viral clearance should be obtained before discontinuing infection control measures in order to prevent nosocomial outbreaks among high-risk patients (71). As the pandemic H1N1 infection has become the major strain of influenza virus (90%) circulating in the local community, the same policy was adopted in that period (123).

Case definition of influenza-like illness (ILI) for influenza surveillance schemes vary widely worldwide. Different ILI definitions will definitely affect the case identification and indeed have implication in implementation of infection control measures. In our unit, we adopt the definition from Centers for Disease Control and Prevention (CDC) which is a simple and highly sensitive (98.4% - 100%) definition. It is also the recommended influenza case definition of the World Health Organization (Department of Communicable Disease Surveillance and Control,1999b). However, the relatively low specificity (7.1% - 12.9%) implies there is low accuracy in identifying influenza activity without laboratory confirmation (124). Thursky *et al* propose a case definition of cough, history of fever and fatigue which has a higher

positive predictive value (PPV) than the CDC definition (124).

Vaccination remains one of the most effective preventive measures to confine infection in the community and especially among the high risk groups. Preliminary immunogenicity data of injectable inactivated monovalent H1N1 vaccine in pediatric population shows that children aged six months to younger than nine years should receive two monthly doses whereas children's age older than nine years can receive one dose (125). To date, there is another intranasal preparation of monovalent H1N1 vaccine which is approved by Food and Drug Administration, USA (126). However, it is a live-attenuated vaccine and is contraindicated in immunocompromised patients.

Wiesik-Szewczyk E *et al* evaluated the response of influenza vaccine in patients with systemic lupus erythamatosus (SLE) who were on immunosuppressive therapies (systemic steroid, chloroquine, cyclophosphamide, azathioprine, methotrexate and cyclosporine A). At one month after immunization, anti-hemagglutinin antibody titres rose in the patient group at least 6.23-fold, compared to 11.90-fold among healthy controls ($p \leq 0.05$). The seroconversion rate range was 53-56% among patients and 72-85% among controls ($p<0.05$ for strains H1N1 and H3N2). The seroprotection rate ranged between 62% and 73% and between 90% and 98% in the patient and control group, respectively ($p<0.05$). At three months after vaccination, the antibody titres were higher at least 3.86-fold in the patient group and 7.65-fold among healthy controls. The seroconversion rate range was 32-40% among patients and 64-70% among controls, while the seroprotection rate ranged between 43% and 50% and between 79% and 94%, respectively ($p< 0.005$ for three strains). The post-vaccination responses were weaker in SLE patients compared to healthy subjects (127). Therefore, there is evidence to suggest the immunogenicity of influenza vaccine is decreased in immunocompromised patients. The data in efficacy of influenza vaccine, especially

the novel H1N1 vaccine, in pediatric oncology and hematopoietic stem cell transplant recipients is still lacking up at this moment.

9.5 Conclusion

Fever, cough and runny nose are the most common presenting symptoms of pandemic H1N1 influenza infection in pediatric oncology patients. The uncomplicated course of pandemic H1N1 infection may be related to the early initiation of antiviral therapy. Persistent symptoms and evidence of prolonged viral excretion which require repeated course of prolonged antiviral therapy are more common, especially among HSCT recipients. The efficacy of H1N1 vaccine in immunocompromised patients still needs further evaluation.

Section 3

Impact of Non-Vaccine Preventable Infectious Diseases and the Potential Use of Adoptive Transfer of Virus-Specific Immunity in Allogeneic Hematopoietic Stem Cell Transplant Recipients

Chapter 10 Impact of Non-vaccine Preventable Viral Infection in HSCT Setting: Epstein-Barr Virus (EBV) in Post-transplant Lymphoproliferative Disorder Complicating Umbilical Cord Blood Transplantation.

10.1 Introduction

Epstein-Barr Virus (EBV) is one of the most successful viruses, infecting more than ninety percent of humans and persisting for the lifetime of the people. EBV was discovered thirty six years ago by electron microscopy of cells cultured from Burkitt lymphoma tissue by Epstein, Achong, and Barr. Four years later, in 1968, EBV was shown to be the etiologic agent of heterophile-positive infectious mononucleosis. EBV DNA was detected in tissues from patients with nasopharyngeal carcinoma in 1970. In the 1980s, EBV was found to be associated with non-Hodgkin lymphoma and oral hairy leukoplakia in patients with the acquired immunodeficiency syndrome (AIDS). Since then, EBV DNA has been found in tissues from other cancers, including T-cell lymphomas and Hodgkin lymphoma (128).

EBV is associated with lymphoproliferative disease in patients with congenital or acquired immunodeficiency syndrome. These include patients with severe combined immunodeficiency, recipients of organ or hematopoietic stem cell transplantation, and patients with AIDS. T-cell mediated immunity is impaired in these patients and they are unable to control the proliferation of EBV-infected B cells. They present with symptoms of infectious mononucleosis or with fever and localized or disseminated lymphoproliferation involving the lymph nodes, liver, lung, kidney, bone marrow, central nervous system, or small intestine (128). Epstein-Barr virus-associated post-transplant lymphoproliferative disorder (EBV-PTLD) has been well studied in solid organ transplantation and allogeneic bone marrow and peripheral blood stem cell

transplantation settings. Patients who have received T-cell–depleted or HLA-mismatched bone marrow, received antilymphocyte antibodies, history of cytomegalovirus diseases, or acquired primary EBV infection after received HSCT are at higher risk for lymphoproliferative disease. However, it is only recently reported after umbilical cord blood transplantation (UCBT) (129, 130). As we know, nearly all neonatal donors are EBV negative and, theoretically, can reduce the risk of the transferral of EBV-infected B lymphocytes from donors to recipients.

X-linked adrenoleukodystrophy (ALD) is a peroxisomal disorder characterized with the accumulation of very long chain fatty acids (VLCFA) in plasma and in tissue. X-linked ALD is due to defect in gene ABCD1 on chromosome Xq28. Childhood cerebral X-linked ALD is the most common phenotype, causing rapid neurodegeneration affecting central nervous system myelin and adrenal cortex. Most patients have evidence of adrenal insufficiency since 2 years of age. Neurological symptoms typically manifest at about 7 years of age and range from frequent falls, personality changes to deterioration of academic performance. It can rapidly progress to vegetative state if no treatment is given (131). Magnetic resonance imaging (MRI) changes usually precede the presentation of neurological symptoms. Timely hematopoietic stem cell transplantation can arrest the progression of childhood cerebral X-linked ALD (132-134).

In this chapter, two patients who developed post-transplant lymphoproliferative disorder associated with EBV infection or reactivation after received unrelated UCBT is described as an illustration of the impact of non-vaccine preventable infectious diseases in hematopoietic stem cell transplant setting.

10.2 Case Illustration

Patient 1

This patient presented at the age of eight years with hyperpigmentation, increased clumsiness and frequent falling. Synacthen test confirmed the presence of adrenal insufficiency. Magnetic resonance imaging (MRI) of the brain showed demyelinating changes consistent with ALD. Diagnosis of adrenoleukodystrophy (ALD) was confirmed by elevation of plasma levels of VLCFA.

Two-antigen mismatched unrelated UCBT was performed in June 1999. Conditioning regimen consisted of busulphan (16 mg/kg), cyclophosphamide (200 mg/kg) and antithymocyte globulin (90 mg/kg). Cyclosporine and prednisolone were used as graft-versus-host disease (GVHD) prophylaxis. Engraftment was achieved on day 13 post-transplant with complete donor chimerism. He developed grade II GVHD on day 43, which progressed to extensive chronic GVHD involved skin and gut which required treatment with systemic steroid and cyclosporine.

A left tonsillar ulcer was first noted on day 74 post-transplant. Bacterial and virologic investigations were all negative. Computer tomography scan (CT scan) showed a mass measuring 1.7 cm in the left tonsillar region, compatible with an enlarged lymph node and other areas were unremarkable. Tonsillectomy was subsequently performed on day 108 post-transplant. Histology showed diffuse monomorphic proliferation of lymphoid cells. Immunohistochemical staining demonstrated positive B cells markers, and EBER was positive by in-situ hybridization. He was diagnosed to have localized early-onset EBV associated monomorphic PTLD.

In order to control PTLD, one of the strategies was reduction of immunosuppressants. Therefore, cyclosporine was withdrawn rapidly, whilst the dose of prednisolone was optimised in order to control chronic GVHD. The dose was slowly reduced over one year, guided by the activity of chronic GVHD. He was also given monthly immunoglobulin infusions as an immunomodulator. The patient is now in remission at nine years follow up without evidence of recurrence of PTLD.

Patient 2

This patient was asymptomatic and screened for ALD, as two elder brothers (deceased) were diagnosed with the condition. His VLCFA was found to be elevated at the age of two years. At the age of five years, he was found to have adrenal insufficiency, and commenced on hydrocortisone. Magnetic Resonance imaging (MRI brain) showed progressive white matter changes between the ages of seven and eignht years old. He also showed some early features of deterioration of psychological test results that prompted early hemoatopoietic stem cell transplantation (HSCT) at the age of eight years old.

He underwent a two-antigen mismatched unrelated UCBT in February 2005. He received conditioning with busulphan (16 mg/kg), cyclophosphamide (200 mg/kg) and antithymocyte globulin (90 mg/kg). Cyclosporine was used as GVHD prophylaxis. Engraftment was achieved on day 20 with complete donor chimerism. He developed grade II GVHD on day 17. Systemic steroid was added to cyclosporine treatment. This progressed to chronic skin GVHD which required steroid and cyclosporine treatment.

The patient developed recurrent episodes of fever, bilateral cervical lymphadenopathies, and hypotension and required fluid resuscitation and on two occasions, inotropes since fourteen months post-transplant. He was treated as sepsis with repeated courses of broad-spectrum antibiotics, and the doses of hydrocortisone were stepped up to stress dose level during these febrile episodes. Cervical lymphadenopathies and fever subsided after treatment. Microbiological investigations were all normal. PTLD was suspected following three similar episodes within two months. He was EBV seropositive before transplantation. His initial EBV DNA was 22000 copies/ml. Computerized tomography (CT) scanning of whole body showed generalized lymphadenopathies in the submandibular, submental, cervical, axillary, mediastinal, abdominal, pelvic and inguinal regions. There was also splenomegaly and bilateral pleural effusions. Cervical lymph node biopsy was performed and showed diffuse, polymorphic proliferation of lymphoid cells, positive for B-cell markers. EBER was positive on in-situ hybridization in 20% – 25% of lymphoid cells. This patient was diagnosed to have late onset EBV-associated polymorphic PTLD.

Cyclosporine was rapidly withdrawn. Rituximab (Rituxan, Genentech, Inc) 375 mg/m^2 was given weekly for 6 weeks. High dose acyclovir (500 mg/m^2 every 8 hours) was given for fourteen days, then reduced to prophylactic dose (250 mg/m^2 every 8 hours). The patient responded well, with resolution of fever, neck swelling, and a rapid reduction of EBV DNA. Ten months following diagnosis, EBV DNA level was undetectable in plasma. He was also given monthly immunoglobulin infusions as an immunomodulator. This patient remains well at three-year follow up without flare up of PTLD.

10.3 Discussion

EBV-associated PTLD is a well-recognised complication following solid organ transplantation and hematopoietic stem cell transplantation (HSCT) using bone marrow or peripheral blood stem cell sources, but rarely reported in umbilical cord blood transplant (UCBT) setting. It represents a heterogeneous group of abnormal lymphoid proliferation. Most cases are B-cell derived, and associated with Epstein-Barr virus (EBV) infection or reactivation. PTLD after allogeneic HSCT which is almost always of donor origin whilst PTLD developed after solid organ transplantation is generally originated from recipient B cells. In UCBT, it is believed that the donors are nearly all EBV-negative and, hence, the risk of EBV-related PTLD is low when compare with other forms of HSCT (135). At that time, our unit did not routinely monitor EBV DNA viral load in plasma due to the rarity of this disease in UCBT recipients.

Barker *et al* and Brunstein *et al* showed that the incidence of EBV-related PTLD in three hundreds and thirty five patients undergoing UCBT with myeloablative or nonmyeloablative conditioning regimens was 4.5% and was actually comparable with other HSCT settings. Unexpectedly, the risk in non-myeloablative regimen was higher than myeloablative regimen (3.3% in myeloablative group and 7.0% in non-myeloablative group). This finding was attributed to the use of antithymocyte globulin in conditioning regimen (136). In our centre, forty five UCBT were performed so far and only two patients with adrenoleukodystrophy developed PTLD. None of the other two hundreds and forty seven bone marrow or peripheral blood stem cell transplants developed this complication. PTLD usually presents in the first three months following transplantation, such a late presentation in patient 2 is unexpected and unprecedented. Delayed in T-cell immune reconstitution and

establishment of EBV-specific cytotoxic T-cell response in UCBT setting may play a role.

Haut *et al* demonstrate the presence of EBV genome in cord blood and make this a likely source of entry into host, or there is possibility of primary EBV infection after UCBT, reactivation of EBV in residual infected host cells or less likely via transfusion of blood products (137).

One of the most important treatment strategies in PTLD is reduction of immunosuppressants as clinical tolerated. A combination of surgical resection and reduction of immunosuppression was successful in inducing complete remission in patient 1 with localized disease.

Rituximab (Rituxan, Genentech, Inc.), a monoclonal antibody therapy (anti-CD 20) can induce complete remission in patients with early onset PTLD. However, early treatment algorithm for PTLD also includes antiviral therapy in an attempt to control EBV infection. It may be successful for treating EBV-associated PTLD with a significant lytic replication of EBV (138, 139). Holmes *et al* showed that the combination of intravenous immunoglobulin (IVIG) with antiviral agent with reduction of immunosuppressants resulted in termination of the process and induced a significant and persistent response, possibly by enabling EBV-specific T cell immunity. The exact mechanism of immunomodulation by IVIG in PTLD still remains to be determined. Holmes *et al* also reported that IVIG might be effective even in patients that did not respond to upfront antiviral therapy in solid organ transplant setting (140). Both of our patients received monthly IVIG as immunomodulatory agent. Patient 2 was treated with rituximab (Rituxan, Genentech, Inc.) and acyclovir. The EBV DNA concentration reduced rapidly once rituximab (Rituxan, Genentech, Inc.) was commenced. Beware that Rituximab (Rituxan,

Genentech, Inc.) was not yet licensed for use in B-cell non-Hodgkin lymphoma at the time when Patient 1 was first diagnosed with PTLD.

Pre-emptive therapy with rituximab (Rituxan, Genentech, Inc.) and regular monitoring of EBV reactivation in allogeneic HSCT results in improvement of outcome for high-risk patients of developing PTLD, with marked decrease in incidence of PTLD and also reduce the incidence of extensive disease and subsequently morbidity and mortality (141-143).

Due to relative small number of subjects described in our case illustration, it is difficult to draw any valid conclusion. But at least, our cases illustrate that: (i) EBV-related PTLD can be presented late in unrelated UCBT setting. Early clinical suspicion can lead to timely and prompt diagnosis; (ii) in contrast to reported literatures that PTLD is a serious post-transplant complication with unfavourable outcome, the clinical course can be relatively benign if treatment is started promptly. Routine EBV surveillance with pre-emptive treatment of anti-CD20 antibody can be used in high risk transplant setting to decrease the incidence of symptomatic EBV diseases.

10.4 Conclusion

EBV-related PTLD can present in recipients of unrelated umbilical cord blood transplantation. Routine EBV monitoring for early diagnosis with pre-emptive treatment may alter and improve patients' outcome.

Chapter 11 Use of Antiviral Agent as Prophylaxis: HHV-6 Encephalitis in Hematopoietic Stem Cell Transplant Recipients as an Illustration

11.1 Introduction

As we know, not all infectious diseases are vaccine-preventable and not all hosts are suitable for receiving vaccination. These infectious diseases can still have significant impact in term of morbidity and mortality. In the previous chapter, I have illustrated the impact of EBV reactivation in transplant setting. In this chapter, I will present the efficacy of using antiviral prophylaxis in preventing non-vaccine preventable infectious diseases in hematopoietic stem cell transplant setting. .

Human herpesvirus 6 (HHV-6) was first being isolated in peripheral blood of patients with acquired immune deficiency syndrome (AIDS) and other lymphoproliferative disorders in 1986 (79). Primary infection occurs in early childhood and has a life-long latency period (80). Reactivation of HHV-6 commonly occurs within the first thirty days of post-transplant period and may cause significant transplant-related morbidity and mortality (144).

Ganciclovir is a nucleoside analogue of guanosine. Through conversion to ganciclovir triphosphate, it competitively inhibits the incorporation of deoxyguanosine triphosphate by viral DNA polymerase and result in termination of elongation of viral DNA. This is the first antiviral agent that is shown to be effective in treatment of cytomegalovirus (CMV) disease in humans. In tissue culture, ganciclovir has excellent antiviral activity against different herpes group of viruses including herpes simplex virus type 1, herpes simplex virus type 2, varicella-zoster virus, Epstein-Barr virus and HHV-6 (145). Most patients diagnosed HHV-6 encephalitis are treated with ganciclovir and/or foscarnet. The treatment outcome remains grave despite there is evidence of clearance of HHV-6 DNA in CSF and

plasma samples after antiviral therapy (81).

There are evidences to suggest antiviral prophylaxis may prevent HHV-6 reactivation in bone marrow transplant recipients (146). Our unit adopted the policy to use ganciclovir as HHV-6 prophylaxis during conditioning for all unrelated hematopoietic stem cell transplant (HSCT) since January, 2002.

In this study, we evaluate the effect of early administration of ganciclovir on HHV-6 encephalitis in pediatric unrelated HSCT with comparison to historic controls.

11.2 Subjects and Methods

Patients

The study was performed in our university teaching hospital in Hong Kong, which is also a tertiary referral center for pediatric cancers and hematopoietic stem cell transplantation in the local region. Clinical and laboratory data of patients who underwent unrelated allogeneic HSCT from January, 2000 to September, 2008 was reviewed. From the year 2000 onwards, our virology laboratory routinely performed HHV-6 PCR in cerebrospinal fluid (CSF) samples in all immunocompromised patients with suspected central nervous system infection.

HHV-6 DNA detection

The presence of HHV-6 DNA was detected by a nested PCR (screening PCR) based on primer sets H6-6/H6-7 and NH-6/NH-7 targeting the major capsid protein gene. The primer sets used in the screening PCR were shown to be consensus and carry the same analytic sensitivity in revealing variants A and B. All HHV-6 screening PCR-positive samples were confirmed by another nested PCR (typing PCR) targeted at the large tegument protein gene. The typing PCR allowed the characterization of HHV-6 into variants A and B by restriction fragment length analysis using Hind III. The PCR product of variant A did not contain any Hind III restriction site whereas the 163-bp PCR product of variant B would be digested into fragments of 97 and 66 base pairs. This Hind III restriction site was presented at position 2945 of the large tegument protein gene of HHV-6B, but not in HHV-6A, had been used to discriminate between the two variants. The detail methodology was described by Chan *et al* (147).

Conditioning regimen and graft-versus-host disease (GVHD) prophylaxis

Conditioning protocols on pretreatment conditioning regimens and graft-versus-host disease (GVHD) prophylaxis following HSCT used for our patients were accorded to previous publicatons. In brief, patients with relapsed ALL would receive TBI-based regimen, relapsed AML and non-malignant condtions would receive busulfan and cyclophosphamide-based regimen. Graft-versus-host disease (GVHD) was diagnosed and scored according to standardized criteria.(148) All patients would receive cyclosporine as prophylaxis from day -1 for malignant conditions, whereas for non-malignant conditions, patients would receive methotrexate and cyclosporine as GVHD prophylaxis.

Criteria of neutrophil engraftment

Day of neutrophil engraftment was defined as the first day of absolute neutrophil count (ANC) of $\geqq$ 0.5 x 10^9/L for three consecutive days.

Case Definition of HHV-6 encephalitis

Case definition of HHV-6 encephalitis was defined as the presence of neurological manifestation namely (i) change of conscious level, including unexplained lethargy and irritability; (ii) change of personality or behavior that persisted for more than 24 hours in conjunction with detection of HHV-6 DNA in cerebrospinal fluid (CSF) samples by polymerase chain reaction (PCR) as previously described and in the absence of any other identifiable etiologies. All cerebrospinal fluid (CSF) specimens had also undergone standard microbiological and virologic examinations to rule out Herpes simplex virus (HSV) 1 and 2, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Human herpesvirus 7 (HHV-7) and Varicella zoster virus (VZV) infections. Other non-infective causes of acute encephalopathy in transplant setting were excluded by electroencephalogram, cranial imaging, measurement of serum glucose, electrolytes and cyclosporine levels.

Virus monitoring and antiviral prophylaxis

Due to a relatively high prevalence of HHV-6 encephalitis in the first 2 years of study period (2 cases out of 16 unrelated HSCT), from January 2002 onward, our unit adopted the policy of using ganciclovir 5 mg/kg twice daily intravenously from day -7 to day -1 during conditioning as prophylaxis against reactivation of HHV-6 for all unrelated HSCT. Acyclovir prophylaxis (250 mg/m^2 every eight hours intravenously) would be given to recipients from day 1 till day 21 who were or whose donors were seropositive for HSV or there were past evidences of HSV infection. Reactivation of CMV was monitored by weekly CMV pp65 antigen assay. Weekly HHV-6 PCR quantitative monitoring was not our unit policy during the study period. Preemptive treatment with ganciclovir (5mg/kg twice daily intravenously for two weeks) would be started if there were evidences of CMV reactivation. There was no change in policies about conditioning regimens and GVHD prophylaxis during the study period. The prevalence of HHV-6 encephalitis was compared before and after the adoption of this policy.

Antiviral treatment for HHV-6 encephalitis

Patients fulfilled the criteria of HHV-6 encephalitis would be treated with ganciclovir (5 mg/kg twice daily intravenously). Patients developed intolerable side effects, namely severe bone marrow suppression or showed no clinical improvement after 72 hours of commencement of neurological states, would switch to foscarnet (60 mg/kg three times daily intravenously). Renal function and electrolytes were monitored serially during the course of foscarnet. Lumber puncture was repeated to monitor the progress of disease and document clearance of virus in CSF. Antiviral therapy would continue for at least 14 days and the treatment endpoint was defined as

clearance of HHV-6 DNA in CSF.

11.3 Results

One hundred and sixteen allogeneic HSCT, which included fifty four unrelated HSCT were performed from January 2000 to September 2008. Unrelated UCB was used as the stem cell source in thirty one cases and the other twenty three cases were from bone marrow (n=19) or peripheral blood stem cells (n=4). Primary diseases for unrelated transplant were acute lymphoblastic leukemia (n=19); acute myeloid leukemia (n=12); other leukemia and myelodysplastic syndrome (n=8); miscellaneous (n=15). The details of the transplant were summarized in Table 14.

Four cases (7.4%) met the diagnostic criteria of HHV-6 encephalitis during the study period. All of them were due to variant B. They were diagnosed to have hematological malignancies and received unrelated umbilical cord blood (UCB) transplantation. The recipients were all seropositive to HHV-6 before transplantation. When compared with historic controls, before the adoption of HHV-6 prophylaxis policy, 12.5% (2 out of 16) unrelated HSCT recipients developed HHV-6 encephalitis and 5.3% (2 out of 38) developed HHV-6 encephalitis after introduction of pre-transplant ganciclovir prophylaxis. If limited to unrelated UCB transplantation, the incidences of HHV-6 encephalitis were 40% and 7.7% before and after ganciclovir prophylaxis respectively. During the same period, no patient who received unrelated bone marrow or peripheral blood stem cell transplantation developed HHV-6 encephalitis.

In our 4 cases, the age ranged from 1.3 years to 9.4 years old (median 7.2 years old). They all received cyclosporine A as GVHD prophylaxis. The most common presentations of HHV-6 encephalitis were depressed conscious state, abnormal behavior, sleep disturbance and seizure. Unexplained hypertension and hyponatremia were also commonly observed in our series. They presented at median 19 days after transplantation (range 18-21 days). All of them showed evidence of bone marrow suppression with marked lymphopneia at presentation. Nearly all patients had normal CSF microbiological and biochemical findings despite being HHV-6 PCR positive. Other non-infective causes of acute encephalopathy in transplant setting were excluded by electroencephalogram, cranial imaging, measurement of serum glucose, electrolytes and cyclosporine levels. They were treated with antiviral agents as described. Lumber puncture was repeated in patient 1, 3 and 4. (Patient 2 was critically ill and was unable to have a repeat lumber puncture done)

HHV-6 was cleared at median of 17 days after starting of antiviral therapy. Despite treatment and demonstration of clearance of HHV-6 PCR after treatment, the outcome remained grave with 50% of cases complicated with significant and persistent neurological deficit after the infection was in control. The others were died from other transplant-related mortalities. The clinical features and outcome of the 4 cases are summarized in Tables 15 and 16.

Table 14. Hematopoietic stem cell transplantation from January, 2000 to September 2008

Type of HSCT	Related*	Unrelated	Total
January, 2000 – December, 2001			
Bone Marrow (BM)	1	11	12
Peripheral blood stem cell (PBSC)	7	0	7
Umbilical cord blood transplant (UCBT)	0	5	5
Subtotal	8	16	24
January, 2002 – September, 2008			
Bone Marrow (BM)	11	8	19
Peripheral blood stem cell (PBSC)	38	4	42
Umbilical cord blood transplant (UCBT)	5	26	31
Subtotal	54	38	92
Total	62	54	116

* HLA-identical siblings and mismatched family member donors

Table 15. Baseline patient characteristics with HHV 6 encephalitis

Patient	Sex / Age (years)	Primary disease	Stem cell source	Conditioning regimen	GVHD prophylaxis	Pre-transplant ganciclovir	HHV-6 Serostatus	Day of engraftment	Overall grading of GVHD
1	M / 9.4	*ALL #CR 4	2Antigen-Mismatch Unrelated UCB Transplant	TBI 9 Gy + Fludarabine 30 mg/m^2 x 4 days + Melphalan 140 mg/m^2	CSA^	No	Positive	19	II
2	F / 6.2	**JMML	1Antigen-Mismatch Unrelated UCB Transplant	Busulphan 16 mg/kg/4 days + Cyclophosphamide 60 mg/kg x 2 days + Melphalan 140 mg/m^2	CSA	No	Positive	- (non-engraftment)	-
3	F / 8.2	ALL ##CR2	2Antigen-Mismatch Unrelated UCB Transplant	TBI 1440cGy + Cyclophosphamide 60 mg/kg x 2 days	CSA	Yes	Positive	33	II
4	M / 1.3	ALL CR2	2Antigen-Mismatch Unrelated UCB Transplant	Busulphan 16 mg/kg/4 days + Etoposide 40 mg/kg + Cyclophosphamide 60 mg/kg x 2 days	CSA	Yes	Positive	13	II

*Acute lymphoblastic leukemia; ** Juvenile Myelomonocytic leukemia; # 4th complete remission'; ## 2nd complete remission; ^ cyclosporine A

Table 16. Outcome of HHV 6 encephalitis infection in hematopoietic stem cell transplantation

Patient	Day of onset (post-transplant)	Peripheral blood count	Immunosuppressant's	CSA trough level (μg/L)	Presentations	CSF cell counts and biochemistry	Treatment	Outcome
1	21	[#]Hb 8.9 [a]WBC 3.0 [b]ANC 2.0 [c]LYM 0.3 [d]PLT 26.0	MP* + CSA**	235	Abnormal behaviour; Seizure; Hyponatremia;	Traumatic tap	Ganciclovir 3 weeks	Alive; Epilepsy; Now 8 years post-transplant
2	20	Hb 9.7 WBC 0.3 ANC 0.2 LYM 0.1 PLT 88.0	MP + CSA	190	Depressed conscious level; Sleep disturbance; Euphoria, Generalised seizure	WBC 1/mm^3; RBC 3/mm^3; Total protein 0.24g/L	Foscarnet 2 weeks (treatment terminated because of renal impairment)	Died on day 38 (non-engraftment, refractory seizure, pneumonia, gastrointestinal bleeding)
3	19	Hb 9.0 WBC 0.2 ANC 0.1 LYM 0.0 PLT 30.0	MP + CSA	217	Fever; Headache; Hypertension; Abnormal behaviour; Hyponatremia; Seizure	WBC 1/mm^3; RBC 2/mm^3; Total protein 0.15g/L	Foscarnet 2 weeks + ganciclovir 2 weeks	Progression of clinical symptoms despite on foscarnet and ganciclovir. Died on day 61 (intracranial bleed)
4	18	Hb 7.0 WBC 1.4 ANC 0.8 LYM 0.0 PLT 3.0	MP + CSA	163	Fever; Focal seizure; Loss of memory; Abnormal behaviour; Hypertension	WBC 2/mm^3; RBC 5/mm^3; Total protein 0.23 g/L	Ganciclovir 2 weeks + Foscarnet 3 weeks	Alive; Refractory epilepsy; Developmental delay

* methylprednisolone; ** cyclosporine A; [#] hemoglobulin, g/dL; [a] white blood cell count, x 10^9/L; [b] absolute neutrophil count, x 10^9/L, [c] lymphocyte count, x 10^9/L, [d] platelet count, x10^9/L

11.4 Discussion

After first being isolated in peripheral blood of HIV infected patients in 1986, HHV-6 has been a well-known pathogen in allogeneic HSCT recipients (79, 149). HHV-6 encephalitis is one of the most significant and serious clinical manifestations of reactivation of HHV-6 infection in HSCT recipients (149, 150). HHV-6B is now established as the cause of exanthema subitum (roseola infantum). In our case series, all of them were due to variant B reactivation. HHV-6A rarely causes infections in infants in the western world but it is shown to be the predominant HHV-6 variant associated with viremic infant infections in the Sub-Sarharan African population (151).

The clinical spectrum of HHV-6 reactivation ranges from asymptomatic viremia, unexplained fever, skin rash, pneumonitis, hepatitis, myelosuppression with delay in neutrophil and platelet engraftment, to graft failure. Due to the neurotropic nature of HHV-6, encephalitis is the most severe form of direct organ damage caused by HHV-6 infection or reactivation (152). With the known predilection to involve the temporal lobe and hippocampi, follow by the amygdala or parahippocampal gyrus, patients usually develop symptoms compatible with acute hippocampal dysfunction namely confusion, abnormal behavior, sleep disturbance and loss of short-term memory. Therefore, if the above mentioned symptoms persist and cannot be explained by other

obvious causes, patients warrant further investigation in the direction of HHV-6 infection (153).

In allogeneic HSCT setting, 25% of recipients develop a wide spectrum of neurological symptoms relate to various infectious agents, drug toxicity and metabolic abnormalities. Moreover, the demonstration of HHV-6 by PCR in asymptomatic subjects may further complicate the picture. Therefore, it is not recommended to test routinely in asymptomatic subjects (154, 155). As in our series, all patients showed persistent unexplained neurological manifestations and apart from presence of HHV-6 in CSF, they were fully evaluated to exclude other possible causes of acute change of conscious state before the diagnosis of HHV-6 encephalitis were made.

After the first successful umbilical cord blood (UCB) transplantation performed in 1989, this has been widely performed in a variety of hematological malignancies, bone marrow failure syndromes and inborn errors of metabolism (156). UCB transplantation has the advantages of allowing a higher degree of HLA disparity between host and graft, with lower incidence and less severe graft-versus-host disease (GVHD). Therefore, UCB provides a readily available source of stem cells with similar survival outcomes as compare with transplant using bone marrow or GCSF mobilized peripheral blood as stem cell source (157). Compare with patients transplanted from matched unrelated bone marrow or peripheral stem cell donors,

UCB transplant recipients have a lower incidence of acute and chronic GVHD, but hematopoietic recovery is delayed, the probability of sustained donor engraftment is less and infectious complications are higher especially in the early post-transplant period. This increased risk of fatal infections is mainly due to the slow neutrophil recovery, transfer of antigen-naïve T cells to recipients with lack of antigen-experienced (memory) T cells in UCB. In fact, memory T cells significantly contribute to early immunological reconstitution of patients after unmanipulated allogeneic bone marrow or peripheral blood stem cell transplant (158). In our series, all cases of HHV-6 encephalitis occurred in patients who received unrelated UCB transplantation. From the experience of CMV infection, CMV seropositive donors may provide additional survival benefit in CMV positive recipients due to transfer of donor-derived immunity; a similar situation may occur in HHV-6 infection (159).

In our series, nearly all infected patients demonstrated normal CSF white cell count and protein level. This might be due to defect in mounting sufficient immune response to invading pathogens in severely immunocompromised patients and sampling of CSF took place at early stage of disease. It was supported by the peripheral blood count at presentation. All of them showed evidence of bone marrow suppression with marked lymphopenia at presentation. Therefore, normal CSF microbiological and biochemical findings did not exclude the diagnosis of

encephalitis in immunocompromised patients. Moreover, in patients with unexplained hypertension or hyponatremia, CNS causes need to be seriously considered as 50% of our cases had persistent hypertension or hyponatremia at the time of presentation.

Overall, HHV-6 encephalitis is still an uncommon but serious complication after HSCT and the prognosis remains very poor. Post-transplant pre-emptive ganciclovir treatment based on the periodic quantitative HHV-6 plasma DNA viral load monitoring is practised in some centers but due to the dynamic kinetics of plasma HHV-6 viral load with published cases of HHV-6 encephalitis develop symptoms even before detection of high level HHV-6 DNA in plasma, the value of pre-emptive antiviral therapy in termination in progression to full blown disease manifestation is still not fully proven (160). In view of the significant morbidity and mortality of HHV-6 encephalitis experienced in our series, prophylactic ganciclovir is used for 7 days during conditioning to reduce the HHV-6 viral load and thus decrease the chance of post-transplant reactivation. Though the incidence of HHV-6 encephalitis appeared to decrease after ganciclovir prophylaxis, we could not prevent two cases in the post-prophylaxis era. Therefore, the exact role of prophylaxis still remains controversial. The future direction will be a prospective randomized controlled trial to formally evaluate the role of antiviral prophylaxis in prevention of HHV-6 reactivation.

Currently routine antiviral prophylaxis for HHV-6 infection is not recommended in HSCT recipients due to low risk of infection in reported series and the significant toxicity of available antiviral agents (161). Whether there is a role of antiviral prophylaxis in the highest risk group, namely unrelated UCB transplant recipients still needs to be evaluated by randomized controlled trial.

One of the main limitations of our study is the lack of plasma DNA profile to correlate the outcome since all the cases were managed before the era of routine quantitative HHV-6 plasma viral load monitoring. Due to the dynamic nature of plasma viral load and lack of correlation among plasma viral load, clinical presentation and outcome, the exact role of regular monitoring remains to be determined.

11.5 Conclusions

In conclusion, HHV-6 encephalitis is still an uncommon but serious complication in unrelated hematopoietic stem cell transplantation. Routine use of pre-transplant ganciclovir prophylaxis in all transplant setting is still not justified based on current data but may have a role in high-risk transplant, namely unrelated umbilical cord blood transplantation.

Chapter 12 Lymphoproliferative Response to Herpes Simplex Virus Type 1, Cytomegalovirus, Epstein-Barr Virus, Varicella Zoster Virus, Human Herpes Virus 6, 7 and 8 antigens stimulation in Pediatric Allogeneic Stem Cell Transplant Recipients.

12.1 Introduction

Hematopoietic stem cell transplantation (HSCT) is now a curative treatment modality for many hematological malignancies, immunodeficiency syndromes, inborn errors of metabolism and hemoglobinopathies. In the modern era of stem cell transplantation with the introduction of umbilical cord blood transplantation and T-cell depleted haploidentical stem cell transplantation from related family donors, nearly all clinically suitable patients can proceed to transplantation if there is a medical indication (57). A major cause of transplant-related mortality is delayed T-cell reconstitution that can result in severe and even life-threatening viral infections. The median time for T-cell recovery is seven to eight months in the setting of CD34+ selected graft with myeloablative conditioning regimen (162).

Reactivation or less commonly primary infections of the herpes group of viruses can cause substantial morbidity and mortality in allogeneic stem cell transplant recipients. Each herpes virus has a unique temporal profile of reactivation. Herpes simplex virus type 1 (HSV-1), Epstein-Barr virus (EBV) and human herpes virus-6 (HHV-6) are detected more frequently in the first 30 days post-transplant whereas varicella zoster virus (VZV) and cytomegalovirus (CMV) tend to be reactivated later

in post-transplant period. In contrast, human herpes virus 7 (HHV-7) reactivation which may manifest as unexplained fever, non-specific rash, delayed engraftment or even encephalitis, does not show consistent temporal predilection (149).

Cell-mediated immunity (CMI) is critical for effective immunity and plays an important role in long-term protection against various viral infections in post-transplant period. The effect of new transplant strategy and the more intensified conditioning regimen on the recovery of CMI to these viruses remains unanswered.

In this chapter, we use a well-established *in vitro* approach to prospectively evaluate the CMI to mitogens and various specific recall antigens derived from herpes simplex virus type 1 (HSV-1), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpes virus-6, 7, 8 (HHV-6,7,8) in allogeneic stem cell transplant recipients within the first 12 months transplant period.

12.2 Subjects and methods

Patient selection

This study included children who underwent allogeneic HSCT in our Lady Pao Children's Cancer Centre, Department of Pediatrics of the Prince of Wales Hospital from 2001 to 2004. The study protocol was approved by the Joint CUHK-NTEC clinical research ethics committee. Written consents were obtained from parents and/or patients before enrollment according to the principles of the Declaration of Helsinki.

Conditioning regimen and graft-versus-host disease (GVHD) prophylaxis

Management protocols on pre-transplant conditioning regimens and graft-versus-host disease (GVHD) prophylaxis following HSCT used for our patients were accorded in previous publications.(163, 164) GVHD was diagnosed and scored according to standardized criteria.(148) In brief, patients with relapsed ALL would receive TBI-based regimen, relapsed AML and non-malignant conditions would receive busulfan and cyclophosphamide-based regimen. Graft-verusus-host disease (GVHD) prophylaxis consisted of cyclosporine from day -1 till day 60 and would slowly taper till day 180 if there was no evidence of chronic GVHD in malignant conditions whereas non-malignant conditions would consist of cyclosporine and methotrexate at day 1, 3, 6 and 11 and would taper from day 180 if no evidence of

GVHD.

Criteria of neutrophil engraftment

Day of neutrophil engraftment was defined as the first day of absolute neutrophil count (ANC) of $\geqq$ 0.5 x 10^9/L for three consecutive days.

Virus monitoring and antiviral prophylaxis

Acyclovir prophylaxis (250 mg/m^2 every 8 hours intravenously) would be given to patients from day 0 till day 21 post-transplant if either the recipients or the donors were seropositive for herpes simplex virus type 1 (HSV-1) or in whom there was a past history of documented HSV-1 infection. Reactivation of CMV was monitored weekly by measuring the level of CMV pp65 antigen in peripheral blood mononuclear cells (PBMC) from day 0 till day 100 post-transplant. Ganciclovir (5mg/kg/day 3 days per week) would be given from day of engraftment till day 100 as cytomegalovirus (CMV) prophylaxis in unrelated donor transplants if recipients or donors were seropositive for CMV. Preemptive treatment with ganciclovir (5mg/kg/dose twice daily intravenously for at least 2 weeks) would be started if there was evidence of CMV reactivation.

All unrelated and HLA-mismatched transplant recipients would receive intravenous immunoglobulin (IVIG) at 500 mg/kg every 2 weeks from day 1 till day 100 as immunomodulatory agent.

Assessment of recovery of cell-mediated immunity by lymphoproliferative assay

Recovery of cell-mediated immunity (CMI) was evaluated by the in-vitro proliferative responses of peripheral blood mononuclear cells (PBMC) to specific purified HSV-1, VZV (Microbix Biosystems, Toronto, ON, Canada), CMV, EBV, HHV-6, HHV-7, HHV-8 (cell culture lysate derived) antigens and mitogens including phytohaemagglutinin (PHA, Gibco BRL, Grand Island, NY, USA), concanavalin A (ConA), pokeweed mitogen (PWM) as positive control.

In brief, PBMC was obtained from heparinized venous blood using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation and washed in RPMI-1640 medium (Celox Laboratories, Inc., St. Paul, MN) supplemented with 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 8% fetal calf serum (Life Technologies, Gaithersburg, MD). The viability of isolated cells ranged from 95% - 98% as determined by the trypan blue exclusion test.

PBMC were washed twice in RPMI-1640 medium and resuspended at a concentration of 2 x 10^6/ml of RPMI-1640, supplemented with penicillin-streptomycin (100 U/ml), 5% autologous serum and 1 mM sodium pyruvate. Aliquots (200 μL) of PBMC containing $5x10^4$ cells were incubated in triplicates in flat-bottom 96-well microtitre tissue culture plate (Costar, Cambridge, MA) with 10 μg/mL PHA, ConA and PWM for 3 days as positive control. A total of $2x10^5$ cells

were cultured separately with HSV-1 and 5 μL of VZV, EBV, HHV-6,7 and 8 antigens for 6 days in 5% CO_2 at 37°C.

Cell cultures were pulsed with [^{3}H]-thymidine (Pharmacia) for 18 hours at the end of incubation, and radioactivity in the samples was then measured by a scintillation counter (Microbeta TriLux; EG & G Wallac, Turku, Finland). Three replicates of counts per minutes (cpm) values for the unstimulated PBMC and three replicates each for PBMC stimulated with HSV-1, CMV, EBV, HHV-6, 7 and 8 antigens. The median cpm for unstimulated PBMC, as well as for PBMC stimulated with various antigens were determined. Results were expressed as stimulation index (SI) that was defined as the ratio of the median cpm in the stimulated samples divided by the cpm in the unstimulated samples. SI $\geqq$ 3 was regarded as positive lymphoproliferative response.(165)

Serial measurements were made at baseline (before transplant), at monthly intervals from the first to sixth month post-transplant and then 3-monthly intervals until 12 months post-transplant for HSV-1, CMV, VZV and HHV-8 antigens. EBV, HHV-6 and HHV-7 were monitored until 5 months after transplantation.

In order to minimize the variability in antigen titers between different batches of viruses, we used a single batch of viruses for LPR monitoring on all subjects throughout the study period.

Statistical analysis

The primary measured outcome was the CMI (lymphoproliferation as measured by stimulation indices) induced by various herpes virus antigens at different time period after HSCT.

Data were descriptively summarized using frequencies and percentages for all categorical variables. Continuous variables were expressed as median and interquartile range (IQR). Stimulation indices between different subgroups were analyzed using Mann-Whitney test. Categorical variables between different diseases or patient groups were analyzed by Chi-square test. *P*-values <0.05 were considered to be statistically significant. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA).

12.3 Results

Characteristics of study population

From 2001-2004, 36 children (M=19; F=17) were recruited. The age ranged from 1.03 – 17.9 years, median 10.5 years old. Malignant conditions (ALL, AML, CML and other hematological malignancies) accounted for 58.3% of cases. Altogether 52.7% of donors were matched sibling donors (MSD). Matched unrelated donors (MUD) accounted for 27.8% of cases. The others received transplants from mismatched family donors (5.6%) and cord blood (13.9%; 1 related, 4 unrelated). Peripheral blood (PBSC), bone marrow (BM) and unrelated cord blood (CB) as the stem cell sources were used in 55.5%, 30.6% and 13.9% of cases, respectively. The mean CD3+ cell dose in PBSC (31.7 x 10^7/kg) were significantly higher than BM (4.2 x 10^7/kg) and CB (1.1 x 10^7/kg) respectively (p=0.009; p=0.03). The mean CD34+ cell dose in PBSC (5.1x10^6/kg) were significantly higher than BM (3.5x10^6/kg, p=0.03) and CB (0.23x10^6/kg, p=0.004) respectively. 16.7% and 9.6% of patients developed severe acute GVHD (grade III/IV) and extensive chronic GVHD, respectively. The 3-month and 12-month survival rate were 86.1% and 71.0% respectively. The characteristics of study patients are shown in Table 17.

Lymphoproliferative response to HSV-1, VZV, CMV, EBV, HHV-6,7 and 8 in study subjects

For analysis on HSV-1, VZV and CMV responses, we further categorized the results according to the serostatus of the donors (D) and recipients (R). For VZV, the number of D+R+, D+R-, D-R+ and D-R- was 17, 8, 7 and 4 respectively. For CMV, the number of D+R+, D+R-, D-R+ and D-R- was 14, 9, 8 and 5 respectively. For HSV-1, the number of D+R+, D+R-, D-R+ and D-R-was 17, 8, 8 and 3 respectively.

Fifty percent of subjects showed SI $\geqq$ 3 to HSV-1 at 2 months after transplantation, an upsurge of median stimulation index (SI) was observed at 5 months after transplant. In subgroup analysis, D+R+ subjects demonstrated the most significant increase in lymphoproliferative response (LPR).

More than half of subjects showed SI index $\geqq$ 3 to CMV at 2 months after transplantation and an upsurge was observed at 5-6 months after transplant. D+R+ subgroups showed the most significant response.

At 4 months post-transplant, more than 50% of patients showed positive SI index to VZV antigen and there was an upsurge observed at 6 months post-transplant. Again, D+R+ showed the most prominent response in LPR.

Although half of the patients showed positive response (SI index $\geqq$ 3) to EBV, HHV-6 and HHV-7 before transplant, the median SI indices were less than 3 to these herpes viruses indicating most of the patients did not show a significant LPR to these antigens during first 6-month post-transplant period.

For HHV-8, only 6.5% subjects showed SI $\geqq$ 3 at baseline and there was no significant lymphoproliferative response to HHV-8 antigen throughout the study period. The details of lymphoproliferative response to various herpes viruses are shown in Tables 18, 19 and Figures 3 and 4A, B and C respectively.

At 3-month post-transplant, patients with matched sibling donor (MSD) transplant had significantly higher stimulation indices (SI) to HSV-1, CMV, EBV, HHV-7 and HHV-8 when compared with patients with matched unrelated donor (MUD) transplant. Patients who received peripheral blood stem cell (PBSC) as the stem cell source also showed higher stimulation indices (SI) to HSV-1, VZV and EBV when compared with patients who received bone marrow (BM). The use of ant-thymocyte globulin (ATG) and total body irradiation (TBI) and severity of acute GVHD did not pose any significant effect on lymphoproliferative response to herpes virus antigen at 3-month post-transplant. At 12-month post-transplant, there was no statistical difference in any parameters in affecting LPR to different herpes viruses. The details are shown in Table 20.

Viral reactivations during the study period

During the study period, 5 patients developed reactivation of CMV virus which required pre-emptive ganciclovir treatment for controlling viral replication at median 4.5 months post-transplant. Three patients developed HSV-1 mucositis. All of them recovered after treatment with appropriate antiviral therapies. There was no virologic-confirmed CMV, EBV, HHV6, 7 and 8 diseases throughout the study period.

Table 17. Characteristics of study patients

Parameters	Number of patients (n=36)
Sex	
Male / Female	19 / 17
Primary Disease for Transplant	
ALL	10 (27.7%)
CR1	2
CR2 / CR 3	7 / 1
AML	5 (13.9%)
CR1 / CR 2	1 / 4
Severe aplastic anemia	6 (16.7%)
Other leukemia/lymphoma (JMML, CML, T-cell lymphoma)	6 (16.7%)
Immunodeficiency syndrome	3 (8.3%)
Hemoglobinopathy	4 (11.1%)
Metabolic disease	2 (5.6%)
Overall survival (OS)	
3-month post-transplant	31 (86.1%)
12-month post-transplant	22 (71.0%)
Cause of death at 3-month post-transplant (n=5)	
Relapse	1 (20.0%)
Transplant-related mortality (TRM)	4 (80.0%)
Cause of death at 12-month post-transplant (n=9)	
Relapse	6 (66.7%)
Treatment-related mortality (TRM)	3 (33.3%)
Type of Transplantation	
Matched sibling donor (MSD)	19 (52.7%)
Matched unrelated donor (MUD)	10 (27.8%)
Mismatched family donor (MMF)	2 (5.6%)
Unrelated Cord blood	4 (11.2%)
Related Cord blood	1 (2.7%)
Conditioning regimen	
Total body irradiation (TBI)	10 (27.7%)
No TBI	26 (72.3%)
Anti-thymocyte globulin (ATG)	20 (55.5%)

No ATG	16 (44.5%)
Stem Cell Source	
Bone Marrow (BM)	11 (30.6%)
Peripheral blood stem cell (PBSC)	20 (55.5%)
Unrelated Cord blood	4 (11.2%)
Related Cord blood	1 (2.7%)
Mean Graft CD3+ count	
Bone Marrow (BM)	4.2×10^7/kg
Peripheral blood stem cell (PBSC)	31.7×10^7/kg
Cord blood (CB)	1.1×10^7/kg
Mean Graft CD34+ count	
Bone Marrow (BM)	3.5×10^6/kg
Peripheral blood stem cell (PBSC)	5.1×10^6/kg
Cord blood (CB)	0.23×10^6/kg
Acute GVHD	
No acute GVHD	11 (30.6%)
Grade I/II	19 (52.7%)
Grade III/IV	6 (16.7%)
Chronic GVHD (n=31)	
No	23 (74.3%)
Limited	5 (16.1%)
Extensive	3 (9.6%)

Table 18. Lymphoproliferative response (SI) to HSV-1, CMV, VZV and HHV-8 at different time points after HSCT.

	Con A#	HSV-1		CMV		VZV		HHV-8	
Time after HSCT	Median (IQR)*	Median (IQR)*	SI≧3 (%)**	Median (IQR)	SI≧3 (%)	Median (IQR)	SI≧3 (%)	Median (IQR)	SI≧3 (%)
Baseline (pre-transplant)	189.0 (56.8-376.3)	15.0 (2.1-47.7)	71.4%	16.4 (3.8-39.5)	80.6%	16.2 (4.4-55.9)	77.8%	1.1 (0.9-1.8)	6.5%
1 month	29.0 (5.0-74.0)	2.3 (0.5-7.8)	50.0%	2.1 (0.75-11.2)	31.7%	1.5 (0.4-3.4)	26.7%	0.9 (0.7-1.4)	0.0%
2 months	40.0 (6.0-94.0)	3.6 (0.9-14.3)	53.6%	3.7 (1.1-9.1)	56.7%	1.3 (0.7-6.3)	32.3%	1.1 (0.7-1.7)	3.8%
3 months	50.0 (19.5-153.8)	3.6 (1.2-26.9)	59.3%	3.1 (0.5-33.9)	51.7%	2.4 (0.7-8.9)	45.2%	1.0 (0.8-1.7)	8.3%
4 months	58.0 (16.0-101.5)	5.7 (1.6-40.2)	72.7%	5.1 (2.4-28.7)	72.7%	2.6 (1.2-8.8)	50.0%	1.3 (1.0-1.5)	0.0%
5 months	69.0 (41.3-109.0)	5.7 (2.0-95.3)	65.2%	8.9 (3.6-137.4)	77.8%	14.4 (2.0-110.9)	66.7%	1.3 (1.0-1.8)	0.0%
6 months	137.0 (41.8-197.0)	21.6 (4.6-89.5)	87.5%	3.7 (1.3-53.7)	54.5%	5.2 (2.2-17.3)	70.8%	1.0 (0.6-1.3)	4.5%
9 months	127.0 (42.0-263.0)	49.5 (19.9-139.1)	81.0%	19.8 (5.4-68.4)	81.8%	15.2 (2.7-83.1)	72.7%	1.0 (0.8-1.6)	4.8%
12 months	178.0 (111.0-350.0)	45.1 (4.4-228.5)	85.7%	25.5 (3.6-40.9)	76.1%	22.1 (4.4-78.4)	76.2%	1.5 (0.9-2.1)	25.0%

#Concanavalin A; *Interquartile range (1st quartile – 3rd quartile); **SI, stimulation index; ≧3 is defined as positive

Table 19. Lymphoproliferative response (SI) to EBV and HHV-6, HHV-7 at different time points after HSCT.

	Con A	EBV		HHV-6		HHV-7	
Time after HSCT	Median (IQR)*	Median (IQR)	SI≧3 (%)**	Median (IQR)	SI≧3 (%)	Median (IQR)*	SI≧3 (%)
aseline (pre-transplant)	189.0 (56.8-376.3)	2.9 (1.4-12.6)	50.0%	2.8 (1.0-9.1)	50.0%	5.9 (1.8-20.4)	57.9%
1 month	29.0 (5.0-74.0)	1.2 (0.4-3.5)	28.6%	1.5 (0.6-6.4)	28.6%	0.9 (0.3-2.8)	20.0%
2 months	40.0 (6.0-94.0)	1.3 (0.5-2.1)	9.1%	1.1 (0.8-4.1)	27.3%	1.4 (0.5-3.8)	30.7%
3 months	50.0 (19.5-153.8)	1.5 (0.7-3.5)	30.0%	1.5 (1.0-26.5)	31.6%	1.5 (0.9-2.9)	23.15
4 months	58.0 (16.0-101.5)	1.6 (1.1-2.1)	0.0%	1.5 (0.5-3.4)	16.7%	1.5 (0.9-4.0)	22.2%
5 months	69.0 (41.3-109.0)	1.2 (0.4-2.8)	14.3%	1.5 (0.3-11.2)	16.7%	0.6 (0.4-2.6)	0.0%

*Concanavalin A; *Interquartile range (1st quartile – 3rd quartile); **SI, stimulation index; ≧3 is defined as positive

Table 20. Lymphoproliferative response (SI) of herpes viruses in different transplant settings at 3-month and 12-month post-transplant

	HSV-1		CMV		EBV		VZV		HHV-6		HHV-7		HHV-8	
	3-month	12-month	3-month	12-month	3-month	12-month	3-month	12-month	3-month	12-month	3-month	12-month	3-month	12-month
MSD (n=18)	4.10*	5.20	5.00	6.50	3.00	ND	2.90	6.70	1.50	ND	1.50	ND	1.30	3.50
MUD (n=12)	0.40 (p<0.01)	3.75 (p=0.46)	0.80 (p=0.03)	4.75 (p=0.16)	0.60 (p=0.03)	ND	1.15 (p=0.58)	5.75 (p=0.95)	1.15 (p=0.73)	ND	0.10 (p=0.01)	ND	0.80 (p=0.04)	2.75 (p=0.67)
BM (n=11)	0.80	5.70	1.30	3.50	0.85	ND	0.9	1.25	1.47	ND	0.50	ND	0.85	5.75
PBSC (n=20)	6.00 (p<0.01)**	8.50 (p=0.30)	3.10 (p=0.92)	4.75 (p=0.63)	3.00 (p=0.03)	ND	7.90 (p=0.05)	3.45 (p=0.09)	1.00 (p=0.39)	ND	1.50 (p=0.13)	ND	1.65 (p=0.07)	4.95 (p=0.90)
CB (n=5)	0.85 (p=0.77)***	6.70 (p=0.72)	5.40 (p=0.30)	4.25 (p=0.72)	0.50 (p=0.13)	ND	1.20 (p=1.00)	1.55 (p=1.00)	1.15 (p=0.24)	ND	0.20 (p=1.00)	ND	1.25 (p=0.16)	4.25 (p=0.72)
No acute GVHD (n=11)	4.80`	5.10	2.20	3.55	2.80	ND	1.00	3.45	1.50	ND	1.50	ND	0.90	3.74
Acute GVHD Grade I/II (n=19)	2.05 (p=0.19)^	3.25 (p=0.55)	4.05 (p=0.70)	4.75 (p=0.84)	1.80 (p=0.59)	ND	3.70 (p=0.98)	2.55 (p=0.75)	1.30 (p=0.81)	ND	1.00 (p=0.36)	ND	1.00 (p=0.89)	2.75 (p=0.54)
Acute GVHD Grade III/IV (n=6)	3.40 (p=0.71)#	4.20 (p=0.94)	2.45 (p=0.57)	2.55 (p=0.45)	1.80 (p=0.49)	ND	3.10 (p=0.67)	2.75 (p=0.54)	1.15 (p=0.21)	ND	1.30 (p=0.88)	ND	1.00 (p=0.60)	3.25 (p=0.64)
ATG (n=20)	4.95	6.70	5.40	3.25	3.00	ND	3.70	4.71	1.00	ND	1.50	ND	1.00	5.45
No ATG (n=16)	1.65 (p=0.06)	4.85 (p=0.75)	1.50 (p=0.16)	2.50 (p=0.34)	0.80 (p=0.07)	ND	2.45 (p=0.51)	5.75 (p=0.32)	1.30 (p=0.83)	ND	0.55 (p=0.08)	ND	1.00 (p=0.75)	4.75 (p=0.50)
TBI (n=10)	4.60	6.80	0.60	1.60	6.60	ND	1.30	4.52	0.80	ND	0.10	N D	10.64	10.94

No TBI (n=26)	2.75	7.55	2.80	3.50	10.62	ND	5.30	5.75	1.40	ND	1.50	ND	11.90	11.25
	(p=0.57)	(p=0.64)	(p<0.01)	(p=0.19)	(p=0.15)		(p=0.24)	(p=0.67)	(p=0.26)		(p=0.03)		(p=0.67)	(p=0.87)

MSD, matched sibling donor; MUD, matched unrelated donor; ND, not done; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood (related + unrelated); GVHD, graft-versus-host disease; ATG, anti-thymocyte globulin; TBI, total body irradiation. Analyzed by Mann Whitney test, statistical significant : $p<0.05$. *median stimulation index; **compare with BM group; ***compare with BM group; ^ compare with no acute GVHD; #compare with grade I/II GVHD

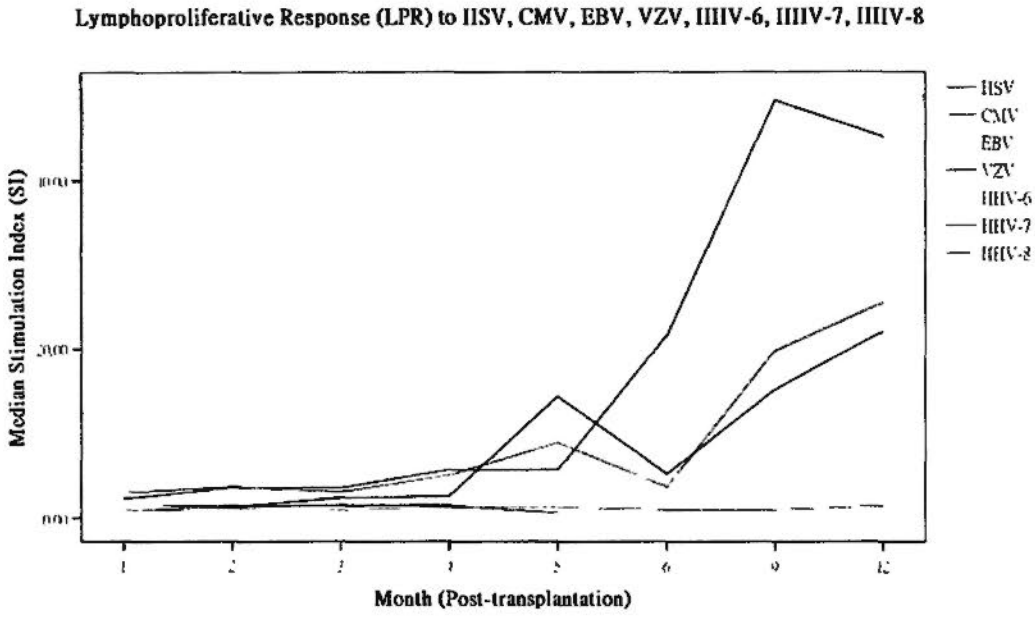


Figure 3. Lymphoproliferative response (LPR) to HSV-1, CMV, EBV, VZV, HHV-6, HHV-7, HHV-8

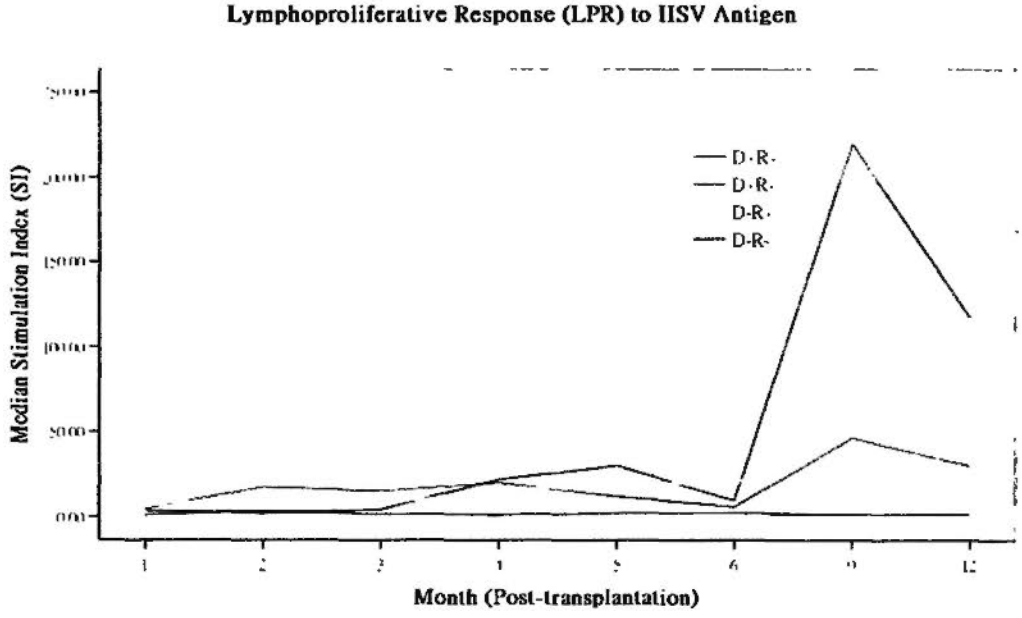


Figure 4A. Lymphoproliferative response (LPR) to HSV-1 – Stratified by donors' and recipients' serostatus

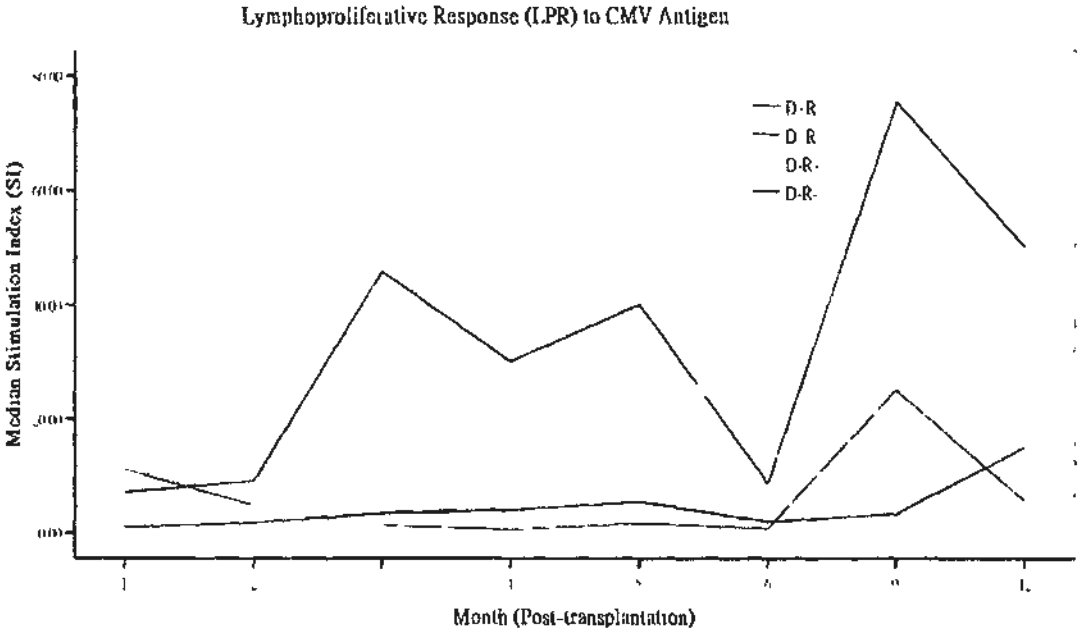


Figure 4B. Lymphoproliferative response (LPR) to CMV – Stratified by donors' and recipients' serostatus

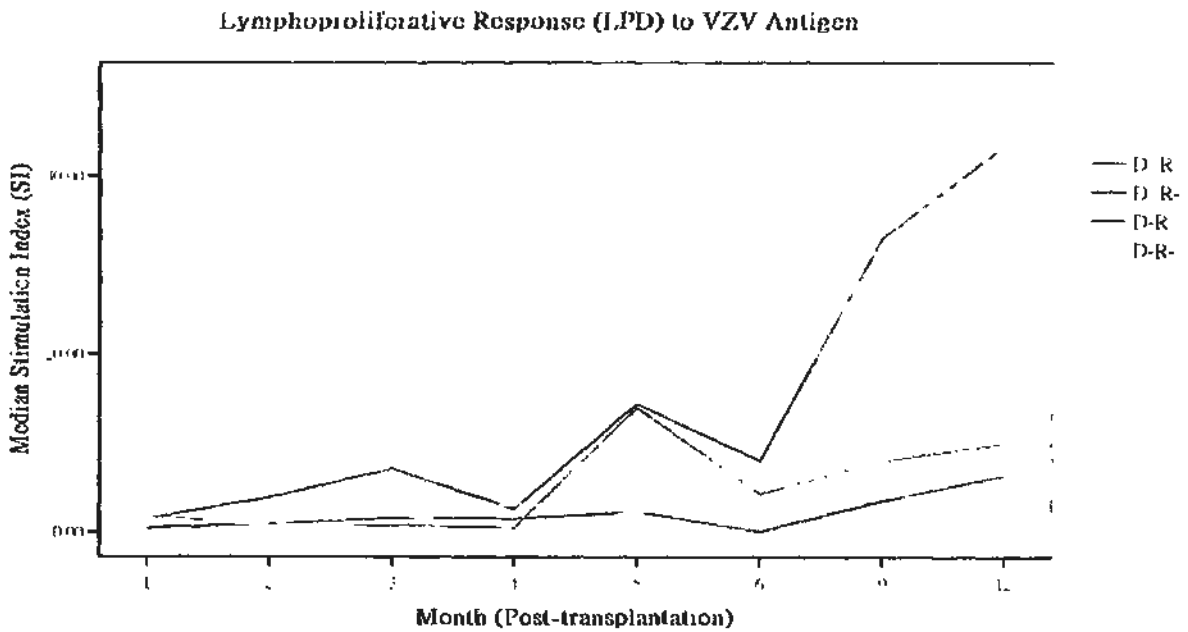


Figure 4C. Lymphoproliferative response (LPR) to VZV – Stratified by donors' and recipients' serostatus

12.4 Discussion

In the allogeneic HSCT setting, immune reconstitution is determined by multiple factors which include: (i) immunosuppressive effect of the conditioning regimen; (ii) graft composition; (iii) presence of acute and/or chronic graft-versus-host (GVHD) disease and (iv) immunosuppressive therapy given after transplantation. In general, natural killer cell (NK) returns to age-matched level at 1-2 months after transplant. B-lymphocyte levels return to the age-matched levels at 3-6 months after transplant. Immunoglobulin isotypes start to normalize at about 6 months after transplant with IgM first, follow by IgG_1, IgG_3, IgG_2, IgG_4 and then IgA. However, IgG subclass deficiency can last up to 18 months or more after transplant (166, 167). The median time for T-cell recovery is 7-8 months in the setting of CD34+ selected graft with myeloablative conditioning regimen (162).

A number of factors which can influence T-cell reconstitution have been identified: (i) type and dose of T-cell antibody used in conditioning; (ii) irradiation as part of the conditioning regimen; (iii) CD34+ cell dose; (iv) choice of stem cell and (v) graft manipulation techniques (168, 169). The delay in immune reconstitution is associated with severe infections. T-cell mediated immunity is specifically important for confining primary and reactivation of viral infections.

There are two sources of T cells in recovering recipients: One is derived from the peripheral expansion of mature and memory T cells which starts as early as 1-2 months after transplantation and reaches the peak at 6 months. The other source is derived from *de novo* maturation of naïve T cells which are originated from transplanted stem cells and are matured in the host's thymic system. This pathway generates a diverse receptor repertoire and is capable of responding to a range of antigens which is important in the reconstitution of CD4+ lymphocytes. Reconstitution to normal levels can occur 1-2 years after transplantation (170, 171). The capacity of the thymic-derived T cell production is diminished in adults due to the involution of thymus after puberty. Irradiation to thymic region, namely in total body irradiation and increase dose intensity of the conditioning regimen can induce tissue damage to epithelial cells of the thymus and result in impairment of its function in maturation of naïve T cells. Therefore, being an adult and the increased dose intensity in the conditioning regimen are risk factors for reduced ability to *de novo* maturation of naïve T cells (172). Incidence of CMV reactivation ranges from 14% - 29% and lethal CMV infections occurs in about 0% - 8%.(173, 174) In general, children have a lower infection death rate than adults which probably reflects impaired thymic function in older patients (175, 176).

Our data showed that although more than 50% of subjects showed a positive lymphoproliferative response to HSV-1, VZV and CMV at the early post-transplant period, an upsurge of stimulation index (SI) could only be demonstrated at 6 months post-transplant. For EBV, HHV-6, HHV-7 and HHV-8, the majority of subjects did not demonstrate a significant response in stimulation index throughout the study period. With the widely use of myeloablative chemotherapy and about 30% of our subjects received total body irradiation (TBI) in conditioning regimen, the process of

immune reconstitution would be delayed. This might account for the delayed major upsurge of stimulation index to HSV-1, CMV, VZV and the suboptimal response to EBV, HHV-6, HHV-7 and HHV-8 antigens in the post-transplant period despite a significant proportion of subjects showed a positive stimulation index before transplantation.

In the subgroup analysis, we found that patients who received matched sibling donor transplantation (MSD) and received GCSF-mobilized peripheral blood stem cell (PBSC) transplantation had a greater degree of rise in stimulation index (SI) when compared with their matched unrelated donor (MUD) transplant counterpart and patients who received bone marrow as the source of stem cells. (Table 20) With the less severe GVHD and thus lesser use of intensive immunosuppressive therapy, the time and extent of immune recovery were more rapid in MSD transplant than MUD transplant recipients.

In our series, the CD3+ and CD34+ cell counts in the PBSC graft were significantly higher than the BM graft. With the higher number of T-cells, both memory and naïve T-cells, the reconstruction of cell-mediated immunity could be faster. This might be the reason for this observation. However, in our series, we could not demonstrate any correlation between lymphoproliferative response to herpes viruses and the use of anti-thymocyte globulin as well as the use of total body irradiation in the conditioning regimen. Since the number of subjects with acute graft-versus-host disease (GVHD) was small, we could not analyze the correlation between lymphoproliferative response and the severity of acute GVHD.

There are several limitations of our study. Firstly, we should recruit healthy non-imunocompromosed children as control and compare with the lymphoproliferative responses of various herpes viruses in immunocompromised subjects. This should be seriously considered in future immune reconstitution studies. The other is the study timpoints for EBV, HHV-6 and HHV-7 should extend to 12 months post-transplant as EBV and HHV-6 are also important pathogens in stem cell transplant setting as described in previous chapters.

Reactivation of herpes viruses in the post-transplant period is common following allogeneic hematopoietic stem cell transplantation and is associated with significant morbidity and mortality. Both CD4+ and CD8+ antigen specific immune reconstitution are required for protection against the reactivation of viral infection. Deficient antigen-specific CD4+ and CD8+ responses within the early post-transplant period was associated with a higher risk of viral reactivation at the late transplant period (177). Therefore, with the infusion of donors' memory T-cells or the presence of recipients' remaining T-memory cells may be associated with early reconstitution of viral-specific immunity. In our series, we showed that seropositivity in both the donor and recipient is an important predictor for the lymphoproliferative response. For HSV-1, CMV and VZV antigens, patients with status (D+; R+) showed a higher stimulation index when compared with other counterparts. Ganepola S *et al* actually recommend CMV seropositive donors for CMV seropositive recipients, as this should lead to an early and durable recovery of CMV-specific T-cell immunity after transplantation with subsequent protection against CMV reactivation and disease (178). This may also account for the current observation of a higher stimulation index in PBSC subgroup since the CD3+ cell dose was significantly higher than in the BM subgroup.

The significance of HHV-8 infection and its reactivation in pediatric allogeneic HSCT setting remains unclear. Rosenzwajg *et al* reported the seropositivity rate in the pediatric population (< 15 years) before transplantation was about 10% (179). HHV-8 is implicated in causing Kaposi's sarcoma (KS) which classically affects elderly men of Mediterranean or Eastern Europe origin. It is also endemic in children and young adults of subequatorial Africa and epidemic in HIV infected individuals. Kaposi's sarcoma has been reported in solid organ transplantation recipients but rarely reported in allogeneic stem cell transplant setting (180). There are no previously published local data of seropositive rate in the Chinese pediatric population. Our data showed that only 6.5% of patients had a stimulation index of $\geqq 3$ before transplant reflected a majority of patients had not encountered this virus before. Our data were consistent with other published series (179). Throughout the 12-month follow-up period, the stimulation index remained low when compared with other antigens. This reflected either primary or reactivation of HHV-8 infection in the post-allogeneic transplant setting was an uncommon event.

There are a number of experimental and clinical approaches which are currently being investigated in boosting the T-cell mediated immune response in the post-transplant period. Regeneration of bone marrow niche and hopefully improvement of the microenvironment of bone marrow is particularly relevant. One of the strategies is to transplant donor bone marrow stem cells with other cells of non-hematopoietic origin, namely stromal cells, mesenchymal stem cells (MSC) or osteoblast progenitors. The mesenchymal stem cells (MSC) are naturally immunosuppressive and have been shown to successfully diminish GVHD in allogeneic stem cell transplantation and may facilitate the induction of mixed donor-recipient chimerism and thus improve engraftment. These measures will lead to

decrease the period of immunosuppression after HSCT and perhaps can decrease the chance and duration of herpes virus reactivation. The other investigational targets are (i) administration of keratinocyte growth factor (KGF) or Fms-like tyrosine kinase 3 ligand (Flt3L) to initiate in-situ thymic recovery and thus reduce the damages may be cytoreductive therapies; (ii) 'De novo" thymic production by ex vivo expanded thymic epithelial cell (TEC) biopsy; (iii) expansion of naïve T-cells by IL-7 or possibly Flt3L (181).

Through our study in the recovery of viral-specific immunity of various herpes viruses (HSV-1, VZV, EBV, CMV, HHV-6, HHV-7 and HHV-8) in patients received allogeneic HSCT, this poses significant implication in term of formulating specific immunomodulatory strategies in controlling these potentially life-threatening infections. Currently, only VZV infection is the only vaccine-preventable disease, the potential use of adoptive transfer of virus-specific immunity in allogeneic HSCT patients can then be further studied.

Varicella-zoster virus (VZV) is a herpes virus that causes chickenpox as a primary infection and herpes zoster when the latent virus is reactivated. Patients who experienced chickenpox will be protected from recurrent attacks by VZV-specific antibody (182). Nonetheless, these subjects may develop herpes zoster when their cell-mediated immunity wanes with advancing ages or diseases that are associated with immunosuppression (183-185). Therefore, patients who underwent HSCT are at high risk of having severe VZV infection because of significant and prolonged immunosuppression in the post-transplant period.

Our unit has performed over 300 HSCT over the past 20 years. Our data showed that 27 (25%) of our first 108 children who received HSCT developed post-transplant herpes zoster. The median time to herpes zoster in our patients was 124 days after

transplant. Twenty-two (81%) children developed herpes zoster within one year after transplantation. Three (11%) children were diagnosed to have herpes zoster with visceral dissemination. The cumulative incidences of post-transplant herpes zoster at 1 and 5 years were 24% and 34% respectively. The median duration of herpes zoster was 5 days (range 3 to 60 days). No mortality was directly related to VZV infection. Therapeutic measures that reduce the occurrence of post-transplant herpes zoster can possibly lower the morbidity and treatment cost for this infection (186).

Sempere *et al* found that acyclovir is effective in controlling VZV proliferation in immunocompromised patients. However, five of their 21 post-transplant adult patients with acute leukemia developed herpes zoster within one month of acyclovir withdrawal (187). In addition, the use of prophylactic acyclovir or ganciclovir did not prevent post-transplant VZV infection in a previous report as well as in our patients. Besides, there is an increasing concern of emergence of acyclovir-resistant strains of varicella zoster virus or herpes simplex virus (188, 189).

Previous study suggested that VZV-specific lymphoproliferative response (LPR) recovered more often in those recipients who developed post-transplant herpes zoster (190). Wilson *et al* also reported that VZV-specific cellular immunity recovered in post-transplant patients as a result of subclinical VZV viremia.(191) This was possibly related to *in vivo* boosting of VZV-specific immune functions of these patients upon natural VZV reactivation. Kato *et al* investigated the transfer of VZV-specific T-cell immunity from 49 marrow donors to their recipients. This report first showed that the 72 healthy subjects who experienced chickenpox previously had a stimulation index (SI) of above 3.0 on VZV-specific LPR that indicated the presence of specific cellular immunity as compared with 21 persons without chickenpox. The authors also concluded that there was no direct transfer of VZV-specific cellular

immunity from transplant donors to recipients. Fourteen (88%) of 16 patients with positive VZV-specific cellular immunity in both donors and recipients developed early post-transplant herpes zoster when their SI dropped to below 3. However, the authors did not analyze the correlation between SI on VZV-specific LPR and development of post-transplant herpes zoster. Two transplant recipients in this study who had VZV infection in the immediate pre-transplant period did not develop herpes zoster following HSCT. This suggests that the presence of adequate number of VZV-specific T-cells early after transplant in the recipients may prevent patients from developing post-transplant herpes zoster (192).

Varicella Vaccine Collaborative Study showed that varicella vaccination resulted in a two-third reduction in herpes zoster in children with leukemia (193). Another clinical trial reported that children with leukemia who received VZV vaccine had a lower incidence of herpes zoster as compared with those after natural infection (3.6% vs 13.1%) (194). A recent study suggested that early post-transplant immunization of recipients with inactivated varicella vaccine resulted in enhancement of VZV-specific cellular immunity. This immunomodulation of transplant recipients was effective in reducing the severity of post-transplant herpes zoster (195). However, this inactivated vaccine is not licensed for clinical use. The live-attenuated varicella vaccine currently available may also be effective in lowering VZV reactivation. However, this vaccine with its live virus may cause severe vaccine-related complication if it is given to transplant recipients too early after transplant when they are still profoundly immunosuppressed. On the other hand, most of the post-transplant herpes zoster (81% in our study) occurs within the first one year following HSCT. Alternatively, varicella vaccine may be used to boost the VZV-specific immunity in recipients before HSCT. Nevertheless, patients with underlying malignant diseases usually cannot tolerate

pre-transplant VZV immunization because most of them are immunocompromised from intensive chemotherapy and radiation treatment before HSCT. Besides, they cannot wait too long for recovery of immune functions before transplant in order to have VZV immunization because of risk of relapse of primary diseases.

Successful transfer of immunity against hepatitis B virus could be demonstrated in recipients of allogeneic HSCT (196, 197). Two post-transplant patients also had sustained clearance of hepatitis B surface antigenemia by anti-HBs positive marrow from their donors (198). Walter *et al* demonstrated that reconstitution of cellular immunity against cytomegalovirus in recipients could be achieved by transfer of T-cell clones from donors or infusion of marrow from seropositive donors (199). Adoptive transfer of immunity against Epstein-Barr virus, tetanus and *Haemophilus influenzae* type b also occurred in recipients of allogeneic transplantation (200, 201). Thus, the adoptive transfer of VZV-specific cell-mediated and humoral immunity from transplant donors to recipients is another feasible approach in the prevention of post-transplant herpes zoster. Surge in specific T- and B-lymphocyte populations could be achieved when healthy individuals are re-immunized with the vaccine. This boost of immune system resulted in less VZV reactivation in the elderly population. It is also possible that the large numbers of VZV specific T- and B-cells present in donors following VZV immunization can be transferred together with stem cells to recipients. These memory lymphocytes can thus protect recipients from post-transplant herpes zoster and subclinical VZV viremia in the early post-transplant period when they are profoundly immunocompromised as a result of the intensive conditioning regimen (190). There is so far no study that investigated whether the adoptive immunity transfer can lower the occurrence or severity of post-transplant VZV reactivation.

There are several reports that showed the development of chickenpox and herpes zoster in normal subjects after VZV immunization (202). The live-attenuated virus administered to the donors before HSCT may transmit with the stem cells to the immunocompromised recipients and introduce active infection early after transplant.

Several previous studies suggested that transplant recipients developed VZV viremia without clinical evidence of herpes zoster (203). Ljungman *et al* found that subclinical VZV reactivation was detected in 26% of post-BMT patients (203). However, the incidence of subclinical VZV viremia may still be underestimated as these trials only involved infrequent testing of patient samples for VZV.

Previous studies have shown that VZV vaccination protected leukemic children from chickenpox when it is given to patients during or after maintenance chemotherapy (204). However, patients who underwent HSCT are much more immunocompromised as compared with children with leukemia in remission. The VZV seropositive transplant recipients may seroconvert after HSCT and thus lose protection against chickenpox following transplant. Hence, these patients may benefit from post-transplant VZV immunization after immune reconstitution occurs. Nonetheless, the immunogenicity, safety and optimal schedule of varicella vaccine in these patients remain unclear. From the literature, it is not certain whether these post-transplant immunocompromised patients have prolonged excretion of VZV following vaccination. The exact role of adoptive transfer of donors' immunity to transplant recipients still needs further evaluation. Our data provides a suitable platform for future studies.

12.5 Conclusion

In this chapter, our group provides the first complete set of data to illustrate the recovery of cell-mediated immunity to various herpes group of viruses over a 12-month study period following allogeneic hematopoietic stem cell transplantation with myeloablative conditioning regimen. The serostatus of the donor and the recipient, and the choice of donors and the source of stem cells are important parameters in determining lymphoproliferative response to herpes viruses in transplant recipients. Our data provides a suitable platform for future studies on the exact role of adoptive transfer of donors' immunity in hastening the recovery of recipients' immune status after hematopoietic stem cell transplantation.

Section 4

Summary and Conclusions

Chapter 13: Summary of Results

Chapter 14: Conclusions

Chapter 13: Summary of Results

Section 1 Background and Overview

Chapter 1 An Overview of Spectrum of Diseases in Pediatric Oncology in Hong Kong

This thesis begins with an overview of the spectrum of pediatric oncology in Hong Kong. Chemotherapies play an important role in treatment of the four commonest pediatric cancers. Although the survival improves significantly from 10% to almost 90% over the past fifty years due to improvement in various treatment protocols, cancer death still ranks the second major causes of death in Hong Kong children (5-14 years old). Infection is one of the most common and often serious treatment-related morbidities and mortalities. This forms the background of the series of original studies.

Chapter 2 An Overview of Different Treatment Modalities in Pediatric Oncology and Impact on Destruction of Immune System

This chapter describes the main treatment modalities in pediatric oncology patients with main focus on chemotherapy and radiotherapy. The impact on destruction of host's immune system is described.

Chapter 3 Current Understanding of Immune Reconstitution in Pediatric Oncology Patients Received Chemotherapy and Hematopoietic Stem Cell Transplant Recipients

This chapter describes the understanding of immune reconstitution in children who had received immunosuppressive therapy. The nadir of bone marrow suppression after chemotherapy is about ten to fourteen days. After the nadir, neutrophils are usually return to normal within a short time (three to four weeks) and then follow by natural killer cells, while B and T lymphocytes take longer to recover and it varies with children's age. Residual deficits may persist up to twelve months after completion of treatment protocol or even longer and the recovery of "immune memory" may not be fully completed especially in young children. This formulates the research idea in performing the following original studies with the aim at improving the immune reconstitution and prevention of infections in our pediatric oncology patients and hematopoietic stem cell transplant recipients.

Section 2 Strategies for Prevention of Infections in Pediatric Oncology Patients

Part 1 Literature Review and Planning of Original Studies

Chapter 4 Literature Review

This chapter reviews the published studies in describing the impact of opportunistic infections in immunocompromised patients and the various established methods in prevention of infection in immunocompromised patients.

Chapter 5 Objectives and Planning of the Original Studies

Although there is presence of various well-established recommendations in term of treatment of infections in this vulnerable group, opportunistic infection continue to cause significant impact in pediatric oncology patients and stem cell transplant recipients. This chapter describes the planning of a series of original studies to postulate various possible strategies in preventing our immunocompromised children from various infectious diseases.

Part 2 Original Studies of Different Strategies in Prevention of Infections in Pediatric Oncology Patients.

Chapter 6 Recovery of Humoral Immune Response to Vaccine-Preventable Infectious Diseases in Pediatric Oncology Patients

This chapter reports our 18-month cohort study which showed a near complete humoral immune recovery in our patients. However, a significant proportion of subjects were still susceptible to various vaccine-preventable infectious diseases up to 18 months after stopping chemotherapy. Post-chemotherapy booster vaccination may have a role in prevention of this potential life-threatening complication. This will evolve to a significant health care problem if no further intervention is implemented as the survival rate of pediatric oncology patients improves significantly with the improvement in various cancer treatment protocols.

Chapter 7 Humoral Immune Response After Post-Chemotherapy Booster Diphtheria-Tetanus-Pertussis Vaccine in Pediatric Patients with Hematological Malignancies.

This chapter reports our randomized-controlled trial to demonstrate intensive chemotherapy led to loss of protective serum antibody titers for different types of vaccine-preventable infectious diseases which could be persistent till 18 months after stopping chemotherapy in patients with hematological malignancies. Booster vaccinations started at six-month after stopping chemotherapy in children with hematological malignancies could be safely administered, and effectively restored a sustained effect in humoral immunity against various types of vaccine-preventable infectious diseases.

Chapter 8 Humoral Immune Response After Post-Chemotherapy Booster Diphtheria-Tetanus-Pertussis Vaccine in Pediatric Patients with Solid Tumors.

This chapter reports our randomized controlled trial to demonstrate chemotherapy for solid tumors led to loss of protective serum antibody titers for different types of vaccine-preventable infectious diseases. Booster vaccinations started at six-month after stopping chemotherapy in children with different solid tumors could be safely administered, and effectively restored a sustained effect in humoral immunity against various types of vaccine-preventable infectious diseases.

Chapter 9 Clinical Presentations and Outcome of Hospitalized Pediatric Oncology Patients with Laboratory-confirmed Pandemic H1N1 Influenza Infection in Hong Kong – Impact of Novel Virus and the Role of Novel Monovalent Influenza Vaccine

This chapter reports our multi-centers territory-wide study to evaluate the clinical course of hospitalized pediatric oncology patients and stem cell transplant recipients with pandemic H1N1 influenza infection in Hong Kong. We found out fever, cough and runny nose were the most common presenting symptoms. Timely initiation of antiviral therapy was likely beneficial and might prevent severe complications in this high risk population group. Persistent symptoms and evidence of prolonged viral excretion which required repeated course of prolonged antiviral therapy were more common among HSCT recipients. The role of monovalent H1N1 vaccine in preventing this novel infection in immunocompromised patients still needs further investigations.

Section 3 Impact of Non-Vaccine Preventable Infectious Diseases and the Potential Use of Adoptive Transfer of Virus-Specific Immunity in Allogeneic Hematopoietic Stem Cell Transplant Recipients

Chapter 10 Impact of Non-vaccine Preventable Viral Infection in HSCT Setting: Epstein-Barr Virus (EBV) in Post-transplant Lymphoproliferative Disorder Complicating Umbilical Cord Blood Transplantation.

EBV-associated post-transplant lymphoproliferative disorder (PTLD) is rarely reported in umbilical cord blood transplant (UCBT) setting. In our two case illustrations, the following new information are added to the literatures: (i) EBV-related PTLD could be presented late in recipients of unrelated UCBT; (ii) in contrast to reported literatures that PTLD, especially in monomorphic form, is a serious complication with unfavorable outcome, our cases showed that the clinical course might be relatively benign if treatment was initiated promptly.

Chapter 11 Use of Antiviral Agents as Prophylaxis: HHV-6 Encephalitis in Hematopoietic Stem Cell Transplant Recipients as an Illustration

For non-vaccine preventable infectious diseases, they can cause significant morbidity and mortality in immunocompromised patients. In this chapter we used HHV-6 infection as an illustration. Reactivation of HHV-6 commonly occurred within the early post-transplant period. We used antiviral prophylaxis at the pre-transplant period to evaluate whether this could reduce the impact and incidence of HHV-6 encephalitis in our unrelated allogeneic stem cell transplant recipients. We showed that routine use of pre-transplant ganciclovir prophylaxis might have a role in high risk transplant setting, namely unrelated umbilical cord blood transplantation.

Chapter 12 Lymphoproliferative Response to Herpes Simplex Virus Type 1, Cytomegalovirus, Epstein-Barr Virus, Varicella Zoster Virus, Human Herpes Virus 6, 7 and 8 Antigen Stimulation in Pediatric Allogeneic Stem Cell Transplant Recipients.

Knowing the recovery pattern of immunity to different viruses is important to predict the causative agents in infective episodes and we can formulate strategies, namely by prophylaxis or transfer of donor's immunity to prevent infection from occurring in transplant setting. Our group provided the first complete set of data to illustrate the recovery of cell-mediated immunity to herpes group of virus in a 12-month study period in children received allogeneic hematopoietic stem cell transplantation with myeloablative conditioning regimen. Our data showed that donors' and recipients' serostatus, choice of donors and stem cell source were important parameters in determining lymphoproliferative response to herpes virus in transplant recipients.

Chapter 14 Conclusions

The following new knowledge is generated from our series of original studies and applied to clinical settings:

1. Although a near complete humoral immune recovery is demonstrated in pediatric oncology patients, a significant proportion of subjects remain susceptible to various vaccine-preventable infectious diseases up to 18 months after stopping chemotherapy. Routine checking of immune recovery after termination of therapy is recommended.
2. Three-dose regimen of DTaP booster vaccine starts at six months after stopping chemotherapy in children with hematological malignancies can be safely administered, and effectively restore a sustained effect in humoral immunity in children with hematological malignancies.
3. Three-dose regimen of DTaP booster vaccine starts at six months after stopping chemotherapy in children with different solid tumors can be safely administered, and effectively restore a sustained effect in humoral immunity in children with solid tumors.
4. Early use of antiviral therapy in patients with clinical suspected or laboratory confirmed H1N1 infection is likely beneficial. Prolonged course of antiviral therapy is used in patients, especially HSCT recipients, with persistent or recurrent symptoms or evidence of prolonged H1N1 excretion.

5. EBV-related lymphoproliferative disorder can present in cord blood transplant setting which require prompt diagnosis and early treatment. Routine monitoring of EBV viral load in plasma and watch out for early signs of EBV-related lymphoproliferative disorder even in recipients of unrelated cord blood transplantation is warranted.
6. Antiviral prophylaxis may have a role in high risk allogeneic stem cell transplant, namely unrelated umbilical cord blood transplant in preventing non-vaccine preventable infectious diseases, namely HHV-6 encephalitis.
7. The full pattern and risk factors of immune reconstitution to various herpes viruses, most of them are non-vaccine preventable, is determined. Timely use of antiviral prophylaxis or use of adoptive transfer of donor's immunity can then be further studied.

Concerning the direction of future studies, our group will further explore the follwing aspects:

1. Humoral response of pneumococcal vaccine (PCV-7 or PCV-13) in pediatric oncology patients.
2. Humoral response of pandemic (2009) H1N1 vaccine in pediatric oncology patients.
3. Role of herpes zoster vaccine in pediatric allogeneic stem cell transplantation in term of trasfer of donor's immunity to recipients to prevent VZV reactivation in HSCT recipients.

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List of Published Works

Cheng FWT, Leung TF, Chan PKS *et al.* Recovery of Humoral and Cellular Immunities to Vaccine-Preventable Infectious Diseases in Pediatric Oncology Patients. *Pediatr Hematol Oncol* 2010;27:195–204.

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RECOVERY OF HUMORAL AND CELLULAR IMMUNITIES TO VACCINE-PREVENTABLE INFECTIOUS DISEASES IN PEDIATRIC ONCOLOGY PATIENTS

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□ *The recovery of antibodies to various vaccine-preventable infectious diseases, humoral and cellular immunity in pediatric oncology patients were evaluated by a prospective longitudinal study for 18 months. Lymphocyte subset (CD3+, CD4+, CD8+, CD16/56+, CD19+), CD4/CD8 ratio, immunoglobulin levels, antibodies to diphtheria, pertussis, tetanus, hepatitis B, measles, mumps, and rubella were measured serially at 6 months till 18 months after stopping all chemotherapy (including maintenance chemotherapy). Twenty-eight children (hematological malignancies, n = 14; solid tumors, n = 14) were studied. The median age was 7.0 ± 3.8 years old (range 2.6–16.2 years old). Although there was significant increase in CD3+, CD4+, CD8+, CD19+ cells, IgG, IgA, and IgM levels (P <.05), CD4+ and CD8+ counts were still below the age-specific normal range at the end of study period. At 18 months after stopping chemotherapy, 11%, 15%, 60%, 30%, 49%, and 30% of subjects remained seronegative against diphtheria, tetanus, hepatitis B, measles, mumps, and rubella. This will evolve to a significant health care problem if no further intervention is implemented, as the survival rate of pediatric oncology patients improves significantly with the improvement in various cancer treatment protocols. Near complete immune recovery was demonstrated in the subjects. Significant proportion of subjects remained susceptible to vaccine-preventable infectious diseases up to 18 months after stopping all chemotherapy.*

Keywords cancer, cellular immunity, humoral immunity, infectious diseases, vaccine

With the improvement of various treatment protocols of pediatric malignancies, the overall and event-free survival rates in this vulnerable group

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improve significantly [1, 2]. However, both primary diseases and their treatments will inevitably cause immunosuppression. In this modern era, various studies have been performed to evaluate the immune recovery after intensive chemotherapy [3, 4]. A number of them showed persistent immune deficit at the end of study period and left the period of complete or satisfactory recovery of immune system unanswered.

The exact duration of immune suppression after chemotherapy remains unanswered. Our group performed this longitudinal study to study the recovery of immune system in children with hematological malignancies and solid tumors up till 18 months after stopping chemotherapy, which is one of the longest ever reported in the English literature. Our group also studied the recovery of antibodies to various vaccine antigens at that period and thus estimated the percentage and duration of subjects that were vulnerable to these vaccine-preventable infections.

METHODS

Study subjects were diagnosed and received treatment in a tertiary referral center for pediatric cancers in Hong Kong. The Clinical Research Ethics Committee of our University approved this clinical trial. Patients and/or their parents gave informed written consent prior to study according to the principles of the Declaration of Helsinki.

Patients aged 1 to 18 years who had been completed treatment for pediatric hematological malignancies and solid tumors for 6 months were recruited consecutively for evaluation of recovery of humoral immunity against 7 vaccine-preventable infectious diseases (hepatitis B, diphtheria, tetanus, pertussis, measles, mumps, and rubella). Hematological malignancies included children with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and other leukemia. They were all treated according to their disease-specific treatment protocols.

Exclusion criteria included (1) patients with past history of those listed vaccine-preventable diseases; (2) evidence of immunodeficiency before diagnosis of malignancy; (3) patients developed relapse of primary disease or secondary malignancies during study period; (4) patients were still receiving systemic steroid for their primary diseases or other conditions with dosage greater or equivalent to prednisolone 2 mg/kg/day; and (5) hematopoietic stem cell transplant recipients.

Immunization status of patients was obtained from the history and vaccination records. Past medical history was reviewed to exclude those with history of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps, and rubella infections before evaluation. They were monitored for development of these vaccine-preventable infectious diseases and other severe infections during the study period. Criteria of severe infection were defined as (i)

culture-proven bacteremia; (ii) culture or histological confirmed or probable invasive fungal infections; and (iii) severe infective episodes that required critical care and/or respiratory support.

Serological Studies

Serum concentrations of antibodies to diphtheria, tetanus, pertussis, measles, mumps, rubella, and hepatitis B were monitored serially started from 6 months (visit 1), 8 months (visit 2), 12 months (visit 3), and 18 months (visit 4) after completion of all chemotherapy including maintenance chemotherapy.

Serum samples from the above 4 visits were stored at −30°C until analysis in one batch. Commercially available kits were used for the determination of antibody titers. Serum levels of specific antibodies to diphtheria, tetanus, and pertussis were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany). The protective antibody levels were defined as 4 IU/mL for diphtheria, 2 IU/mL for tetanus, and 24 IU/mL for pertussis [5]. Titer to hepatitis B surface antibody (anti-HBs) was measured by using an enzyme-linked immunosorbent assay. (BioSupply, UK). Antibody level $\geq$ 10 mIU/mL was considered as protective. Measles and mumps immunoglobulin G (IgG) antibodies were tested by a modification of the NOVUM measles/mumps virus IgG ELISA test kits (Novum Diagnostica, Germany) and rubella by Imx Rubella IgG2.0 antibody assay (Abbott Laboratories, Chicago, IL, USA). Antibody levels exceeding a cutoff value of 10 IU/mL were interpreted as seropositive [6].

Assessment of Cellular and Humoral Immunities

Lymphocyte subset and serum immunoglobulin level were checked at 6 months (visit 1) and 18 months (visit 4) after stopping chemotherapy. Subsets of circulating lymphocytes were quantified by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA, USA) using fluorescence-labeled monoclonal antibodies CD3+, CD4+ (helper T lymphocyte), CD8+ (cytotoxic T lymphocyte), CD19+ (mature B lymphocyte), and CD16/56+ (natural killer cells). Serum IgG, IgA, and IgM concentrations were measured by nephelometry (Binding Site, Birmingham, UK).

Statistical Analysis

Data were expressed as median and standard deviation unless otherwise stated. Demographic data between different subgroups were analyzed using Student t test for independent parametric variables and χ^2 for categorical variables between different disease or patient groups. Wilcoxon signed ranks test was used to analyze between values at visit 1 (6 months after stopping

chemotherapy) and visit 4 (18 months after stopping all chemotherapy). Statistical significance was defined as $P < .05$.

Data analysis was performed by SPSS for Windows (version 14.0; SPSS, Chicago, IL, USA).

RESULTS

Twenty-eight patients with hematological and solid tumors were recruited (18 males and 10 females); median age was 7.0 ± 3.8 years old (range: 2.6–16.2 years old). All patients had completed primary series of childhood immunization schedules before malignancy developed. Primary diagnosis included 14 cases of hematological malignancies and 14 cases of solid tumors. Hematological malignancies included acute lymphoblastic leukemia (ALL), $n = 12$; acute myeloid leukemia (AML), $n = 2$. Solid tumors group included central nervous system (CNS) tumor, $n = 3$; Hodgkin lymphoma, $n = 3$; osteosarcoma, $n = 2$; and 1 case of germ cell tumor, rhabdomyosarcoma, neuroblastoma, nasopharyngeal carcinoma, clear cell sarcoma, and Ewing's sarcoma respectively. The median duration of chemotherapy was 22.4 ± 7.7 months in hematological groups, which included cumulative dose of prednisolone 1680 mg/m^2 and dexamethasone 210 to 740 mg/m^2 during intensive phase of treatment of ALL and 10.0 ± 4.0 months in solid tumors groups. No radiation was given to the mediastinal region as part of their therapies.

Recovery of Humoral Immunity

At 6 months after stopping chemotherapy (visit 1), the median absolute neutrophnils, CD3+ cells (1.43 ± 0.52 × 10^9/L), CD4/CD8 ratio, CD16/56+ cells, and immunoglobulin levels were within normal limit, but CD4+ cells (0.39 × 10^9/L), CD8+ cells (0.39 × 10^9/L), and CD19+ cells (0.37 × 10^9/L) were low when compared with age-specific normal lymphocyte subset values [7].

Throughout the 18-month study period, there was an overall statistical significant increase in various lymphocyte subsets and immunoglobulin levels. The median CD3+ lymphocyte raised from 1.43 ± 0.52 × 10^9/L at visit 1 to 1.76 ± 0.68 × 10^9/L ($P = .01$) at visit 4; CD4+ raised from 0.39 ± 0.17 × 10^9/L to 0.54 ± 0.28 × 10^9/L ($P = .01$); CD8+ raised from 0.39 ± 0.20 × 10^9/L to 0.47 ± 0.20 × 10^9/L ($P = .04$); CD19+ increased from 0.37 ± 0.17 × 10^9/L to 0.45 ± 0.20 × 10^9/L ($P = .03$). CD4/CD8 ratio was >1.0 throughout the study period (visit 1: 1.09 ± 0.44; visit 4: 1.14 ± 0.33). IgG increased from 9.52 ± 2.43 to 10.28 ± 2.73 g/L ($P = .02$); IgA raised from 1.27 ± 0.59 to 1.57 ± 0.6 g/L ($P = .01$); and IgM changes from 0.92 ± 0.41 to 1.22 ± 0.66 g/L ($P = .01$). Overall, all immune parameters were nearly normal at the end of study period (18 months after stopping

TABLE 1 Recovery of humoral and cellular immunities in pediatric oncology patients ($n = 28$)

	6 months after stopping chemotherapy (Visit 1)	18 months after stopping chemotherapy (Visit 4)	P^*
Absolute neutrophil count (10^9/L)	4.13 ± 1.81	4.47 ± 1.99	.38
CD3+ (10^9/L) 2.39 (1.40–3.70)^	1.43 ± 0.52^^	1.76 ± 0.68	.01**
CD3+CD4+ (10^9/L) 1.38 (0.70–2.20)	0.39 ± 0.17	0.54 ± 0.28	.01**
CD3+CD8+ (10^9/L) 0.84 (0.49–1.30)	0.39 ± 0.20	0.47 ± 0.20	.04**
CD4+/CD8+ ratio	1.09 ± 0.44	1.14 ± 0.33	.43
CD19+ (10^9/L) 0.75 (0.39–1.40)^	0.37 ± 0.17	0.45 ± 0.20	.03**
CD16/56+ (10^9/L) 0.30 (0.13–0.72)	0.15 ± 0.08	0.14 ± 0.06	.43
IgG (g/L) (5.49–15.84)	9.52 ± 2.43	10.28 ± 2.73	.02**
IgA (g/L) (0.61–3.48)	1.27 ± 0.59	1.57 ± 0.60	.01**
IgM (g/L) (0.23–2.59)	0.92 ± 0.41	1.22 ± 0.66	.01**

*Analyzed by Wilcoxon signed ranks test between values at visit 1 (6 months after stopping chemotherapy) and visit 4 (18 months after stopping all chemotherapy).

**Statistical significance: $P < .05$.

^Normal median value (10th percentile–90th percentile); ^^expressed as median ± standard deviation.

all chemotherapy) except median CD4+ and CD8+ cell counts, which were still lower than age-specific normal values [7].

No subjects developed infections due to these vaccine-preventable pathogens during the study period. None of them fulfilled the criteria of severe infection. The detail is shown in Table 1.

Antibodies to Vaccine-Preventable Infectious Diseases

Concerning the recovery of protective antibody levels to 7 major vaccine-preventable infectious diseases, only 82% of subjects had protective anti-diphtheria antibody levels, the percentages to hepatitis B, measles, mumps, and rubella were 29.6%, 70.4%, 55.6%, and 70.4%, respectively, at 6 months after stopping chemotherapy. The seropositive rate of pertussis and tetanus were 96.5% at baseline.

Throughout the study period, there was no significant increase in various antibody levels. Up till 18 months after stopping chemotherapy, there were still 11%, 15%, 60%, 30%, 49%, and 30% of subjects who remained susceptible to diphtheria, tetanus, hepatitis B, measles, mumps, and rubella infections. The detail is shown in Table 2.

DISCUSSION

Various studies have shown that immune impairment can persist up to 12 months after stopping intensive chemotherapy. The degree of immune reconstitution was dependent on the treatment intensity. The most heavily treated patients, namely survivors of high-risk group of ALL and AML, have persistent abnormalities in cellular and humoral immunities for at least 6

TABLE 2. Seropositivity of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps, and rubella in pediatric oncology patients

Vaccine-preventable infectious diseases	Visit 1	Visit 2	Visit 3	Visit 4
Diphtheria (total)	82.1%	78.6%	75.0%	89.3%
Diphtheria (hematology)	78.6%	71.4%	71.4%	78.6%
Diphtheria (solid tumor)	85.7%	85.7%	78.6%	100%
Tetanus (total)	96.4%	96.4%	92.9%	85.2%
Tetanus (hematology)	92.3%	96.4%	92.9%	78.6%
Tetanus (solid tumor)	100%	96.4%	92.9%	92.3%
Pertussis (total)	96.5%	96.2%	100%	100%
Pertussis (hematology)	92.9%	100%	100%	100%
Pertussis (solid tumor)	100%	92.3%	100%	100%
Hepatitis B (total)	29.6%	25.9%	32.1%	40.7%
Hepatitis b (hematology)	15.4%	14.3%	35.7%	42.9%
Hepatitis b (solid tumor)	42.9%	38.5%	28.6%	38.5%
Measles (total)	70.4%	—	71.4%	70.4%
Measles (hematology)	76.9%	—	71.4%	71.4%
Measles (solid tumor)	54.3%	—	71.4%	69.2%
Mumps (total)	55.6%	—	60.7%	51.9%
Mumps (hematology)	53.9%	—	57.1%	42.9%
Mumps (solid tumor)	57.1%	—	64.3%	61.5%
Rubella (total)	70.4%	—	64.3%	70.4%
Rubella (hematology)	69.2%	—	64.3%	69.2%
Rubella (solid tumor)	71.4%	—	64.3%	71.4%

months following treatment. Despite normalization of T- and B-cell functions in vitro, they respond poorly to immunization with T cell–dependent antigens, indicating residual in vivo immune deficit [8, 9]. Our study prospectively evaluated the natural recovery of humoral and cellular immunities and the recovery of antibodies to various vaccine antigens in pediatric oncology patients longitudinally from 6 months till 18 months after stopping chemotherapy, which is one of the longest periods of evaluation in reported the English literature.

In our cohort, although T-helper cells (CD4+), cytotoxic T cells (CD8+), and B lymphocytes (CD19+) remained low at 6 months after stopping chemotherapy, they had relatively normal median absolute neutrophil count, lymphocyte count, and CD4/CD8 ratio. This may be the reason for the absence of severe infections, namely bacteremia, invasive fungal infections, or septic episodes that required critical care and/or respiratory support, despite the extent of the depression of cellular and humoral immunities.

We demonstrate there was satisfactory recovery of humoral and cellular immunities throughout the study period and the immune parameters of both groups were nearly normal at the end of study. However, the CD4+ and CD8+ cells remained low at the end of study period, indicating residual immune deficit persisted up to 18 months after stopping chemotherapy.

T lymphocytes are composed of a heterogenous group of short- and long-lived cells. Under normal circumstances, the "long-lived" cells typically

contain the "naïve" subset, which are quiescent and remain in a noncycling state for months or even years while awaiting encounter with antigens. "Short-lived" cells generally contain the effectors and memory subsets, undergo variable levels of cell cycling in response to antigen, and result in ongoing modulation of their contribution to overall T-cell repertoire. When the T-cells are acutely depleted after chemotherapy, restoration of heterogenous populations of T cells and reestablishment of T-cell immunocompetence is a slow and frequent incomplete process [10, 11]. Therefore, despite the persistence of memory T cells, loss of immunological memory is still noted [12, 13]. In our study, loss of immunological memory is demonstrated by persistent loss of previous antibodies to various vaccine antigens up to 18 months after stopping chemotherapy, although other parameters indicating a good recovery to humoral immunity in vivo.

Vaccine-preventable infectious diseases are always one of the major threats in immunocompromised patients. Kaplan et al showed that severe complications occurred in about 80% of patients who acquired measles infection. The case fatality rate of measles infection was about 70% for oncology patients and about 40% for human immunodeficiency virus (HIV)-infected patients. Most important, rash could be absent in 30% of cases, which posed significant implications in patient management and infection control policy in oncology centers [14].

Our study measured serially the recovery of antibodies and estimated the duration of vulnerable periods to 7 major vaccine-preventable infectious diseases that the children would be exposed to. Our results clearly showed that there was no significant increase in percentage of seropositivity to various vaccine antigens, although immunological studies demonstrated a good recovery of humoral immunity in vivo. A significant number of subjects, ranging from 15% to 60%, still have excessive risk of acquiring potentially life-threatening vaccine-preventable infectious diseases up to 18 months after stopping chemotherapy. Our data are compatible with other published series [15, 16]. This reflects that there is residual deficit in memory T-cell function despite the other aspects demonstrated satisfactory or nearly complete recovery. This will become a significant public health problem if no further intervention is implemented, as the survival rate of pediatric oncology patients improves significantly with the improvement in various cancer treatment protocols.

Herd effect is defined as the reduction of infection or disease in the unimmunized segment as a result of immunizing a proportion of the population [17]. The estimated herd immunity thresholds for vaccine preventable infectious diseases are as follow: diphtheria: 85%, pertussis: 92–94%, poliomyelitis: 80–86%, measles: 83–94%, mumps: 75–86%, rubella: 80–85% [11, 18]. Therefore, our cohort was below the herd immunity thresholds for diphtheria, measles, mumps, and rubella during the whole 18-month study period. Booster vaccinations started after stopping chemotherapy may have

a role to decrease the proportion of unprotected subjects in the community. Patel et al. showed that children who had completed treatment of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia developed satisfactorily to 1 dose of diphtheria, tetanus, acellular pertussis, *Haemophilus influenza* type b, meningococcus C, poliomyelitis, measles, mumps, and rubella vaccines that were given at median 6 months after stopping chemotherapy. The response sustained up to 12 months after vaccinations [19]. Ek et al also showed that children who were intensively treated with chemotherapy, namely children with high-risk ALL, showed insufficient immune response to revaccination and the suboptimal response was correlated with the numbers of memory B cells and antibody-secreting cells [20].

Therefore, according to our study result and other published data, 6 months after stopping chemotherapy, including maintenance therapy, seems to be a reasonable time to start revaccination [5]. Currently, our unit adopts the following policies in our pediatric oncology patients: (i) Revaccinate with 3 doses of diphtheria-tetanus-acellular pertussis vaccines starting at 6 months after stopping all chemotherapy, each dose is 2 months apart. We do not routinely perform antibody testing of diphtheria, tetanus, and pertussis because this is not part of the routine service of our virology laboratory. (ii) Evaluate the serostatus of measles, mumps, rubella, and hepatitis B at 6 months after stopping all chemotherapy and revaccinate with 1 dose of measles-mump-rubella and 3 doses of hepatitis B vaccines for those seronegative patients. (iii) Patients can return to school 3 to 6 months after stopping chemotherapy.

We should admit that there are still not enough data to suggest the detailed strategy of revaccination, namely, (i) What is the optimal number of doses of revaccination? (ii) Should we adopt a universal approach or only revaccinate those unprotected subjects after immune check-up? (iii) Do we need to have a different revaccination schedules for standard and high risk patients?

One limitation of our study is the lack of complete set of pretreatment serology data to strongly support that the low seropositivity rate to various vaccine antigens is solely due to immunosuppressive effect of chemotherapy. We did have pretreatment data for hepatitis B and measles to support our argument. For hepatitis B, overall 68% were seropositive before starting chemotherapy (53.9% in hematology malignancies group and 84.4% in solid tumors group); after completion of treatment, the overall seropositivity dropped to about 30% and stayed at 40% up to 18 months after stopping chemotherapeutic agents. For measles, 85.7% of subjects were protective but the level was 70% after treatment completed. Despite this, our study result was compatible with other published literature that evaluated antibody levels from the time of diagnosis of malignancies [16]. Immunosuppressive effect of chemotherapeutic agents played an important role in this aspect. Moreover, due to the limitation of the design of the study, it is difficult to separate

the effect of age on absolute counts of lymphocyte subset from the effect of recovery of immune system.

CONCLUSION

Our study showed that although there was near complete immune recovery in our subjects, a significant proportion of subjects remained susceptible to various vaccine-preventable infectious diseases up to 18 months after stopping chemotherapy, which can evolve to significant public health problem if no interventions are implemented.

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Humoral Immune Response After Post-Chemotherapy Booster Diphtheria–Tetanus–Pertussis Vaccine in Pediatric Oncology Patients

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Background. The role of post-chemotherapy booster vaccination in pediatric oncology children remains to be established. In this randomized controlled study, we studied the effect of immune responses to diphtheria–tetanus–pertussis (DTP) booster vaccination in children 6 months after completing chemotherapy. ***Methods.*** Children 1–18 years old with chemotherapy completed for 6 months (baseline) were eligible. Subjects were randomized into vaccine and control group. In the former, three doses of DTP vaccine ((Aventis Pasteur Inc., Lyon, France) were administered. IgG antibody titers against diphtheria, tetanus, pertussis, hepatitis B, measles, mumps, and rubella antibodies were measured serially in vaccine and control groups. Subsets of circulating lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD16/56^+$) were quantified by flow cytometry using fluorescence-labeled monoclonal antibodies. ***Results.*** Fifty-six children (28 vaccinees; 28 controls) were enrolled. Protective antibody levels against diphtheria, tetanus, pertussis were found at baseline in 83.6%, 96.5%, 96.1% of them respectively. After three doses of DTP, all vaccinees demonstrated a sustain rise in antibody levels and the antibody titers were significantly higher than control group. 35.8% of subjects were susceptible to measles mumps and rubella infection and 69% showed anti-HBs antibody titer less than protective level up to 18 months after stopping chemotherapy. ***Conclusions.*** Post-chemotherapy booster vaccinations produced a strong and sustained effect in humoral immunity against vaccine-preventable infectious diseases. Pediatr Blood Cancer 2009;52:248–253. © 2008 Wiley-Liss, Inc.

Key words: chemotherapy; humoral immunity; oncology; vaccine

INTRODUCTION

Vaccination plays a key role in preventing infectious diseases. Effective vaccines have curtailed dramatically or almost eliminated diphtheria, measles, mumps, poliomyelitis, rubella, and tetanus in developed countries [1]. Consistent high level of vaccine coverage accompanied with intensive surveillance and effective public health disease control measures provided basis for effective prevention and possibly eradication of infectious diseases.

In children with malignancy, chemotherapy, and radiotherapy are the mainstay of treatment. Vaccination of these children presents challenges due to efficacy and safety concerns [2]. There are well-established and validated recommendation for vaccination in transplant patients [3,4]. There are very few guidelines in revaccination of children after intensive chemotherapy. Most of them were established based on data from limited published studies and expert opinions [5–7].

Restoration of normal number of B-lymphocytes and achievement of normal serum immunoglobin levels by 6 months after stopping chemotherapy in most children that have completed treatment of acute lymphoblastic leukemia (ALL) provided the basis of starting revaccination program in these patients [8,9]. The administration of booster vaccines to these patients has resulted in a good response rate. Advance in treatment protocols and improvement in drug efficacy had led to significant improvement in disease-free survival. On the other hand, intensive chemotherapy resulted in marked impairment in humoral and cellular immunities that could last for up to 6 months after stopping chemotherapy. Recent cohort studies also demonstrated different rates of loss of protective serum antibodies against poliomyelitis, tetanus, hepatitis B, measles, mumps, and rubella in pediatric oncology patients who had received intensive chemotherapy [10,11].

We conducted a randomized control trial to evaluate the antibody levels of diphtheria, pertussis, tetanus, hepatitis B, measles, mumps, and rubella after completion of treatment in pediatric oncology and to investigate their humoral immune responses to booster diphtheria, tetanus, and pertussis (DTP) vaccinations. This study was designed to answer (1) whether booster vaccination is absolutely necessary as the antibody levels may return to protective level with time after immune recovery from intensive chemotherapy and (2) whether the antibody levels produced after booster vaccination can be sustained or further booster vaccines are needed to be given afterward.

METHODS

Patients and Samples

From the period June 1, 2003 to October 31, 2004, 68 consecutive patients 1–18 years who had been treated successfully for pediatric hematological malignancy and solid tumors were recruited for evaluation of humoral immunity against vaccine-preventable diseases (hepatitis B, diphtheria, tetanus, pertussis, measles, mumps, and rubella) and their responses to booster DTP vaccine. They were all diagnosed and received treatment at the Children's Cancer Center of a university teaching hospital in Hong Kong, which is also a tertiary referral center for pediatric cancers in Hong Kong. All patients were in remission. The Clinical Research Ethics Committee of our University approved this clinical trial, and

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patients and/or their parents gave informed written consent prior to study.

Exclusion criteria included: (1) patients with past history of those listed vaccine-preventable diseases, (2) evidence of immunodeficiency before diagnosis of malignancy, (3) history of allergic or severe reactions to DTP vaccine or its components, and (4) development of relapse or secondary malignancy during the study period. A total of twelve patients did not enter the study. Eight of them did not consent for the study, two due to history of severe febrile reaction to previous DTP vaccines, and the other two were excluded because of relapse of primary disease during study period.

The acute leukemia (AL) group included acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and lymphoma; the solid tumor (ST) group included all other malignancies that arose from solid organs. Patients in each group were randomized in blocks of five for the vaccine or control group. Immunization status of patients was obtained from the history and vaccination records. Past medical history was reviewed to exclude those with history of diphtheria, tetanus, pertussis, hepatitis B, measles, mumps, and rubella infections before enrollment.

Randomization

Eligible patients were randomly assigned at 1:1 ratio into treatment and control groups in this randomized clinical trial. An independent research staff formed computer-generated randomization sequence.

Surveillance of Post-Vaccination Humoral Immunity

Serum concentrations of antibodies to diphtheria, tetanus, pertussis, measles, mumps, rubella, and hepatitis B were monitored serially started from 6 months after completion of intensive chemotherapy (visit 1). In the vaccination group, three doses of booster DTP vaccines were given at 6 months (visit 1), 8 months (visit 2), and 10 months (visit 3) after completion of chemotherapy. Each 0.5 ml dose of DTP vaccine (Aventis Pasteur, Lyon, France) contained ≥30 IU purified diphtheria toxoid, ≥60 IU purified tetanus toxoid and ≥4 IU heat-inactivated *Bordetella perutssis*. These vaccines were stored at 4°C until use. No intervention was performed for the control group. Blood for diphtheria, tetanus, pertussis, hepatitis B, measles, mumps, and rubella antibodies were checked at visit 1, visit 2, visit 4, and visit 5. Table I illustrates the study timeline.

Serological Studies

Serum samples from the above four visits were stored at −30°C until analysis in one batch. Commercially available kits were used for the determination of antibody titers. Serum levels of specific antibodies to diphtheria, tetanus, and pertussis were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany). The protective antibody levels were defined as 4 IU/mL for diphtheria, 2 IU/mL for tetanus, and 24 IU/mL for pertussis. Titer to Hepatitis B surface antibody (anti-HBs) was measured by using an enzyme-linked fluorescent immunoassay. Antibody level ≥10 mIU/ml was considered as protective. Titers to measles, mumps, and rubella were measured by using enzyme-linked fluorescent immunoassay for IgG antibodies against each of the respective pathogens.

Assessment of Cellular and Humoral Immunity

Subsets of circulating lymphocytes were quantified by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA) using fluorescence-labeled monoclonal antibodies to $CD3^+$, $CD4^+$ and $CD8^+$ (T lymphocytes), $CD19^+$ (mature B lymphocytes) and $CD16/56^-$ (natural killer cells). Serum IgG concentration was measured by nephelometry (Binding Site, Birmingham, UK).

Statistical Analysis

The primary outcomes were the differences in serum concentrations of specific antibodies to diphtheria, tetanus, and pertussis between vaccine and control groups at the end of the 12-month study period. Secondary outcomes included the differences in serum concentrations of total IgG and anti-HBs, measles, mumps, and rubella, as well as circulating lymphocyte subpopulations, between the two groups, and longitudinal changes in serum diphtheria, tetanus, and pertussis antibody levels in the patients and controls at baseline and 12 months following vaccination.

Data were expressed as mean and standard deviation unless otherwise stated. Serum concentrations of specific antibodies were $\log_{10}$-transformed to achieve normal distribution of data before analysis. Demographic data between different subgroups were analyzed using Student's *t*-test for independent parametric variables and χ^2 for categorical variables between different disease or patient groups. Statistical analysis was performed only for subjects who had received at least two doses of DTP vaccines. Patients who relapsed

TABLE I. Study Flowchart

	Time Following Completion of Chemotherapy				
	6 months (baseline, visit 1)	8 months (visit 2)	10 months (visit 3)	12 months (visit 4)	18 months (visit 5)
Immunological surveillance					
Complete blood count with differential's	+	+	−	+	+
Lymphocyte subsets by flow cytometry	+	−	−	+/-	+/−
Serum IgG, IgM and IgA levels	+	−	−	+	+
Antibodies to diphtheria, tetanus, and pertussis	+	+	−	+	+
Antibody to hepatitis B virus surface antigen	+	+	−	+	+
Antibodies to mumps, measles, rubella	+	−	−	+	+
Booster vaccination					
DTP vaccine	+	+	+	−	−

during follow-up were excluded from analysis. Following DTP vaccination, the longitudinal changes in serum antibody levels between study visits were analyzed using paired *t*-test. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL), and $P < 0.05$ was considered to be statistically significant.

RESULTS

Study Populations

During the study period, 68 consecutive children fulfilled the inclusion criteria. Thirty-seven suffered from acute leukemia (AL) and 31 had solid tumors (ST).Twenty-nine patients with AL (24 ALL; 5 AML) were recruited. Fourteen were randomized to the vaccination group and 15 to the control group. Twenty-seven ST patients were recruited with 14 and 13 being randomized into the vaccination and control groups respectively. In total, 28 patients received three doses of booster DTP vaccine and another 28 patients were in the control group. All patients were followed up until the end of study. Eighty-four booster vaccines were given. No significant vaccine-related adverse reactions were noted. The baseline characteristics of subjects in vaccination and control groups were comparable (Table II).

Baseline Antibody Titer

At baseline, 16.4% patients had diphtheria antibody titer below protective level, and 3.5% and 3.9% of patients were seronegative for tetanus and pertussis, respectively. For measles, mumps, and rubella, 35.8% of patients did not have protective levels of these specific antibodies. Sixty-nine percent of patients were susceptible to hepatitis B infection (Table III).

Humoral Immunity to Booster DTP Vaccinations

After three doses of DTP vaccine, 100% of patients demonstrated seropositivity against diphtheria, tetanus, and pertussis. The responses were sustained till 1 year after first dose of DTP vaccine (Table III). The geometric mean of antibody titers against diphtheria, tetanus, and pertussis are significantly higher than baseline and the response were sustained till 1 year after vaccinations. The longitudinal changes in serum diphtheria, tetanus, and pertussis antibody levels during the study period are shown in Figure 1. Booster DTP vaccinations were efficacious in increasing the respective specific antibodies. During the 12-month follow up, the seropositivity rates were similar among unvaccinated patients. The seropositive rate of mumps, measles, rubella and hepatitis B in both groups were unchanged during this study. Circulating numbers of both total T lymphocytes ($CD3^+$) and T-helper lymphocytes ($CD3^+CD4^+$) increased during the study period in both vaccine and control groups ($P < 0.05$ for all), but there were no differences in these parameters at either baseline or end-of-study between the two groups (Table IV).

DISCUSSION

The safe and effective use of vaccines has always been a major challenge in immunocompromised patients. The key concerns are the safety and the ability of patients to first mount and then sustain protective immune response. There are well-established and validated recommendations for revaccination patients who have undergone hematopoietic stem cell transplantation but such information is still missing for post-chemotherapy patients [3,4]. Although a number of cohort studies were published to address the change of antibody titers and response to booster vaccine after chemotherapy. There are no validated recommendations for booster vaccination program for post-chemotherapy patients [12–14].

Thirty years ago, de Vaan et al. [15] conducted a study to investigate the serial changes in antibody titers to diphtheria, pertussis, tetanus, and poliomyelitis (types I, II, and III) after stopping chemotherapy in 49 children with acute lymphoblastic leukemia (ALL) and their response to revaccination with DT-Polio vaccine 1 year after cessation of treatment. The antibody titers were lower than in healthy controls but still at protective levels in most patients. No spontaneous rise in antibody titer was shown before revaccination in the first year post-treatment, but a rise in antibody titers was demonstrated which was similar to healthy controls after revaccination.

With the increase in dose intensity of treatment of ALL and other malignancies, the disease-free survival has improved; however,

TABLE II. Baseline Clinical and Laboratory Characteristics of Subjects

Parameter	Vaccine group (n = 28)	Control group (n = 28)	*P*
Age, years	8.8 ± 3.7	8.0 ± 3.8	0.442
Male, n (%)	20 (71.4)	18 (50.0)	0.567
Duration of prior chemotherapy, months	15.5 ± 8.6	16.1 ± 8.9	0.796
Serum total IgG level, g/L	10.02 ± 2.43	9.53 ± 2.39	0.451
Total white cell count, 10^9/L	7.2 ± 2.2	7.8 ± 2.5	0.382
Absolute neutrophil count, 10^9/L	4.15 ± 1.93	4.45 ± 1.95	0.565
Absolute lymphocyte count, 10^9/L	2.35 ± 0.99	2.43 ± 0.79	0.744
Circulating lymphocyte subsets			
Total T cells ($CD3^+$), 10^9/L	1.39 ± 0.66	1.42 ± 0.52	0.827
T-suppressor cells ($CD3^+CD8^+$), 10^9/L	0.62 ± 0.29	0.65 ± 0.31	0.685
T-helper cells ($CD3^+CD4^+$), 10^9/L	0.68 ± 0.38	0.66 ± 0.27	0.784
$CD4^+$:$CD8^+$ ratio	1.15 ± 0.39	1.11 ± 0.43	0.696
Mature B cells ($CD19^+$), 10^9/L	0.67 ± 0.35	0.66 ± 0.28	0.861
Natural killer cells ($CD16/56^+$), 10^9/L	0.23 ± 0.12	0.27 ± 0.17	0.261

Results expressed as mean ± standard deviation unless otherwise stated.

TABLE III. Changes in Seropositivity Rates for Vaccine-Preventable Diseases at Baseline and at Visit 5

	Vaccine group			Control group		
Disease	Baseline[a] (%)	Visit 5[b] (%)	P^c	Baseline (%)	Visit 5 (%)	P^c
Diphtheria	85.7	100	0.125	81.5	92.6	0.500
Tetanus	92.9	100	0.500	100	96.3	1.000
Pertussis	100	100	—	92.6	100	0.500
Measles	63.0	54.5	1.000	70.4	76.9	1.000
Mumps	66.7	58.3	1.000	57.7	33.3	1.000
Rubella	53.6	66.7	1.000	74.1	75.0	1.000
Hepatitis B	32.1	18.2	1.000	29.6	60.0	0.375

[a]Six months after stopping chemotherapy; [b]Twelve months after first dose of DTP vaccine; [c]Analyzed by McNemar test for paired binomial variables.

immune reconstitution after treatment may be severely prolonged [16–20]. In our study, chemotherapy induced a loss of protective serum antibody titers for diphtheria, tetanus, pertussis, measles, mumps, rubella, and hepatitis B at 6 months after stopping chemotherapy respectively. Hepatitis B, measles, mumps, and rubella antibody titers were most affected by the immunosuppressive effect of cytotoxic therapy. These findings were consistent with other published data [10,12,19].

The administration of booster DTP vaccines starting at 6 months after stopping chemotherapy enabled 100% of patients in treatment group to recover protective antibody at a persistently high titers. The antibody titers were also sustained above the protective level throughout the 1-year follow up period. This finding suggests that chemotherapy did not entirely abolish the specific humoral immune memory in patients with undetectable serum antibody titers after chemotherapy. The impaired capacity to mount an immune response

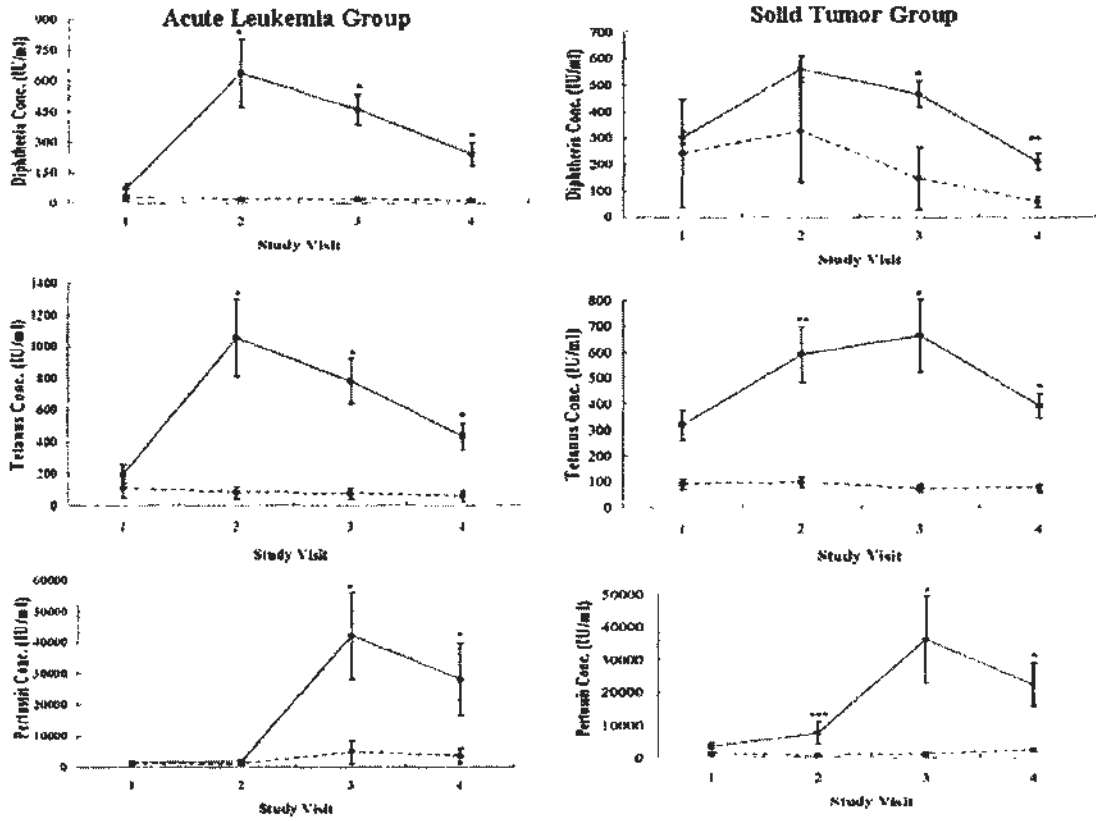

Fig. 1. Longitudinal changes in serum concentrations of diphtheria, tetanus, and pertussis antibodies in patients with acute leukemia and solid tumors from the vaccine (solid lines) and control (dashed lines) groups. Error bars represent standard errors of mean. Asterisks are significant P-values for comparisons between vaccine and control groups at respective study visits: $^*P < 0.001$; $^{**}P < 0.005$; $^{***}P < 0.01$.

TABLE IV. Changes in Circulating Lymphocyte Subpopulations in our Cancer Children With and Without DTP Vaccination

		Vaccine group		Control group		
Lymphocyte subset	Study visit	Count (10^9/L)	P*	Count (10^9/L)	P*	P**
$CD3^+$	1	1.41 ± 0.65	—	1.43 ± 0.52	—	0.827
	5	1.72 ± 0.68	0.015	1.76 ± 0.68	0.010	0.825
$CD3^+CD4^+$	1	0.70 ± 0.38	—	0.67 ± 0.27	—	0.784
	5	0.83 ± 0.33	0.019	0.84 ± 0.38	0.014	0.908
$CD3^+CD8^+$	1	0.63 ± 0.29	—	0.66 ± 0.31	—	0.685
	5	0.73 ± 0.33	0.078	0.74 ± 0.29	0.122	0.886
$CD4^+$:$CD8^+$ ratio	1	1.14 ± 0.40	—	1.10 ± 0.44	—	0.696
	5	1.19 ± 0.33	0.314	1.15 ± 0.32	0.330	0.771
$CD19^+$	1	0.69 ± 0.35	—	0.65 ± 0.28	—	0.861
	5	0.73 ± 0.28	0.467	0.72 ± 0.34	0.171	0.935
$CD16/56^+$	1	0.23 ± 0.12	—	0.28 ± 0.17	—	0.261
	5	0.25 ± 0.11	0.390	0.23 ± 0.10	0.152	0.443

*Analyzed by paired t-test between values at visit 1 (baseline) and visit 5 (post-vaccination); **Analyzed by Student's t-test between the two groups at each visit.

is temporary and it tends to return to normal 6 months after the end ofchemotherapy. In the control group, although the process of immune reconstruction continued, there was no significant increase in percentage of patients become seropositive and there is no significant change in the absolute antibodies titers throughout 1 year indicating they are still vulnerable to these vaccine-preventable diseases, indicating there is a role for post-chemotherapy booster vaccination. A total of 84 booster vaccinations were given. No significant adverse events were noted during the entire study period.

Important questions that remain unanswered include: (1) What is the optimal number of doses of DTP vaccine to produce sustained immune response? (2) What is the optimal timing and number of booster dose of MMR and hepatitis B vaccines?

In conclusion, our study demonstrated that intensive chemotherapy leads to loss of protective serum antibody titers for different types of vaccine-preventable infectious diseases. Booster vaccinations started at 6-month after stopping chemotherapy can be safely administered and effectively restore a sustained effect in humoral immunity against various types of vaccine-preventable infectious diseases.

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Pandemic influenza A (2009-H1N1) infection in paediatric oncology patients in Hong Kong

The overall clinical picture of pandemic H1N1 infection in two of the most vulnerable patient groups – paediatric oncology and haematopoietic stem cell transplant (HSCT) recipients is not fully understood. Traditionally, severely immunocompromised patients are well-known to suffer from complications of influenza infection. A recent study described the clinical presentation of pandemic influenza A (2009-H1N1) in children with acute lymphoblastic leukaemia, demonstrating similar findings (Launes *et al*, 2010).

In Hong Kong, which has a population of seven million and more than 34 000 reported cases of H1N1 in 2009, we performed a territory-wide study to evaluate the clinical course of this novel infection in hospitalized paediatric oncology patients with laboratory-confirmed pandemic influenza (2009-H1N1) infection. Contrary to the results of Launes *et al* (2010), we report a different finding.

The following case definitions were used to recruit patients for review: (i) children aged ≤18 years old with oncological conditions or HSCT recipients who were receiving active treatment presenting with influenza-like illness (ILI) and laboratory-confirmed pandemic H1N1 influenza infection or (ii) children aged ≤ 18 years old with oncological conditions or HSCT recipients who had completed chemotherapy or discontinued immunosuppressants less than 12 months earlier, presenting with ILI and laboratory-confirmed pandemic H1N1 infection. The definition of ILI was fever (temperature ≥38°C) with either cough, sore throat or both for at least 24 h. Laboratory-confirmed pandemic H1N1 infection was defined as the detection of H1N1-H1 gene by real-time reverse-transcription polymerase chain reaction (RT-PCR) in nasopharyngeal aspirate (NPA) or nasopharyngeal swab or combined oropharyngeal and nasal swab sample.

Table I. Patient characteristics.

Patient	Sex/Age (years)	Primary disease	Treatment phase
1	F/3·4	Acute lymphoblastic leukaemia (ALL)	Intensive chemotherapy
2	M/5·7	Cerebellar medulloblastoma	Intensive chemotherapy
3	M/13·7	Relapsed ALL	Intensive chemotherapy
4	M/8·3	Relapsed ALL	Maintenance chemotherapy
5	M/7·8	ALL	Maintenance chemotherapy
6	M/11·8	T-cell lymphoma	Maintenance chemotherapy
7	M/12·3	ALL	Maintenance chemotherapy
8	M/12·4	Oligodendroglioma	Intensive chemotherapy
9	M/13·2	CNS* germinoma	Intensive chemotherapy
10	F/7·1	Beta-thalassaemia	Post-unrelated cord blood transplant (CBT) 25 months with chronic GVHD† of skin and lung on cyclosporine A
11	F/7·2	T-cell lymphoma, MDS‡	Post-unrelated CBT 23 months with chronic skin GVHD on cyclosporine A
12	M/12·5	Ph§+ve ALL, choroid plexus tumour	Post-unrelated CBT 36 months with chronic GVHD
13	M/13·3	Adrenoleucodystrophy	Post-unrelated CBT 45 months with chronic skin GVHD on prednisolone
14	F/14·1	Relapsed acute myeloid leukaemia (AML)	HLA-identical sibling post-bone marrow transplant (BMT) 9 months
15	M/14·2	Relapsed Ph+ve ALL	HLA-identical sibling PBSC¶ transplant 20 months with extensive chronic GVHD of skin, lung (bronchiolitis, obliterans), gut and mucosa on prednisolone, cyclosporine A, sirolimus and mycophenolate mofetil
16	M/14·6	Refractory AML	Post-unrelated CBT 16 months with chronic skin GVHD on cyclosporine A

*Central nervous system.
†Graft-*versus*-host disease.
‡Myelodysplastic syndrome.
§Philadelphia chromosome.
¶Peripheral blood stem cell.

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Table II Clinical presentation and outcome of pandemic H1N1 infection

Patient	Presenting symptoms	ANC*	LYM†	CXR‡ changes	Duration of fever (d)	Treatment	Antiviral therapy started on days of fever (d)	Duration of PCR remained positive (d)	Outcome
1	Fever, cough, runny nose, sore throat	5·7	1·8	No	6	Oseltamivir 30 mg BD for 5 d, Empirical antibiotics for 2 d	6	Remained positive on day 14 of illness	Discharge on day 7 of illness
2	Fever, cough	0·7	0·1	No	3	Oseltamivir 45 mg BD for 5 d, Empirical antibiotics for 7 d	3	10	Discharge on day 8 of illness
3	Fever, runny nose, cough, sore throat	0·1	0·3	Yes (bilateral hilar streakiness)	5	Oseltamivir 75 mg BD for 3 d then 150 mg BD for 7 d Empirical antibiotics for 7 d	3	ND§	Required 2 l/min O supplement Discharge on day 9 of illness
4	Fever, runny nose, cough	1·5	0·5	Yes (bilateral hilar streakiness)	1·2	Oseltamivir 60 mg BD for 5 d	1	ND	Discharge on day 3 of illness
5	Fever	3·1	1·1	No	3	Oseltamivir 60 mg BD for 5 d	1	ND	Discharge on day 4 of illness
6	Fever, cough, runny nose, sore throat	4·4	0·2	No	2	Oseltamivir 60 mg BD for 5 d	1	Remained positive on day 14 of illness	Discharge on day 4 of illness
7	Fever, cough, runny nose, sore throat	3·4	0·75	No	3	Oseltamivir 60 mg BD for 7 d, Empirical antibiotics for 5 d	2	ND	Discharge on day 6 of illness
8	Fever, cough, runny nose, sore throat, chills, rigour, vomiting	3·1	0·4	No	2	Oseltamivir 60 mg BD for 5 d	2	8	Discharge on day 4 of illness
9	Fever, sore throat, cough	0·2	0·4	No	4	Oseltamivir 75 mg BD for 5 d	2	7	Discharge on day 7 of illness
10	Fever, cough, myalgia, chills, rigour, malaise	4·4	1·2	No	4	2 courses of oseltamivir 60 mg BD for 5 d, Empirical antibiotics for 5 d	1	ND	Discharge on day 6 of illness
11	Fever, runny nose	11·0	1·4	No	1	Oseltamivir 60 mg BD for 10 d, Empirical antibiotics for 5 d	1	ND	Discharge on day 4 of illness
12	Fever	4·3	2·2	No	9	Oseltamivir 75 mg BD for 10 d	1	ND	Discharge on day 10 of illness
13	Fever, cough, runny nose, tonic clonic seizure	7·6	3·8	No	4	Oseltamivir 150 mg BD for 10 d	1	ND	Discharge on day 6 of illness
14	Fever, sore throat, malaise	4·0	3·1	No	4	Oseltamivir 75 mg BD for 10 d	1	ND	Discharge on day 5 of illness
15	Fever, cough, runny nose	2·3	0·9	No	2	2 courses of oseltamivir 75 mg BD for 5 d, Empirical antibiotics	1	Remained positive on day 14 of illness	Required 2 l/min O_2 supplement, discharge on day 12 of illness

Table II (Continued)

Patient	Presenting symptoms	ANC*	LYM†	CXR‡ changes	Duration of fever (d)	Treatment	Antiviral therapy started on days of fever (d)	Duration of PCR remained positive (d)	Outcome
16	Fever, cough, sore throat, chills	3·2	0·9	No	4	Oseltamivir 75 mg BD for 10 d	1	ND	Discharge on day 6 of illness

*Absolute neutrophil count (10^9/l)
†Absolute lymphocyte count (10^9/l)
‡Chest X ray
§Not done

The following data were retrieved from patients' records: (i) baseline demographic data, (ii) presenting symptoms/signs and laboratory results at diagnosis; (iii) clinical course; (iv) treatment offered; (v) clinical outcome.

Sixteen consecutive patients (12 male: 4 female) fulfilled the case definition and were recruited. The median age was 12·4 years (range 3·4–14·6 years). The primary diseases included: (i) acute lymphoblastic leukaemia (n = 7), (ii) brain tumour (n = 4); (iii) acute myeloid leukaemia (n = 2), (iv) lymphoma, (n = 1); (v) others (n = 2). Nine patients were receiving chemotherapy according to their disease-specific protocols. Seven patients have received HSCT, of whom six had chronic graft-versus-host disease (GVHD).

The most common presenting symptoms were fever (100%), cough (75·0%) and runny nose (56·3%) and sore throat (50·0%). One patient (Patient 13), with a history of epilepsy presented with generalized convulsion and fever. One patient (Patient 8) presented with vomiting but none presented with diarrhoea.

Concerning antiviral therapy, oseltamivir was started at median 1 d after fever onset (range from 1 to 6 d). Eight patients (50·0%) received one course of 5–7 d of oseltamvir. Patients 3, 10 and 15 had recurrence of fever and worsening of respiratory symptoms after a 5-d course of oseltamivir and required an extra 5-d of high dose antiviral therapy.

Seven patients had repeated virological studies, four had become negative by day 14 from diagnosis but three showed persistent positive PCR results up to day 24 of illness. No laboratory-confirmed secondary nosocomial spread of infection was recorded during the 5-month study period. The details are shown in Tables I and II.

Two patients (Patients 3, 15) developed tachypnea and required oxygen supplement via nasal cannula. They did not require intensive care or requirement of mechanical ventilation. All of our patients recovered fully and none of them had residual morbidity complications.

In our cohort of immunocompromised cancer children, the most commonly reported symptoms included fever, cough and runny nose. Gastrointestinal symptoms (nausea, vomiting and/or diarrhoea) previously reported with pandemic H1N1 influenza were not common in our series.

Although the clinical presentation of patients who were hospitalized with pandemic (2009-H1N1) influenza were generally similar to those reported during peak periods of seasonal influenza, the epidemiology of mostly affected population is quite different. Jain *et al* (2009) studied 272 hospitalized patients with pandemic H1N1 infection in United States. Up to 45% of hospitalizations involved persons under the age of 18 years, more than one-third of the patients were aged between 18 and 49 years, and only 5% were 65 years of age or older. Seventy-three percent of the patients had at least one underlying medical condition, which included asthma; diabetes; heart, lung, and neurological diseases, and pregnancy (Jain *et al*, 2009).

Cao *et al* (2009) described the clinical features of 426 cases of pandemic H1N1 infection in China, the majority of which ran a mild clinical course. The virus could be detected by real time RT-PCR for 6 d. The duration of infection might be shortened if antiviral agent was administered. Webb *et al* (2010) reported that about 93% of the patients who required intensive care were younger than 65 years old and 10% were pregnant women. All these data suggest that young patients constitute one of the highest risk groups of pandemic H1N1 infection. However, our patient series, traditionally believed to be one of the highest risk groups for severe influenza infection, ran a relative uncomplicated clinical course and all of them recovered uneventfully from this infection. This might be probably due to the early start of antiviral therapy (median 1 d after onset of fever), which halted the propagation of virus and decreased the viral load in the immunocompromised hosts. However, three patients (Patients 3, 10 and 15) required an additional course of high-dose antiviral therapy due to recurrence of fever and respiratory symptoms. We also observed that HSCT recipients more commonly to suffered from prolonged or recurrent respiratory symptoms, therefore, we routinely prescribed a 10-d course of antiviral therapy in these patients in the latter half of the study period. Jain *et al* (2009) also demonstrated that

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BRIEF REPORT
Post-Transplant EBV-Related Lymphoproliferative Disorder Complicating Umbilical Cord Blood Transplantation in Patients of Adrenoleukodystrophy

Frankie Wai Tsoi Cheng, MRCPCH,[1]* Vincent Lee, MRCP,[1] Ka Fai To, FRCP,[2] K.C. Allen Chan, FRCPA,[3] Ming Kong Shing, MRCP,[1] and Chi Kong Li, MD[1]

EBV-associated post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication following solid organ transplantation and hematopoietic stem cell transplantation (HSCT) using bone marrow or peripheral blood as stem cell sources, but rarely reported in umbilical cord blood transplantation (UCBT). We report two cases in unrelated UCBT setting and added the following new information to the literature: (i) EBV-related PTLD can be presented late in recipients of unrelated UCBT; (ii) in contrast to reported literatures that PTLD is a serious complication with unfavorable outcome, especially in monomorphic form, our cases showed that the clinical course may be relatively benign if treatment is initiated promptly. Pediatr Blood Cancer 2009;53:1329–1331.

Key words: lymphoproliferative disease; umbilical cord blood transplantation; X-linked adrenoleukodystrophy

INTRODUCTION

Epstein–Barr virus-associated post-transplant lymphoproliferative disorder (EBV-PTLD) has been well studied in solid organ transplantation and allogeneic bone marrow and peripheral blood stem cell transplantation settings but it is only recently reported after umbilical cord blood transplantation (UCBT) [1,2].

X-linked ALD is a peroxisomal disorder due to a defect in gene ABCD1 on chromosome Xq28. Childhood cerebral X-linked ALD is the most common phenotype, causing rapid neurodegeneration affecting central nervous system myelin and adrenal cortex. It can rapidly progress to vegetative state if left untreated [3]. Early hematopoietic stem cell transplant can arrest progression of childhood cerebral X-linked ALD [4–6].

In this report, we describe two patients who developed PTLD associated with EBV infection after receiving UCBT.

CASE REPORT

Patient 1

This patient presented at the age of 8 years with hyperpigmentation, increased clumsiness, and frequent falls. Synacthen test showed adrenal insufficiency, and a diagnosis of adrenoleukodystrophy (ALD) was confirmed by elevated plasma levels of VLCFA. Magnetic resonance imaging (MRI) of the brain showed demyelinating changes consistent with ALD.

A two-antigen mismatched unrelated UCBT was performed with conditioning regimen consisted of oral busulfan (16 mg/kg), intravenous cyclophosphamide (200 mg/kg), and antithymocyte globulin (90 mg/kg). Cyclosporine and prednisolone were used as graft-versus-host disease (GVHD) prophylaxis. He was EBV (IgG EBV VCA) seropositive before transplant. Engraftment was achieved on day 13 post-transplant with complete donor chimerism. He developed grade II GVHD on day 43, which progressed to extensive chronic GVHD involved skin and gut which required treatment with steroid and cyclosporine. Immune surveillance showed decreased CD4+ level (0.14×10^9/L) and was eventually normalized at 9 months post-transplant indicating a prolonged period of immune reconstitution.

A left tonsillar ulcer was first noted on day 74 post-transplant. Bacterial and virologic investigations were all negative. Computer tomography scan (CT scan) showed a mass measuring 1.7 cm in the left tonsillar region, compatible with an enlarged lymph node and other areas were unremarkable. Tonsillectomy was subsequently performed on day 108 post-transplant. Histology showed diffuse monomorphic proliferation of lymphoid cells, B-cell markers were positive, and EBER was positive by in-situ hybridization. He was diagnosed to have localized early-onset EBV-associated monomorphic PTLD.

Cyclosporine was withdrawn rapidly trying to reduce immunosuppression, while prednisolone dose was optimized in order to control chronic GVHD. The dose was slowly reduced over 1 year, guided by the activity of chronic GVHD. He was also given monthly immunoglobulin infusions as an immunomodulator. The patient is now in remission at 9 years follow-up without evidence of recurrence of PTLD.

Patient 2

This patient was asymptomatic and screened for ALD, as two elder brothers, both deceased, had also been diagnosed with this condition. His VLCFA was found to be elevated at the age of 2 years. At the age of 5 years, he was found to have adrenal insufficiency, and commenced on hydrocortisone. MRI brain imaging showed progressive white matter changes between the ages of 7 and 8 years. He also showed some early features of deteriorating psychological

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tests that prompted early hematopoietic stem cell transplantation (HSCT) at the age of 8.

He underwent a two-antigen mismatched unrelated UCBT with conditioning regimen consisted of oral busulfan (16 mg/kg), intravenous cyclophosphamide (200 mg/kg), and antithymocyte globulin (90 mg/kg). Cyclosporine was used as GVHD prophylaxis. Engraftment was achieved on day 20 with complete donor chimerism. He developed grade II GVHD on day 17. Steroid was added to cyclosporine treatment. This progressed to chronic skin GVHD which required steroid and cyclosporine treatment. His T-cell surveillance showed decreased CD4+ (0.11×10^9/L) and CD8+ (0.27×10^9/L) till 12 months after UCBT indicating a prolonged period of impaired T-cell reconstitution and immunosuppression after transplant.

The patient developed recurrent episodes of fever, bilateral cervical lymphadenopathies, and hypotension requiring fluid resuscitation and on two occasions, inotropes since 14 months post-transplant. He was treated as sepsis with repeated courses of broad-spectrum antibiotics, and also given stress doses of hydrocortisone during these febrile episodes. Cervical lymphadenopathies and fever subsided after treatment. Microbiological investigations were all normal. PTLD was suspected following three similar episodes within 2 months. He was EBV seropositive (IgG EBV VCA) before transplant. His initial EBV DNA was 22,000 copies/ml. CT scan of whole body showed generalized lymphadenopathies in the submandibular, submental, cervical, axillary, mediastinal, abdominal, pelvic, and inguinal regions. There was also splenomegaly and bilateral pleural effusions. Cervical lymph node biopsy was performed and showed diffuse, polymorphic proliferation of lymphoid cells, positive for B-cell markers. EBER was positive on in-situ hybridization in 20–25% of lymphoid cells. This patient was diagnosed of having late onset EBV-associated polymorphic PTLD.

Cyclosporine was rapidly withdrawn. Rituximab 375 mg/m^2 was given weekly for 6 weeks. High-dose acyclovir (500 mg/m^2 every 8 hr) was given for 14 days, then reduced to prophylactic dose (250 mg/m^2 every 8 hr). The patient responded well, with resolution of fever, neck swelling, and a rapid reduction of EBV DNA. Ten months following diagnosis, EBV DNA level was undetectable in plasma. He was also given monthly immunoglobulin infusions as an immunomodulator. This patient remains well at 3-year follow-up without flare up of PTLD.

DISCUSSION

EBV-associated PTLD is a well-recognized complication following solid organ transplantation and HSCT using bone marrow or peripheral blood stem cell sources, but rarely reported in UCBT. It represents a heterogeneous group of abnormal lymphoid proliferation. Most cases are B-cell derived, and associated with EBV infection. In UCBT, it is believed that the donors are nearly all EBV negative and, hence, the risk of EBV-related PTLD is low when compared with other forms of HSCT [7]. At that time, our unit did not adopt the policy to routinely screen for EBV DNA due to the rarity of disease in UCBT recipients.

Barker et al. and Brunstein et al. showed that the incidence of EBV-related PTLD in unrelated UCBT with myeloablative or nonmyeloablative conditioning regimens was 2.0–4.5% and was actually comparable with other HSCT settings. This complication occurred at median 6 months after transplant [1,8]. This was attributed to the use of antithymocyte globulin [1,8]. In our center, 45 UCBT were performed and only 2 patients with ALD developed PTLD. None of the other 247 bone marrow or peripheral blood stem cell transplants developed this complication. PTLD commonly presents in the first 3–6 months following transplantation, such a late presentation (patient 2) was unexpected and unprecedented. Delayed in T-cell immune reconstitution and establishment of EBV-specific cytotoxic T-cell response in UCBT setting may play a role.

There are reports demonstrating presence of EBV genome in cord blood and make this a likely source of entry into host, or there is possibility of primary EBV infection in engrafted donor cells by residual infected host cells, or less likely via transfusion of blood products [9]. In our two cases, there was no evidence to suggest the presence of EBV genome in cord blood samples or of primary EBV infection before the onset of PTLD, indicating the origin of EBV-PTLD may be of recipient origin.

One of the most important treatments in PTLD is reduction of immunosuppressants if feasible. A combination of surgical resection and reduction of immunosuppression was successful in inducing complete remission for Patient 1 with localized disease.

Rituximab, a monoclonal antibody therapy (anti-CD 20), can induce complete remission in patients with early onset PTLD. However, early treatment algorithm for PTLD also includes antiviral therapy in an attempt to control EBV infection. It may be successful for treating EBV-associated PTLD with a significant lytic replication of EBV [10,11]. There is a report of using of IVIG in combination with antiviral agent and reduction of immunosuppressants leading to termination of the process and inducing a significant and persistent response, possibly by enabling EBV-specific T-cell control [12]. The mechanism of immunomodulation by IVIG in PTLD still remains to be determined and there are reports to suggest that IVIG may be active even in patients who do not respond to upfront antiviral therapy in solid organ transplantation setting [12]. Both patients received monthly IVIG as immunomodulatory agent. Patient 2 was treated with rituximab and acyclovir. The EBV DNA concentration reduced rapidly once rituximab was commenced. Rituximab was not yet licensed for use in B-cell non-Hodgkin's lymphoma at the time when Patient 1 was first diagnosed with PTLD.

Preemptive therapy with rituximab and regular monitoring of EBV reactivation in allogeneic HSCT result in improved outcome for patients at high risk of developing PTLD, with marked decrease in incidence of PTLD and also reduction in extensive disease and subsequently mortality [13–15].

Our case reports add the following new information to the literature: (i) EBV-related PTLD can be presented late in UCBT setting. Early clinical suspicion will lead to timely and prompt diagnosis; (ii) in contrast to reported literature that PTLD is a serious complication with unfavorable outcome, especially in monomorphic form, our case report showed that the clinical course may be relatively benign if treatment is initiated promptly [7]. Routine EBV surveillance with preemptive treatment of anti-CD20 antibody can be used when there is evidence of EBV reactivation. EBV-related PTLD can present late in recipients of UCBT. Routine EBV monitoring for early diagnosis with preemptive treatment may improve patients' outcome.

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the early use of antiviral drugs was beneficial in hospitalized patients.

In summary, fever, cough, runny nose and sore throat were the most common presenting symptoms. In contrast to the findings of Launes *et al* (2010), the uncomplicated course of pandemic H1N1 infection in our cohort of paediatric oncology and HSCT recipients may have been related to the early initiation of antiviral therapy.

Conflict of interest statement

None to declare.

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HHV-6 encephalitis in pediatric unrelated umbilical cord transplantation: A role for ganciclovir prophylaxis?

Cheng FWT, Lee V, Leung WK, Chan PKS, Leung TF, Shing MK, Li CK. HHV-6 encephalitis in pediatric unrelated umbilical cord transplantation: A role for ganciclovir prophylaxis?
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Abstract: The role of ganciclovir as HHV-6 prophylaxis in unrelated HSCT setting remains controversial. We performed an eight-yr retrospective review of patients received unrelated HSCT from January 2000 to September 2008. From January 2002, ganciclovir prophylaxis 5 mg/kg twice daily for seven days for all unrelated HSCT before transplant was adopted. The prevalence of HHV-6 encephalitis was studied before and after the change in policy. Fifty-four unrelated HSCT were performed from January 2000 to September 2008. Four cases (7.4%) of HHV-6 encephalitis were diagnosed. All of them were due to variant B infection. Two cases out of 16 cases (12.5%) were diagnosed before adoption of the policy; two cases out of 38 cases (5.3%) were diagnosed afterward. All of them were unrelated UCB transplant recipients. They were all seropositive to HHV-6 before transplant. Two cases complicated with significant residual neurological deficit and refractory seizure. The other two cases died of other transplant-related mortalities. We conclude that HHV-6 encephalitis is still a rare complication of unrelated HSCT and may be more common in unrelated UCB transplant. Routine use of ganciclovir as HHV-6 prophylaxis in all unrelated HSCT recipients may not be justified but may have a role in unrelated UCB transplant.

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HHV-6 was first isolated in peripheral blood of patients with acquired immune deficiency syndrome and other lymphoproliferative disorders in 1986 (1). Primary infection occurs in early childhood and has a lifelong latency period (2). Reactivation of HHV-6 commonly occurs within the first 30 days of post-transplant period and may cause significant transplant-related morbidity and mortality (3).

Ganciclovir is a nucleoside analogue of guanosine. Through conversion to ganciclovir triphosphate, it competitively inhibits the incorporation of deoxyguanosine triphosphate by viral DNA polymerase and result in termination of elongation of viral DNA. This is the first antiviral agent to be shown effective in treatment of CMV disease in human. In tissue culture, ganciclovir has excellent antiviral activity against different herpes group of viruses including HSV type 1, HSV type 2, varicella-zoster virus, EBV, and HHV-6 (4). Most patients diagnosed HHV-6 encephalitis were treated with ganciclovir and/or foscarnet. The treatment outcome remained poor despite showing clearance of HHV-6 DNA in CSF and plasma samples after antiviral therapy (5).

There was evidence to suggest antiviral prophylaxis may prevent HHV-6 reactivation in bone marrow transplant recipients (6). Our unit adopted the policy to use ganciclovir as HHV-6 prophylaxis during conditioning for all unrelated HSCT since January 2002.

Abbreviations: CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; EBV, Epstein–Barr virus; GVHD, graft-versus-host disease; HHV, human herpesvirus; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; HSV, herpes simplex virus; PCR, polymerase chain reaction; UCB, umbilical cord blood.

In this study, we investigate the effect of this policy on HHV-6 encephalitis in pediatric unrelated HSCT.

Materials and methods

Patients

The study was performed in a university teaching hospital in Hong Kong, which is also a tertiary referral center for pediatric cancers and stem cell transplant in the local region. Clinical and laboratory data of patients who underwent unrelated allogeneic HSCT from January 2000 to September 2008 were reviewed. From the year 2000 onwards, our virology laboratory routinely performed HHV-6 PCR in CSF samples in all immunocompromised patients with suspected central nervous system infection.

HHV-6 DNA detection

The presence of HHV-6 DNA was detected by a nested PCR (screening PCR) based on primer sets H6-6/H6-7 and NH-6/NH-7 targeting the major capsid protein gene. The primer sets used in the screening PCR have shown to be consensus and carry the same analytic sensitivity in revealing variants A and B. All HHV-6 screening PCR-positive samples were confirmed by another nested PCR (typing PCR) targeting the large tegument protein gene. The typing PCR allows the characterization of HHV-6 into variants A and B by restriction fragment length analysis using Hind III. The PCR product of variant A does not contain any Hind III restriction site, whereas the 163-bp PCR product of variant B will be digested into fragments of 97 and 66 bp. This Hind III restriction site present at position 2945 of the large tegument protein gene of HHV-6B, but not in HHV-6A, has been used discriminate between the two variants. The detail methodology has been described by Chan et al. (7).

Conditioning regimen and GVHD prophylaxis

The detail of the conditioning regimen was described in Table 1. GVHD was diagnosed and scored according to standardized criteria (8). All patients received cyclosporine as prophylaxis from day-1 for malignant conditions, for non-malignant conditions, patients would receive methotrexate and cyclosporine.

Criteria of neutrophil engraftment

Day of neutrophil engraftment was defined as the first day of absolute neutrophil count of $\geq 0.5 \times 10^9$/L for three consecutive days.

Case definition of HHV-6 encephalitis

Case definition of HHV-6 encephalitis was based on the presence of neurological manifestation namely change of conscious level, including lethargy, irritability, change of personality or behavior that persisted for more than 24 h in conjunction with detection of HHV-6 DNA in CSF samples by PCR as previously described and in the absence of any other identifiable etiology. All CSF specimens had also undergone standard microbiological and virologic examinations to exclude HSV 1 and 2, CMV, EBV, HHV-7, and Varicella-zoster virus infections. Other non-infective causes of acute encephalopathy in a transplant setting were excluded by electroencephalogram, cranial imaging, measurement of serum electrolytes, and cyclosporine levels.

Virus monitoring and antiviral prophylaxis

Because of a relatively high prevalence of HHV-6 encephalitis in the first two yr (2/16 unrelated HSCT), from January 2002, our unit adopted the policy of using ganciclovir 5 mg/kg twice daily intravenously from day-7 to day-1 during conditioning as prophylaxis against reactivation of HHV-6 for all unrelated HSCT. Acyclovir prophylaxis (250 mg/m^2 every 8 h intravenously) was given to recipients who were or whose donors were HSV seropositive or there was past evidence of HSV infection. Reactivation of CMV was monitored by weekly CMV pp65 antigen assay. Weekly HHV-6 PCR quantitative monitoring was not our unit policy during the study period. Preemptive treatment with ganciclovir (5 mg/kg twice daily intravenously for two wk)

Table 1 Baseline patient characteristics

Patient	Sex/age at transplant (yr)	Primary disease	Stem cell source	Conditioning regimen	GVHD prophylaxis	Pretransplant ganciclovir	HHV-6 Serostatus before transplant	Day of engraftment	Overall grading of GVHD
1	M/9.4	ALL CR 4	2 Antigen-mismatch unrelated UCB transplant	TBI 9 Gy + fludarabine 30 mg/m^2 × 4 days + melphalan 140 mg/m^2	CSA	No	Positive	19	II
2	F/6.2	JMML	1 Antigen-mismatch unrelated UCB transplant	Busulphan 16 mg/kg/4 days + cyclophosphamide 60 mg/kg × 2 days + melphalan 140 mg/m^2	CSA	No	Positive	– (non-engraftment)	–
3	F/8.2	ALL CR2	2 Antigen-mismatch unrelated UCB transplant	TBI 1440 cGy + cyclophosphamide 60 mg/kg × 2 days	CSA	Yes	Positive	33	II
4	M/1.3	ALL CR2	2 Antigen-mismatch unrelated UCB transplant	Busulphan 16 mg/kg/4 days + etoposide 40 mg/kg + cyclophosphamide 60 mg/kg × 2 days	CSA	Yes	Positive	13	II

ALL, acute lymphoblastic leukemia, CSA, cyclosporine A, JMML, juvenile myelomonocytic leukemia, CR 4, 4th complete remission, CR 2, 2nd complete remission

would be started if there was evidence of CMV reactivation. There was no change in conditioning regimens and GVHD prophylaxis and treatment protocols. The prevalence of HHV-6 encephalitis was compared before and after the adoption of the policy.

Antiviral treatment for HHV-6 encephalitis

Patients fulfilled the criteria of HHV-6 encephalitis would be treated with ganciclovir (5 mg/kg twice daily intravenously). Patients with intolerable side effects, namely severe bone marrow suppression or no clinical improvement after 72 h of commencement of neurological states, would switch to foscarnet (60 mg/kg three times daily intravenously). Renal function and electrolytes were monitored serially during the course of foscarnet. Lumbar puncture was repeated to monitor the progress of disease and document clearance of virus in CSF. Antiviral therapy would continue for at least 14 days and treatment endpoint was clearance of HHV-6 DNA in CSF.

Results

One hundred and sixteen allogeneic HSCT, which included 54 unrelated HSCT, were performed from January 2000 to September 2008. Unrelated UCB was used as the stem cell source in 31 cases and the other 23 cases were from bone marrow (n = 19) or peripheral blood stem cells (n = 4). Primary diseases for unrelated transplant were acute lymphoblastic leukemia (n = 19); acute myeloid leukemia (n = 12); other leukemia and myelodysplastic syndrome (n = 8); miscellaneous (n = 15).

Four cases (7.4%) met the diagnostic criteria of HHV-6 encephalitis during the study period. All of them were because of variant B. They were diagnosed to have hematological malignancies and received unrelated UCB transplant. They were all seropositive to HHV-6 before transplant. Before the adoption of HHV-6 prophylaxis policy, 12.5% (two out of 16) unrelated HSCT developed HHV-6 encephalitis and 5.3% (two out of 38) developed HHV-6 encephalitis after introduction of pretransplant ganciclovir prophylaxis. If limited to unrelated UCB transplant, the incidences of HHV-6 encephalitis were 40% and 7.7% before and after ganciclovir prophylaxis, respectively. During the same period, no patient who received unrelated bone marrow or peripheral blood stem cell transplant developed HHV-6 encephalitis.

In our four cases, the age ranged from 1.3 to 9.4 yr old (median 7.2 yr old). They all received CSA as GVHD prophylaxis. The most common presentations of HHV-6 encephalitis were depressed conscious state, abnormal behavior, sleep disturbance, and seizure. Unexplained hypertension and hyponatremia were also commonly observed in our series. They presented at median 19 days after transplant (range 18–21 days). All of them showed evidence of bone marrow suppression with marked lymphopenia at presentation. Nearly all patients had normal CSF microbiological and biochemical findings despite being HHV-6 PCR positive. Other non-infective causes of acute encephalopathy in a transplant setting were excluded by electroencephalogram, cranial imaging, measurement of serum electrolytes, and cyclosporine levels. They were treated with antiviral agents as described. Lumber puncture was repeated in patient 1, 3, and 4 (patient 2 was critically ill and unable to have a repeat lumber puncture). HHV-6 was cleared at median of 17 days after starting of antiviral therapy. Despite treatment and demonstration of clearance of HHV-6 PCR after treatment, the outcome remained poor with 50% of cases complicated with significant neurological deficit after the infection was in control and the rest died of other transplant-related mortalities. The clinical features and outcome of the four cases are summarized in Tables 1 and 2.

Discussion

After first being isolated in peripheral blood of HIV-infected patients in 1986, HHV-6 has been a known pathogen in allogeneic HSCT recipients (1, 9). HHV-6 encephalitis is one of the most significant clinical manifestations of reactivation of HHV-6 infection in HSCT recipients (9, 10). HHV-6B is now established as the cause of exanthema subitum (roseola infantum). In our case series, all of them were because of variant B reactivation. HHV-6A rarely causes infections in infants in the Western world but it was shown to be the predominant HHV-6 variant associated with viremic infant infections in the sub-Sarharan African population (11).

The clinical spectrum of HHV-6 reactivation ranged from asymptomatic viremia, unexplained fever, skin rash, pneumonitis, myelosuppression with delay in neutrophil and platelet engraftment, and graft failure. Because of the neurotropic nature of HHV-6, encephalitis is the most severe form of direct organ damage caused by HHV-6 reactivation (12). With the known predilection to involve the temporal lobe and hippocampi, followed by the amygdala or parahippocampal gyrus, patients usually develop symptoms compatible with acute hippocampal dysfunction namely confusion, abnormal behavior, sleep disturbance, and loss of short-term memory. If the earlier mentioned symptoms persist, patients warrant further investigation (13).

Table 2 Outcome of HHV-6B encephalitis

Patient	Day of onset (post-transplant)	Peripheral blood count	Immunosuppressants	CSA trough level (μg/L)	Presentations	CSF cell counts and biochemistry	Treatment	Outcome
1	21	Hb 8 9 WBC 3 0 ANC 2 0 LYM 0 3 PLT 26 0	MP + CSA	235	Abnormal behavior, Seizure, Hyponatremia,	Traumatic tap	Ganciclovir 3 wk	Alive, Epilepsy, Now 8 yr post-transplant
2	20	Hb 9 7 WBC 0 3 ANC 0 2 LYM 0 1 PLT 88 0	MP + CSA	190	Depressed conscious level, Sleep disturbance, Euphoria, Generalized seizure	WBC 1/mm^3, RBC 3/mm^3, Total protein 0 24 g/l	Foscarnet 2 wk (treatment terminated because of renal impairment)	Died on day 38 (non-engraftment, refractory seizure, pneumonia, gastrointestinal bleeding)
3	19	Hb 9 0 WBC 0 2 ANC 0 1 LYM 0 0 PLT 30 0	MP + CSA	217	Fever, Headache Hypertension, Abnormal behavior, Hyponatremia, Seizure	WBC 1/mm^3, RBC 2/mm^3, Total protein 0 15 g/L	Foscarnet 2 wk + ganciclovir 2 wk	Progression of clinical symptoms despite on foscarnet and ganciclovir Died on day 61 (intracranial bleed)
4	18	Hb 7 0 WBC 1 4 ANC 0 8 LYM 0 0 PLT 3 0	MP + CSA	163	Fever, Focal seizure, Loss of memory, Abnormal behavior, Hypertension	WBC 2/mm^3, RBC 5/mm^3, Total protein 0 23 g/L	Ganciclovir 2 wk + foscarnet 3 wk	Alive, Refractory epilepsy, Developmental delay

Hb, hemoglobulin (g/dL), MP, methylprednisolone, CSA, cyclosporine A, WBC, white blood cell count (× 10^9/L), ANC, absolute neutrophil count (× 10^9/L), LYM, lymphocyte count (× 10^9/L), PLT, platelet count (× 10^9/L)

In allogeneic HSCT setting, 25% of recipients develop a wide spectrum of neurological symptoms related to various infectious agents, drug toxicity, and metabolic abnormalities. Moreover, the demonstration of HHV-6 by PCR in asymptomatic subjects may further complicate the picture. Therefore, it is not recommended to be tested routinely in asymptomatic subjects (14, 15). As in our series, all patients showed persistent unexplained neurological manifestations and apart from presence of HHV-6 in CSF, they were fully evaluated to exclude other possible causes of acute change of conscious state before the diagnosis of HHV-6 encephalitis was made.

After the first successful UCB transplant in 1989, this has been widely performed in a variety of hematological malignancies, bone marrow failure syndromes, and inborn errors of metabolism (16). UCB transplant has the advantages of allowing a higher degree of HLA disparity between host and graft, with lower incidence and less severe GVHD. Therefore, UCB provides a readily available source of stem cells with similar survival outcomes as compared with transplant using bone marrow as stem cell source (17). Compared with patients transplanted from matched unrelated bone marrow or peripheral stem cell donors, UCB transplant recipients have a lower incidence of acute and chronic GVHD, but hematopoietic recovery is delayed, the probability of sustained donor engraftment is less and infectious complications are higher especially in the early post-transplant period. This increased risk of fatal infections is mainly because of the slow neutrophil recovery, transfer of antigen-naïve T cells to recipients with lack of antigen-experienced (memory) T cells in UCB. In fact, memory T cells significantly contribute to early immunological reconstitution of patients after unmanipulated allogeneic bone marrow or peripheral blood stem cell transplant (18). In our series, all cases of HHV-6 encephalitis occurred in patients who received unrelated UCB transplant. From the experience of CMV infection, CMV seropositive donors may provide additional survival benefit in CMV positive recipients because of transfer of donor-derived immunity; a similar situation may occur with HHV-6 infection (19).

In our series, nearly all patients demonstrated normal CSF white cell count and protein level. This may be because of defect in mounting sufficient immune response to invading pathogens in severely immunocompromised patients and sampling of CSF at early stage of disease. It was supported by the peripheral blood count at presentation. All of them showed evidence of bone marrow suppression with marked lymphopenia at presentation. Therefore, normal CSF microbiological and biochemical findings do not exclude the diagnosis of encephalitis in immunocompromised patients. Moreover, in patients with

unexplained hypertension or hyponatremia. CNS causes need to be excluded as 50% of our cases had persistent hypertension or hyponatremia.

Overall, HHV-6 encephalitis is an uncommon complication after HSCT but the prognosis remained very poor. Post-transplant preemptive ganciclovir treatment based on the periodic quantitative HHV-6 plasma DNA viral load monitoring was practiced in some centers but because of the dynamic kinetics of plasma HHV-6 viral load with published cases of HHV-6 encephalitis developed symptoms before detection of high level HHV-6 DNA in plasma, the value of preemptive antiviral therapy in termination in progression to full blown disease manifestation is not fully proven (20). In view of the significant morbidity and mortality of HHV-6 encephalitis experienced in our series, we adopt a prophylactic ganciclovir policy during conditioning to reduce the HHV-6 viral load and thus decrease the chance of post-transplant reactivation. Though the incidence of HHV-6 encephalitis appeared to decrease after ganciclovir prophylaxis, we could not prevent two cases. The role of prophylaxis still remains debatable.

Currently routine antiviral prophylaxis for HHV-6 infection is not recommended in HSCT recipients because of low risk of infection in reported series and the significant toxicity of available antiviral agents (21). Whether there is a role of antiviral prophylaxis in the highest risk group, namely unrelated UCB transplant recipients, still needs to be evaluated by randomized controlled trial.

One of the main limitations of our study is the lack of plasma DNA profile to correlate the outcome because all the cases were managed before the era of routine quantitative HHV-6 plasma viral load monitoring. Because of the dynamic nature of plasma viral load, some cases actually presented before demonstration of viral DNA in plasma. The exact role of regular monitoring remains to be determined.

In conclusion, HHV-6 encephalitis is uncommon in unrelated HSCT. Routine use of pre-transplant ganciclovir prophylaxis is not justified based on current data but may have a role in unrelated UCB transplant.

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Lymphoproliferative response to herpes simplex virus type 1, cytomegalovirus, Epstein–Barr virus, varicella zoster virus, human herpes virus 6, 7, and 8 antigen stimulation in pediatric allogeneic stem cell transplant recipients

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Abstract: We evaluate the recovery of CMI to various herpes viruses by measuring *in vitro* LPR to specific recall antigens. CMI was evaluated by the *in vitro* LPR of PBMC to specific purified HSV-1, VZV, CMV, EBV, HHV-6, -7, -8, antigens. Results were expressed as SI. SI ≧ 3 was regarded as positive LPR. Serial measurements were taken prospectively from pretransplant till 12-month post-transplant. Thirty-six patients (M = 19; F = 17) with median age 10.5 yr old were recruited. Most transplants were from MSD with PBSC as the stem cell source. Altogether 50% of subjects started to show positive LPR to HSV-1, CMV, and VZV antigens at two-month post-transplant, major upsurges were noted until 6-month post-transplant. Subjects showed positive LPR to EBV, HHV-6, HHV-7, and HHV-8 antigens were all along < 50% throughout the study period. The antibody status of donor and recipient for HSV-1, CMV, and VZV were associated with the timing of recovery of CMI. Choice of donor and stem cell source were important determinants of eventual LPR to various herpes viruses at 3-month post-transplant. At 12-month post-transplant, there was no statistical difference in any parameters in affecting LPR to different herpes viruses.

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HSCT is now a curative treatment modality for many hematological malignancies, immunodeficiency syndromes, inborn errors of metabolism and hemoglobinopathies. With the introduction of umbilical CB transplant and T-cell-depleted haploidentical stem cell transplant from related

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ANC, absolute neutrophil count; ATG, anti-thymocyte globulin; BM, bone marrow; CB, cord blood; CMI, cell-mediated immunity; CML, chronic myeloid leukemia; CMV, cytomegalovirus; ConA, concanavalin A; EBV, Epstein Barr virus; GCSF, granulocyte colony stimulating factor; GVHD, graft-versus-host disease; HHV-6, human herpes virus-6; HHV-7, human herpes virus 7; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; HSV-1, herpes simplex virus type 1; IQR, interquartile range; IVIG, intravenous immunoglobulin; KGF, keratinocyte growth factor; KS, Kaposi's sarcoma; LPR, lymphoproliferative response; MSD, matched siblings donor, MUD, matched unrelated donors; PBMC, peripheral blood mononuclear cells; PBSC, peripheral blood stem cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SI, stimulation indices; TBI, total body irradiation; TEC, thymic epithelial cell; VZV, varicella zoster virus.

family donors, nearly all patients can proceed to transplant if there is a medical indication (1). A major cause of transplant-related mortality is delayed T-cell reconstitution that can result in severe and even life-threatening viral infections. The median time for T-cell recovery is 7–8 months in the setting of CD34+ selected graft with myeloablative conditioning regimen (2).

Reactivation or less common primary infections of the herpes group of viruses can cause substantial morbidity and mortality in allogeneic stem cell transplant recipients. Each herpes virus has a unique temporal profile of reactivation. HSV-1, EBV, and HHV-6 are detected most frequently in the first 30 days post-transplant, whereas VZV and CMV tend to be reactivated later in post-transplant period. In contrast, HHV-7 reactivation that may manifest as unexplained fever, non-specific rash, delayed engraftment, or even encephalitis does not show consistent temporal predilection (3).

CMI is critical for effective immunity and plays an important role in long-term protection against various viral infections in post-transplant period. The effect of new transplant strategy on the recovery of CMI to these viruses remains unanswered. This study used a well-established *in vitro* approach to prospectively evaluate the CMI to mitogens and specific recall antigens derived from HSV-1, VZV, CMV, EBV, and HHV-6,7,8 in a series of allogeneic stem cell transplant recipients within the first 12-month transplant period.

Materials and methods

Patient selection

This study included children who underwent allogeneic HSCT in Children's Cancer Centre, Department of Pediatrics of the Prince of Wales Hospital from 2001 to 2004. The study protocol was approved by the Joint CUHK-NTEC clinical research ethics committee. Written consents were obtained from parents and/or patients before enrollment according to the principles of the Declaration of Helsinki.

Conditioning regimen and GVHD prophylaxis

Management protocols on pretransplant conditioning regimens and GVHD prophylaxis following HSCT used for our patients were according to previous publications (4, 5). In brief, patients with relapsed ALL received TBI-based regimen, relapsed AML, and non-malignant conditions received busulfan and cyclophosphamide-based regimen. GHVD prophylaxis consisted of cyclosporine from day 1 till day 60 and slowly tapered till day 180 if no evidence of chronic GVHD in malignant conditions, whereas non-malignant conditions would consist of cyclosporine and methotrexate at days 1, 3, 6, and 11. GVHD was diagnosed and scored according to standardized criteria (6).

Criteria of neutrophil engraftment

Day of neutrophil engraftment was defined as the first day of ANC of $\geq 0.5 \times 10^9$/L for three consecutive days

Virus monitoring and antiviral prophylaxis

Acyclovir prophylaxis (250 mg/m^2 every 8 h intravenously) was given either to the recipients or to the donors who were seropositive for HSV-1 or in whom there was a history of HSV-1 infection from day 0 till day 21 post-transplant. Reactivation of CMV was monitored weekly by measuring the level of CMV pp65 antigen in PBMC from day 0 till day 100. Ganciclovir (5 mg/kg per day 3 days per week) was given from day of engraftment till day 100 as CMV prophylaxis in unrelated donor transplants. Preemptive treatment with ganciclovir (5 mg/kg per dose twice daily intravenously for at least 2 wk) would be started if there was evidence of CMV reactivation.

All unrelated and HLA-mismatched transplants received IVIG at 500 mg/kg every 2 wk from day 1 till day 100.

Lymphoproliferative Assay

CMI was evaluated by the *in vitro* proliferative responses of PBMC to specific purified HSV-1, VZV (Microbix Biosystems, Toronto, ON, Canada), CMV, EBV, HHV-6, HHV-7, HHV-8 (cell culture lysate derived) antigens and mitogens including PHA (Gibco BRL, Grand Island, NY, USA), ConA, PWM as positive control.

In brief, PBMC was obtained from heparinised venous blood using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation and washed in RPMI-1640 medium (Celox Laboratories, Inc., St. Paul, MN, USA) supplemented with 2 mM L-glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, and 8% fetal calf serum (Life Technologies, Gaithersburg, MD, USA). The viability of isolated cells ranged from 95% to 98% as determined by the trypan blue exclusion test.

PBMC were washed twice in RPMI-1640 medium and resuspended at a concentration of 2×10^6/mL of RPMI-1640, supplemented with penicillin-streptomycin (100 U/mL), 5% autologous serum, and 1 mM sodium pyruvate. Aliquots (200 μL) of PBMC containing 5×10^4 cells were incubated in triplicates in flat-bottom 96-well microtiter tissue culture plate (Costar, Cambridge, MA, USA) with 10 μg/mL PHA, ConA, and PWM for 3 days as positive control. A total of 2×10^5 cells were cultured separately with HSV-1 and 5 μL of VZV, EBV, HHV-6, -7, and -8 antigens for 6 days in 5% CO_2 at 37 °C.

Cell cultures were pulsed with [^{3}H]-thymidine (Pharmacia) for 18 h at the end of incubation, and radioactivity in the samples was then measured by a scintillation counter (Microbeta TriLux; EG & G Wallac, Turku, Finland). Three replicates of counts per minutes (cpm) values for the unstimulated PBMC and three replicates each for PBMC stimulated with HSV-1, CMV, EBV, HHV-6, -7, and -8 antigens. The median cpm for unstimulated PBMC as well as for PBMC stimulated with various antigens was determined. Results were expressed as SI that was defined as the ratio of the median cpm in the stimulated samples divided by the cpm in the unstimulated samples. SI $\geq$ 3 was regarded as positive LPR (7).

Serial measurements were made at baseline (before transplant), at monthly intervals from the first to sixth month post-transplant and then 3-monthly intervals until 12-month post-transplant for HSV-1, CMV, VZV, and

HHV-8 antigens. EBV, HHV-6, and HHV-7 were monitored until 5 months after transplantation.

To minimize the variability in antigen titers between different batches of viruses, we used a single batch of viruses for LPR monitoring on all subjects throughout the study period.

Statistical analysis

The primary measured outcome was the CMI (lymphoproliferation as measured by SI) induced by various herpes virus antigens at different time period after HSCT.

Data were descriptively summarized using frequencies and percentages for all categorical variables. Continuous variables were expressed as median and IQR. Stimulation indices between different subgroups were analyzed using Mann–Whitney *U*-test. Categorical variables between different diseases or patient groups were analyzed by Chi-square test. p-values < 0.05 were considered to be statistically significant. Data analysis was performed by SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA).

Results

Characteristics of study population

From 2001 to 2004, 36 children ($M = 19$; $F = 17$) were recruited. The age ranged from 1.03 to 17.9 yr, median 10.5 yr. Malignant conditions (ALL, AML, CML, and other hematological malignancies) accounted for 58.3% of cases. Altogether 52.7% of donors were MSD. MUD accounted for 27.8% of cases. The others received transplants from mismatched family donors (5.6%) and CB (13.9%; one related, four unrelated). PBSC, BM, and unrelated CB as the stem cell sources were used in 55.5%, 30.6%, and 13.9% of cases, respectively. The mean CD3+ cell dose in PBSC (31.7×10^7/kg) was significantly higher than BM (4.2×10^7/kg) and CB (1.1×10^7/kg), respectively ($p = 0.009$; $p = 0.03$). The mean CD34+ cell dose in PBSC (5.1×10^6/kg) was significantly higher than BM (3.5×10^6/kg, $p = 0.03$) and CB (0.23×10^6/kg, $p = 0.004$), respectively. Of the patients, 16.7% and 9.6% developed severe acute GVHD (grade III/IV) and extensive chronic GVHD, respectively. The 3-month and 12-month survival rates were 86.1% and 71.0%, respectively. The characteristics of study patients are listed in Table 1.

Table 1. Characteristics of study patients

Parameters	Number of patients (n = 36)
Sex	
Male/female	19/17
Primary disease for transplant	
ALL	10 (27.7%)
CR1	2
CR2/CR 3	7/1
AML	5 (13.9%)
CR1/CR 2	1/4
Severe aplastic anemia	6 (16.7%)
Other leukemia/lymphoma	
(JMML, CML, T-cell lymphoma)	6 (16.7%)
Immunodeficiency syndrome	3 (8.3%)
Hemoglobinopathy	4 (11.1%)
Metabolic disease	2 (5.6%)
Survival	
3-month post-transplant	31 (86.1%)
12-month post-transplant	22 (71.0%)
Cause of death at 3-month post-transplant (n = 5)	
Relapse	1 (20.0%)
Transplant-related mortality (TRM)	4 (80.0%)
Cause of death at 12-month post-transplant (n = 9)	
Relapse	6 (66.7%)
Treatment-related mortality (TRM)	3 (33.3%)
Type of transplant	
Matched sibling donor (MSD)	19 (52.7%)
Matched unrelated donor (MUD)	10 (27.8%)
Mismatched family donor (MMF)	2 (5.6%)
Unrelated cord blood	4 (11.2%)
Related cord blood	1 (2.7%)
Conditioning	
Total body irradiation (TBI)	10 (27.7%)
No TBI	26 (72.3%)
Anti-thymocyte globulin (ATG)	20 (55.5%)
No ATG	16 (44.5%)
Stem cell source	
Bone marrow (BM)	11 (30.6%)
Peripheral blood stem cell (PBSC)	20 (55.5%)
Unrelated cord blood	4 (11.2%)
Related cord blood	1 (2.7%)
Mean graft CD3+ count	
Bone marrow (BM)	4.2×10^7/kg
Peripheral blood stem cell (PBSC)	31.7×10^7/kg
Cord blood (CB)	1.1×10^7/kg
Mean graft CD34+ count	
Bone marrow (BM)	3.5×10^6/kg
Peripheral blood stem cell (PBSC)	5.1×10^6/kg
Cord blood (CB)	0.23×10^6/kg
Acute GVHD	
No acute GVHD	11 (30.6%)
Grade I/II	19 (52.7%)
Grade III/IV	6 (16.7%)
Chronic GVHD (n = 31)	
No	23 (74.3%)
Limited	5 (16.1%)
Extensive	3 (9.6%)

LPR to HSV-1, VZV, CMV, EBV, HHV-6, -7, and -8 in study subjects

For analysis on HSV-1, VZV, and CMV responses, we further categorized the results according to the serostatus of the donors (D) and recipients (R). For VZV, the number of D+R+, D+R−, D−R+, and D−R− was 17, 8, 7, and 4, respectively. For CMV, the number of D+R+, D+R-, D−R+, and D−R− was 14, 9, 8, and 5, respectively. For HSV-1, the number of D+R+, D+R−, D−R+, and D-R-was 17, 8, 8, and 3, respectively.

Fifty percent of subjects showed SI ≥ 3 to HSV-1 at two months after transplantation, an upsurge of median SI was observed at five

months after transplant. In subgroup analysis, D+R+ subjects demonstrated the most significant increase in LPR.

More than half of subjects showed SI index ≧3 to CMV at two months after transplantation, and an upsurge was observed at 5–6 months after transplant. D+R+ subgroups showed the most significant response.

At 4-month post-transplant, more than 50% of patients showed positive SI index to VZV antigen, and there was an upsurge observed at 6-month post-transplant. Again, D+R+ showed the most prominent response in LPR.

Although half of the patients showed positive response (SI index ≧3) to EBV, HHV-6, and HHV-7 before transplant, the median SI indices were <3 to these herpes viruses indicating most of the patients did not show a significant LPR to these antigens during first 6-month post-transplant period.

For HHV-8, only 6.5% subjects showed SI ≧ 3 at baseline, and there was no significant LPR to HHV-8 antigen throughout the study period. The details of LPR to various herpes viruses are given in Tables 2, 3, and Figs. 1 and 2.

At 3-month post-transplant, patients with MSD transplant had significantly higher SI to HSV-1, CMV, EBV, HHV-7, and HHV-8 when compared with patients with MUD transplant. Patients who received PBSC as the stem cell source also showed higher SI to HSV-1, VZV, and EBV when compared with patients who received BM. Use of ATG and TBI and severity of acute GVHD did not pose any significant effect on LPR to herpes virus antigen at 3-month post-transplant. At 12-month post-transplant, there was no statistical difference in any parameters in affecting LPR to different herpes viruses. The details are provided in Table 4.

Viral reactivations during the study period

During the study period, five patients developed reactivation of CMV virus that required preemptive ganciclovir treatment for controlling viral replication at median 4.5-month post-transplant. Three patients developed HSV-1 mucositis. All

Table 2 Lymphoproliferative responses to HSV-1, CMV, VZV, and HHV-8 at different time points after HSCT

	Con A	HSV-1		CMV		VZV		HHV-8	
Time after HSCT	Median (IQR)*	Median (IQR)*	SI ≥ 3 (%)†	Median (IQR)	SI ≥ 3 (%)	Median (IQR)	SI ≥ 3 (%)	Median (IQR)	SI≥3 (%)
Baseline (pretransplant)	189 0 (56 8–376 3)	15 0 (2 1–47 7)	71 4	16 4 (3 8–39 5)	80 6	16 2 (4 4–55 9)	77 8	1 1 (0 9–1 8)	6 5%
1 month	29 0 (5 0–74 0)	2 3 (0 5–7 8)	50 0	2 1 (0 75–11 2)	31 7	1 5 (0 4–3 4)	26 7	0 9 (0 7–1 4)	0 0%
2 months	40 0 (6 0–94 0)	3 6 (0 9–14 3)	53 6	3 7 (1 1–9 1)	56 7	1 3 (0 7–6 3)	32 3	1 1 (0 7–1 7)	3 8%
3 months	50 0 (19 5–153 8)	3 6 (1 2–26 9)	59 3	3 1 (0 5–33 9)	51 7	2 4 (0 7–8 9)	45 2	1 0 (0 8–1 7)	8 3%
4 months	58 0 (16 0–101 5)	5 7 (1 6–40 2)	72 7	5 1 (2 4–28 7)	72 7	2 6 (1 2–8 8)	50 0	1 3 (1 0–1 5)	0 0%
5 months	69 0 (41 3–109 0)	5 7 (2 0–95 3)	65 2	8 9 (3 6–137 4)	77 8	14 4 (2 0–110 9)	66 7	1 3 (1 0–1 8)	0 0%
6 months	137 0 (41 8–197 0)	21 6 (4 6–89 5)	87 5	3 7 (1 3–53 7)	54 5	5 2 (2 2–17 3)	70 8	1 0 (0 6–1 3)	4 5%
9 months	127 0 (42 0–263 0)	49 5 (19 9–139 1)	81 0	19 8 (5 4–68 4)	81 8	15 2 (2 7–83 1)	72 7	1 0 (0 8–1 6)	4 8%
12 months	178 0 (111 0–350 0)	45 1 (4 4–228 5)	85 7	25 5 (3 6–40 9)	76 1	22 1 (4 4–78 4)	76 2	1 5 (0 9–2 1)	25 0%

*Interquartile range (1st quartile – 3rd quartile)
†SI, stimulation index, ≧3 is defined as positive
Con A, Concanavalin A

Table 3 Lymphoproliferative responses to EBV and HHV-6, -7 at different time points after HSCT

	Con A	EBV		HHV-6		HHV-7	
Time after HSCT	Median (IQR)*	Median (IQR)	SI ≥ 3 (%)†	Median (IQR)	SI ≥ 3 (%)	Median (IQR)*	SI ≥ 3 (%)
Baseline (pretransplant)	189 0 (56 8–376 3)	2 9 (1 4–12 6)	50 0	2 8 (1 0–9 1)	50 0	5 9 (1 8–20 4)	57 9
1 month	29 0 (5 0–74 0)	1 2 (0 4–3 5)	28 6	1 5 (0 6–6 4)	28 6	0 9 (0 3–2 8)	20 0
2 months	40 0 (6 0–94 0)	1 3 (0 5–2 1)	9 1	1 1 (0 8–4 1)	27 3	1 4 (0 5–3 8)	30 7
3 months	50 0 (19 5–153 8)	1 5 (0 7–3 5)	30 0	1 5 (1 0–26 5)	31 6	1 5 (0 9–2 9)	23 15
4 months	58 0 (16 0–101 5)	1 6 (1 1–2 1)	0 0	1 5 (0 5–3 4)	16 7	1 5 (0 9–4 0)	22 2
5 months	69 0 (41 3–109 0)	1 2 (0 4–2 8)	14 3	1 5 (0 3–11 2)	16 7	0 6 (0 4–2 6)	0 0

*Interquartile range (1st quartile – 3rd quartile)
†SI, stimulation index, ≧3 is defined as positive
Con A, Concanavalin A

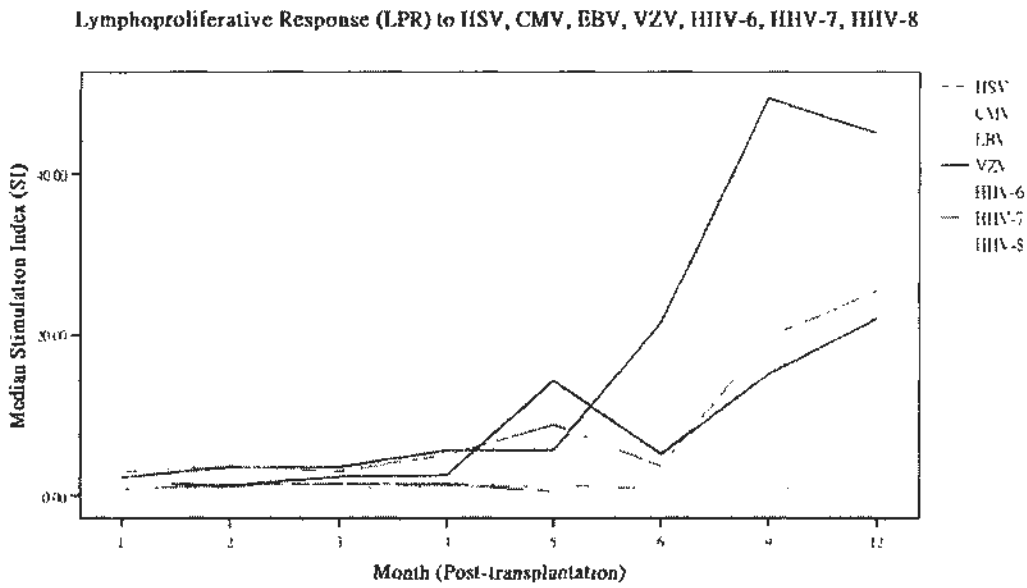

Fig 1. Lymphoproliferative responses to HSV, CMV, EBV, VZV, HHV-6, -7, and -8

of them recovered after treatment with appropriate antiviral therapies. There was no virologic-confirmed CMV, EBV, HHV-6, -7, and -8 diseases throughout the study period.

Discussion

In the allogeneic HSCT setting, immune reconstitution is determined by multiple factors including immunosuppressive effect of the conditioning regimen, graft manipulation, presence of acute and/or chronic GVHD, and the immunosuppressive therapy given after transplantation as variables. In general, B-lymphocyte levels return to the age-matched levels at 3–6 months after transplant. Immunoglobulin isotypes start to normalize at about 6 months after transplant with IgM first, followed by IgG_1, IgG_3, IgG_2, IgG_4, and IgA. However, IgG subclass deficiency can last up to 18 months or more after transplantation (8, 9).

A number of factors that can influence T-cell reconstitution have been identified: (i) type and dose of T-cell antibody used in conditioning; (ii) irradiation as part of the conditioning regimen; (iii) stem cell dose; (iv) choice of stem cell and (v) graft manipulation techniques (10, 11). The delay in immune reconstitution is associated with severe infections. T-cell-mediated immunity is specifically important for confining primary viral infection and reactivation.

There are two sources of T cells in recovering recipients: one from the peripheral expansion of mature and memory T cells that starts at 1- to 2-month post-transplant and reaches the peak at 6 months, and the other source from *de novo* maturation of naïve T cells derives from transplanted stem cells and produced in the host's thymic system. This pathway generates a diverse receptor repertoire and is capable of responding to a range of antigens, which is important in the reconstitution of CD4+ lymphocytes. Reconstitution to normal levels can occur 1–2 yr after transplantation (12, 13). The capacity of the thymic-derived T-cell production is diminished in adults because of the involution of thymus after puberty. Irradiation to thymic region, namely in TBI and increase dose intensity of the conditioning regimen can induce tissue damage to epithelial cells of the thymus and impair its function in T-cell maturation. Therefore, being adult and the increased dose intensity in the conditioning regimen are risk factors for reduced ability to regenerate new T cells (14). Incidence of CMV reactivation ranges from 14% to 29% and lethal CMV infections occurs in about 0–8% (15, 16). In general, children have a lower infection death rate than adults, which probably reflects impaired thymic function in older patients (17, 18).

Our data showed that although more than 50% of subjects showed a positive LPR to HSV-1, VZV, and CMV at the early post-transplant period, an upsurge of stimulation index could only be demonstrated at 6-month post-transplant. For EBV, HHV-6, HHV-7, and HHV-8, the majority of subjects did not demonstrate a significant response in stimulation index throughout the study period. With the use of a myeloablative conditioning regimen, especially in

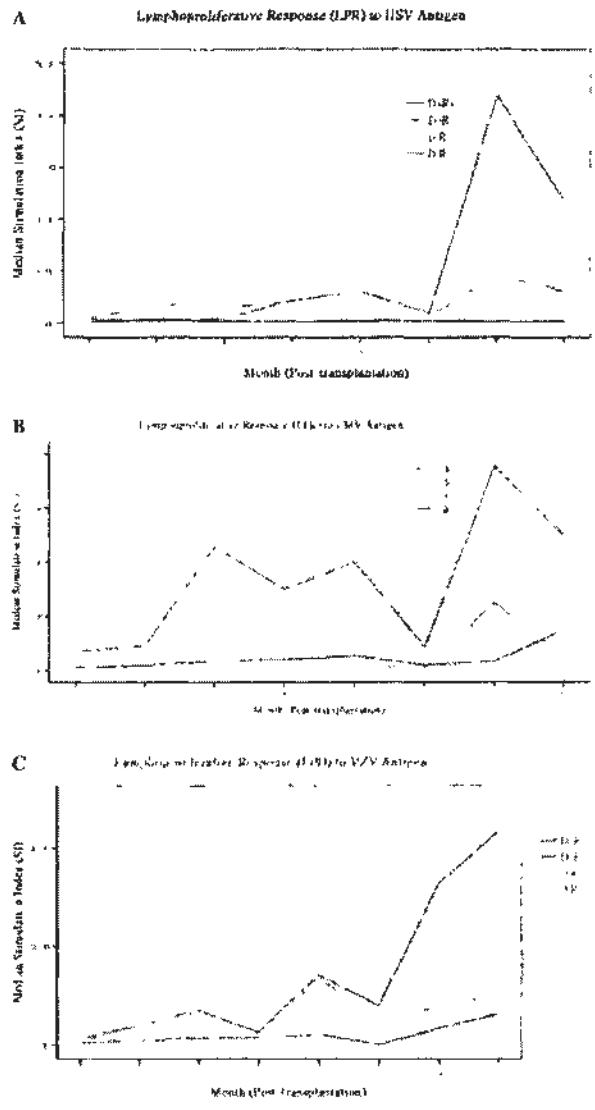

Fig. 2. A: Lymphoproliferative response to HSV. B: Lymphoproliferative response to CMV. C: Lymphoproliferative response to VZV.

malignant conditions, the process of immune reconstitution will be delayed. This may account for the delayed major upsurge of stimulation index to HSV-1, CMV, VZV, and the suboptimal response to EBV, HHV-6, HHV-7, and HHV-8 antigens in the post-transplant period despite a significant proportion of subjects showing a positive stimulation index before transplant.

In the subgroup analysis, we found that patients who received a, MSD transplant and received GCSF-mobilized PBSC transplant had a greater degree of rise in stimulation index when compared with their MUD transplant counterpart and patients who received BM as the source of stem cells (Table 4). With the less severe GVHD and thus less use of intensive immunosuppressive therapy, the time and extent of immune recovery is more rapid in MSD transplant than MUD transplant. In our series, the CD3+ and CD34+ cell count in the PBSC graft is significantly higher than the BM graft, with the higher number of T-cells, both memory and naïve T-cells, the reconstruction of CMI will be faster. This may be the reason for this observation. However, in our series, we could not demonstrate any correlation between LPR to herpes viruses and the use of ATG as well as the use of TBI in the conditioning regimen. Because the number of subjects with acute GVHD was small, we could not analyze the correlation between LPR and the severity of acute GVHD.

Reactivation of herpes viruses in the post-transplant period is common following allogeneic HSCT and is associated with significant morbidity and mortality. Both CD4+ and CD8+ antigen-specific immune reconstitutions are required for protection against the reactivation of viral infection. Deficient antigen-specific CD4+ and CD8+ responses within the early post-transplant period was associated with a higher risk of viral reactivation at the late transplant period (19). Therefore, with the infusion of donors' memory T cells or the presence of recipients' remaining T-memory cells may be associated with early reconstitution of viral-specific immunity. In our series, we showed that seropositivity in both the donor and recipient is an important predictor for the LPR. For HSV-1, CMV, and VZV antigens, patients with status (D+; R+) showed a higher stimulation index when compared with other counterparts. Ganepola et al. actually recommend preferring CMV seropositive donors for CMV seropositive recipients, as this should lead to an early and durable CMV-specific T-cell immunity after transplant with subsequent protection against CMV disease (20). This may also account for the current observation of a higher stimulation index in PBSC subgroup, since the CD3+ cell dose was significantly higher than in the BM subgroup.

The significance of HHV-8 infection and its reactivation in pediatric allogeneic HSCT setting remains unclear. Rosenzwajg et al. (21) reported that the seropositivity rate in the pediatric population (< 15 yr) before transplantation was about 10%. HHV-8 is implicated in causing KS, which classically affects elderly men of Mediterranean or Eastern Europe origin. It is also endemic in children and young adults of subequatorial Africa and epidemic in individuals with HIV infection. KS has been reported in solid organ transplantation recipients but rarely

Table 4 Lymphoproliferative responses (SI) of herpes viruses in different transplant settings at 3 months and 12 months post transplant

	HSV-1		CMV		EBV		VZV		HHV-6		HHV-7		HHV-8	
	3 month	12 month	3 month	12 month	3-month	12 month	3 month	12 month	3 month	12-month	3 month	12 month	3 month	12 month
MSD (n = 18)	4 10*	5 20	5 00	6 50	3 00	ND	2 90	6 70	1 50	ND	1 50	ND	1 30	3 50
MUD (n = 12)	0 40	3 75	0 80	4 75	0 60	ND	1 15	5 75	1 15	ND	0 10	ND	0 80	2 75
	(p ≤ 0 01)	(p = 0 46)	(p = 0 03)	(p = 0 16)	(p = 0 03)		(p = 0 58)	(p = 0 95)	(p = 0 73)		(p = 0 01)		(p = 0 04)	(p = 0 67)
BM (n = 11)	0 80	5 70	1 30	3 50	0 85	ND	0 9	1 25	1 47	ND	0 50	ND	0 85	5 75
PBSC (n = 20)	6 00	8 50	3 10	4 75	3 00	ND	7 90	3 45	1 00	ND	1 50	ND	1 65	4 95
	(p ≤ 0 01)†	(p = 0 30)	(p = 0 92)	(p = 0 63)	(p = 0 03)		(p = 0 05)	(p = 0 09)	(p = 0 39)		(p = 0 13)		(p = 0 07)	(p = 0 90)
CB (n = 5)	0 85	6 70	5 40	4 25	0 50	ND	1 20	1 55	1 15	ND	0 20	ND	1 25	4 25
	(p = 0 77)‡	(p = 0 72)	(p = 0 30)	(p = 0 72)	(p = 0 13)		(p = 1 00)	(p = 1 00)	(p = 0 24)		(p = 1 00)		(p = 0 16)	(p = 0 72)
No acute GVHD (n = 11)	4 80	5 10	2 20	3 55	2 80	ND	1 00	3 45	1 50	ND	1 50	ND	0 90	3 74
Acute GVHD Grade I/II (n = 19)	2 05	3 25	4 05	4 75	1 80	ND	3 70	2 55	1 30	ND	1 00	ND	1 00	2 75
	(p = 0 19)§	(p = 0 55)	(p = 0 70)	(p = 0 84)	(p = 0 59)		(p = 0 98)	(p = 0 75)	(p = 0 81)		(p = 0 36)		(p = 0 89)	(p = 0 54)
Acute GVHD Grade III/IV (n = 6)	3 40	4 20	2 45	2 55	1 80	ND	3 10	2 75	1 15	ND	1 30	ND	1 00	3 25
	(p = 0 71)¶	(p = 0 94)	(p = 0 57)	(p = 0 45)	(p = 0 49)		(p = 0 67)	(p = 0 54)	(p = 0 21)		(p = 0 88)		(p = 0 60)	(p = 0 64)
ATG (n = 20)	4 95	6 70	5 40	3 25	3 00	ND	3 70	4 71	1 00	ND	1 50	ND	1 00	5 45
No ATG (n = 16)	1 65	4 85	1 50	2 50	0 80	ND	2 45	5 75	1 30	ND	0 55	ND	1 00	4 75
	(p = 0 06)	(p = 0 75)	(p = 0 16)	(p = 0 34)	(p = 0 07)		(p = 0 51)	(p = 0 32)	(p = 0 83)		(p = 0 08)		(p = 0 75)	(p = 0 50)
TBI (n = 10)	4 60	6 80	0 60	1 60	6 60	ND	1 30	4 52	0 80	ND	0 10	ND	10 64	10 94
No TBI (n = 26)	2 75	7 55	2 80	3 50	10 62	ND	5 30	5 75	1 40	ND	1 50	ND	11 90	11 25
	(p = 0 57)	(p = 0 64)	(p < 0 01)	(p = 0 19)	(p = 0 15)		(p = 0 24)	(p = 0 67)	(p = 0 76)		(p = 0 03)		(p = 0 67)	(p = 0 87)

Analyzed by Mann–Whitney *U* test statistical significant $p < 0.05$

*Median stimulation index

†Compare with BM group

‡Compare with BM group

§Compare with no acute GVHD

¶Compare with grade I/II GVHD

MSD matched sibling donor MUD matched unrelated donor ND not done BM bone marrow PBSC peripheral blood stem cell CB cord blood (related + unrelated) GVHD graft versus host disease ATG anti thymocyte globulin TBI total body irradiation

reported in allogeneic stem cell transplantation setting (22). There are no previously published local data of seropositive rate in the Chinese pediatric population. Our data show that only 6.5% of patients had a stimulation index of ≧3 before transplant reflecting that a majority of patients had not encountered this virus before. Our data were consistent with other published series (21). Throughout the 12-month follow-up period, the stimulation index remained low when compared with other antigens. This reflects either primary or reactivation of HHV-8 infection in the post-allogeneic transplant setting is an uncommon event.

There are a number of experimental and clinical approaches that are currently being investigated in boosting the T-cell-mediated immune response in the post-transplant period. Regeneration of BM niche and hopefully improvement on the microenvironment of BM is particularly relevant. One of the strategies may be to transplant donor BM stem cells with other donors' cells of non-hematopoietic origin, namely stromal cells, mesenchymal stem cells, or osteoblast progenitors. The mesenchymal stem cells are naturally immunosuppressive and have been shown to successfully diminish GVHD in allogeneic stem cell transplantation and may facilitate the induction of mixed donor–recipient chimerism and thus improve engraftment. These measures will lead to decrease the period of immunosuppression after HSCT and decrease the chance and duration of herpes virus reactivation. The other investigational targets are (i) administration of KGF or Fms-like tyrosine kinase 3 ligand (Flt3L) to initiate *in situ* thymic recovery and thus reduce the damages may be cytoreductive therapies; (ii) *de novo* thymic production by ex vivo expanded TEC biopsy; (iii) expansion of naïve T-cells by IL-7 or possibly Flt3L (23).

Our group provides the first complete set of data to illustrate the recovery of CMI to the herpes group of viruses over the 12-month study period following allogeneic hematopoietic stem cell transplant with myeloablative conditioning regimen. The serostatus of the donor and the recipient, the choice of donors, and the source of stem cells are important parameters in determining LPR to herpes viruses in transplant recipients at 3-month post-transplant.

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