SCRIPTA MEDICA (BRNO) – 76 (3): 127–132, *June 2003*

MOLECULAR APPROACHES TO PEDIATRIC HEMATOONCOLOGY.

REVIEW

HRSTKOVÁ H.

First Department of Pediatrics, Faculty of Medicine, Masaryk University, Brno

A b s t r a c t

This review summarizes an experience of the 1st Department of Pediatrics at Masaryk University with recent molecular approaches to pediatric hematooncology. It is mainly focused on detection of minimal residual disease in patients with acute leukemia and detection of opportunistic pathogens in immunocompromised children – two main areas of great importance in pediatric hematooncology. Molecular techniques, such as polymerase chain reaction and restriction fragment length polymorphism serve as a tool for better, reliable and rapid diagnosis and detailed description of processes that may be otherwise undetectable.

K e y w o r d s

Children, Oncology, Leukemia, Polymerase chain reaction

A b b r e v i a t i o n s u s e d

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; FN, febrile neutropenia; HHV-6, human herpesvirus 6; MRD, minimal residual disease; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism;

INTRODUCTION

Molecular biology techniques are fast developing and applicable in all fields of medicine for better diagnosis of diseases and for better monitoring of treatment. In paediatric haematooncology there is a broad spectrum of applications of molecular techniques (*1,2,3*). In this review article I summarize our experience using two such examples that may significantly improve outcome of children treated for haematological malignancy: 1) monitoring of residual disease by reverse transcription polymerase chain reaction (PCR) (*3,4,5*); and 2) detection of different opportunistic pathogens in immunocompromised children using PCR and restriction fragment length polymorphism (RFLP) (*6,7,8*).

Minimal residual disease

Monitoring of acute leukemia patiens during and after treatment for the presence of remaining leukemic cells (minimal residual disease, MRD) has been shown to give major insight into the effectiveness of treatment *(1,3,4,5)*. In particular the measurement of the decrease of the leukemic cell load during the first phases of treatment has high prognostic value. Patients with acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) may have a total of approximately 1012 malignant cells at the time of diagnosis *(2)*. The disease is considered to be in complete remission when less than 5% of malignant cells in bone marrow are morphologically detectable *(2,5)*. These patients, however, still harbour as many as 1010 leukaemic cells. From that point, the level of malignant cells is largely unknown. Tremendous effort has been made in past 20 years to describe and characterize residual leukaemic cells regarding prognosis and therapy. Leukaemic cells can be distinguished from normal haematopoietic progenitors on the basis of morphologic and cytochemical properties, karyotypic or genetic abnormalities, antigen-receptor gene rearrangements, cell growth requirements in vitro, and immunophenotype *(1,2)*. Combinations of these techniques can detect small numbers of leukemic blasts among normal cells. At the First Department of Pediatrics, Masaryk university, Brno we focussed on children with AML *(3,9)*. Their prognosis is not satisfactory; long-term remission can be achieved only in 25–40% of children *(4)*. The aim of the proposed study was to improve diagnosis of AML in cases characterized by specific chromosomal translocations t(8;21) and t(15;17) *(10)*. These translocations can be efficiently detected using reverse transcriptase PCR *(2,3)*. To determine optimal conditions for detection of the t(8;21) and t(15;17) translocations in human cells, a detail study of experimental conditions for reverse transcription PCR on human cell lines NB-4 and KASUMI-1 was performed. The results proved to be valid for clinical diagnostics of these chromosomal aberrations in blood and/or bone marrow samples of AML patients and 8 patients diagnosed at the 1st Department of Pediatrics, Masaryk University, Brno with AML were monitored. Higher sensitivity of reverse transcription PCR in comparison to flow cytometry and malignant cell morphology has been noticed in all cases during AML treatment *(10)*.

Opportunistic infections

Extensive bone marrow infiltration with cancer cells and anticancer therapy lead to immune incompetence in children with cancer (*8,11*). Since the prognosis and overall survival of children with cancer have dramatically improved during past 30 years, problems remain related to infections, mainly during leukopenic periods (*11,12*). Causal pathogens of febrile neutropenia (FN), most frequently bacterial, are identified and confirmed by culture in 25–35% of the cases (*11,12*).

In other 15–25% of patients with FN, bacterial or fungal pathogens are suspected clinically (*11,12,13*). The remaining 50% of cases are classified as a fever of unknown origin and may be caused by other pathogens, namely viruses and fungi, that are more difficult to detect by conventional diagnostic methods (*13,14,15*).

Lymphotropic herpesviruses Epstein-Barr virus (EBV), cytomegalovirus (CMV) and human herpesvirus-6 (HHV-6) establish a lifelong persistent infection in a great majority of humans. They usually produce inaparent infection or transient immune compromise in otherwise healthy hosts but are able to cause life-threatening primary or reactivated infections in individuals with congenital or acquired T-cell immunodeficiencies (*14–18*). The spectrum of diseases caused by lymphotropic herpesviruses is well documented in patients undergoing bone marrow transplantation or organ transplantation and in individuals infected with human immunodeficiency virus (*15–21*). In patients undergoing conventional anticancer therapy without BMT, the data focusing on EBV, CMV or HHV-6 infection are scarcely documented and mostly reported as individual cases (*22–24*). With a combined diagnostic approach of serology and viral DNA detection, a prospective study of children undergoing non-BMT therapy for cancer was performed at the First Department of Pediatrics, Masaryk University, Brno to demostrate the incidence of infections caused by lymphotropic herepesviruses (*25,26*).

The group of 186 children was examined for the presence of EBV, CMV and HHV-6 before, during, and 2 months after the end of anticancer treatment. Serology and viral DNA analysis by polymerase chain reaction of these viruses was monitored in all children. At the time of cancer diagnosis, there was no difference in seropositivity between cancer patients and age-matched healthy controls. During anticancer therapy, EBV and CMV infection was present in 65 (34.9%) and 66 (35.4%) of 186 patients, respectively (*25,26*). HHV-6 infection was present in 23 (32.9%) of 70 patients (*18,25*). The infection occurred independently from blood transfusions given (*25*). Combination of serology and DNA analysis in detection of symptomatic EBV, CMV, and HHV-6 infection was superior to serology alone and viral DNA positivity closely correlated with early clinical symptoms. EBV, CMV and HHV-6 infections were frequently present during therapy of paediatric malignancy (*25*).

Another opportunistic pathogens that are hardly diagnosed by standard cultivation or serological techniques are fungal pathogens (*13*). The early diagnosis of fungal infection (namely the most frequent candidosis and aspergillosis) can prevent dissemination and systemic spread of fungi if early treatment is instituted (*27,28*). This is not possible with standard techniques since fungal pathogens are usually slow-growing in artificial cultivation media and serology is frequently unreliable (*29,30,31*). Using a combination of PCR and RFLP techniques to detect highly conservative fungus-specific DNA regions

enables precise, rapid and unbiased diagnosis from blood or any other tissue from patients with suspected fungal infection (*32,33*). In a group of 24 children aged 1–18 years with cancer and febrile neutropenia treated at the First Department of Pediatrics, Masaryk University, Brno the invasive mycotic infection was monitored using standard cultivation in combination with fungus-specific DNA detection by PCR and RFLP (*33*). Fungal DNA was found in peripheral blood in 11 episodes of febrile neutropenia in 10 patients while cultivation from blood cultures (*C. albicans, C. tropicalis, C. glabrata*) was positive in only 5 out of 11 PCR-positive episodes of febrile neutropenia. In a patient with bronchopneumonia, the aspergiloma has developed with both histomorphological and PCR confirmation (*33,34*). Thus, the examination of fungal DNA by molecular techniques was reliable and highly sensitive method for the detection of invasive mycotic infection enabling early targeted antifungal therapy in patients with cancer, especially during an episode of febrile neutropenia (*33–36*).

CONCLUSIONS

The introduction of molecular techniques and their translation from basic research laboratory to the clinic represents one of the major recent improvements in the care of our patients. I believe that providing just a brief summary of an ongoing clinical research at the 1st Department of Pediatrics at the Masaryk University in Brno will provide the reader with sufficient information about the clinical applications of molecular biology techniques.

Acknowledgements

I acknowledge Drs. Jan Šmarda, Radek Horváth and Jaroslav Michálek for their contribution in introducing molecular approaches at the 1st Department of Pediatrics.

Hrstková H.

MOLEKULÁRNÍ PŘÍSTUPY K DĚTSKÉ HEMATOONKOLOGII

Souhrn

Tento přehled shrnuje zkušenosti I. dětské kliniky Lékařské fakulty Masarykovy university v Brně se současnými molekulárními přístupy k dětské hematoonkologii. Je hlavně zaměřen na detekci mimální zbytkové choroby u pacientů s akutní leukémií a na detekci oportunních infekcí u imunokompromitovaných dětí – tedy na dvě oblasti zásadního významu v dětské hematoonkologii. Molekulární techniky jako polymerázová řetězová reakce a polymorfismus délky restrikčních fragmentů slouží jako účinný prostředek pro spolehlivou a rychlou diagnostiku a podrobný popis procesů, které by jinými standardními diagnostickými metodami byly nedetekovatelné.

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