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Professional paper

CD20 Positive Childhood B-non Hodgkin Lymphoma (B-NHL): Morphology, Immunophenotype and a Novel Treatment Approach: A Single Center Experience

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A B S T R A C T

Lymphomas represent the third most common group of cancers in childhood and adolescence, mature B non Hodgkin's lymphoma (B-NHL) accounting for up to 60% of newly diagnosed patients. The diagnosis of specific entities of B-NHL is based on well-defined morphologic analysis, immunophenotyping, cytogenetics and molecular genetics, which determine the optimal treatment strategy. In adult population a major turning point in treatment of B-NHL has been achieved since rituximab, in combination with CHOP has improved the survival rate up to 19%. Rituximab is a chimeric monoclonal antibody that targets CD20, a transmembrane calcium channel expressed on normal and malignant B-cells that mediates cytotoxic, apoptotic and anti-proliferative effects. The effect of rituximab in pediatric population is still not well enough investigated. Based on morphology and immunophenotype of malignant cells, seven children with B-NHL in our institution were eligible for treatment with modified B-NHL-Berlin-Frankfurt-Münster (BFM)-95-based protocol with rituximab administered on day -5. The complete remission was achieved in all seven patients. Six patients are still in complete remission at least 12 months after having finished chemotherapy and one patient relapsed two months after the last cycle and subsequently died. Major adverse effects observed during treatment were prolonged B-cell depletion and myelosupression. Rituximab in combination with B-NHL-BFM-95 protocol was otherwise well tolerated and proved to be effective in children and adolescents with B-NHL. The number of our patients is too small and the follow-up of a larger group of patients will help in defining the role of rituximab in the treatment of childhood B-NHL.

Key words: *non Hodgkin lymphoma, children, rituximab*

Introduction

In pediatric population the mature B cell-non-Hodgkin lymphomas (NHL) represent approximately 60% of all diagnosed NHL, with Burkitt's lymphoma (BL)/Burkitt's-like lymphoma (BLL) and diffuse large B-cell lymphomas (DLBCL) accounting for up to 40% and 10–20% of cases, respectively[1,2].

The diagnostic criteria for B-NHL according the new World Health Organization (WHO) classification include pathology, immunophenotype, cytogenetics and molecular genetics, which determine the approach to therapy and prognosis.

During the last decade the 5-year event–free survival (EFS) of children with both localized and advanced B--NHL has improved significantly, mainly due to intensive multi-agent chemotherapy protocols applied as standard treatment approach. The EFS for stages I and II now reaches ≥90–95% and for stages III and IV 80–90%, respectively[3–5].

In spite of this success, a proportion of patients do not respond to therapy. High-dose therapy induces toxicity in a majority of patients, resulting in numerous complications, increased demand for supportive care and pro-

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longed hospitalization periods[5]. Patients with relapsed B-NHL represent an especially vulnerable group, as their prognosis remains poor despite aggressive therapy approach, with EFS being 30%[6]. Therefore, further studies are needed to optimize the therapeutic approach in this group of patients.

Rituximab is a chimeric monoclonal antibody targeting CD20, a transmembrane calcium channel which is expressed on all normal B-cells and ≥98% of malignant B-NHL cells[7]. It mediates complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, sensitizes B-NHL cells to cytotoxic chemotherapy and has direct apoptotic and anti-proliferative effects[8]. Due to encouraging results obtained from two pivotal trials[9,10], regimen consisting of rituximab and CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) is now well established as a standard first-line treatment for diffuse large B cell lymphoma (DLBCL) in adult patients and is a putative viable treatment in patients with relapsed or refractory indolent B-NHL.

Data on combined therapy with rituximab in pediatric population with B-NHL are still scarce and many of the trials have been published only as abstracts or brief reports.

The aim of this study was to evaluate the therapeutic effect and toxicity of the modified B-Non-Hodgkin's Lymphoma (NHL)-Berlin-Frankfurt-Munster (BFM)-95-based protocol + rituximab in 7 children with diffuse large B cell lymphoma (DLBCL), Burkitt (BL) and Burkitt-like lymphoma (BLL), with emphasis on diagnostic criteria and molecular characteristics of these entities.

Methods

Patients and eligibility

All patients met the following study criteria: age below 18 years, newly diagnosed with either CD20+ Burkitt or Burkitt-like lymphoma and DLBCL (according to the Revised European-American Lymphoma Classification or the World Health Organization Classification), stage III or IV according to Ann Harbour staging system, and stage II if LDH levels were at least two-fold the institutional upper value; they have not received any previous treatment; active hepatitis infection had to be excluded by serologic tests; the patients had to be available for follow-up for at least two years after beginning of the therapy. A written informed consent was required from the patients' parents.

Diagnosis

The diagnosis was established according to physical examination, a full blood count (FBC), a metabolic evaluation, chest X-ray scan, a MSCT scan of neck, thorax, abdomen and pelvis to evaluate the extent of the spreading of the disease and staging, a CT brain scan where indicated, a percutaneous sternal bone marrow (BM) aspiration and a lumbar puncture prior to treatment.

All patients underwent surgery to obtain a sample for histology and radical surgery was performed in two patients with stage II disease. The diagnosis of B-NHL was based on both histology and cytology in all cases, including PCR and FISH analysis. Fine needle aspiration material of bone marrow was air dried and stained for morphological analysis with May-Grünwald-Giemsa (Figures 1 and 2).

The expression of CD20 on lymphoma cells was identified by immunohistochemistry in all cases. In addition, in two patients with bone marrow involvement, CD20 positivity was verified by flow cytometry (Figure 3). The patients in whom specific translocations were identified were included in the study.

Treatment

All patients received intravenous infusions of rituximab at a dose of 375 mg/m^2 5 days prior to each cycle of chemotherapy according to the standard NHL-BFM 95 treatment protocol, with a modification of prolonging the

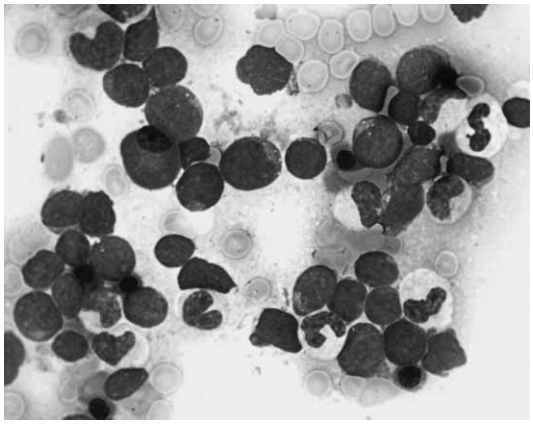

Fig. 1. Fine needle aspiration material of bone marrow in patient with B-ALL, stained for morphological analysis with May-Grünwald-Giemsa, large magnification. Pathognomonic cytoplasmatic vacuoles visible inside lymphocytes.

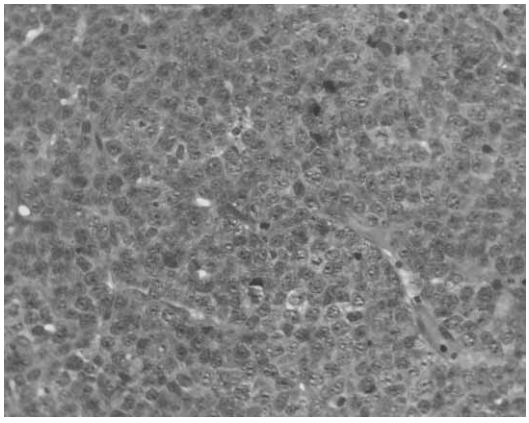

Fig. 2. Fine needle aspiration material of bone marrow in patient with DLBCL, stained for morphological analysis with May-Grünwald-Giemsa, small magnification. Typical morphological findings.

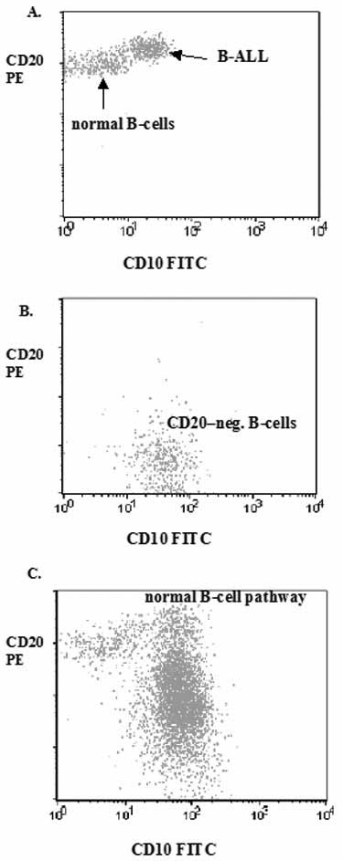

Fig. 3. Follow-up of CD20+ bone marrow B-cells in patient with B-ALL detected by flow cytometry at diagnosis, with clear grouping in the area typical for B-ALL (A). Absence of CD20+ B-cells during treatment with rituximab (B). Normalization of CD20+ population of B-cells 12 months after completing the treatment (C).

time interval between the cycles to 28 days. Prior to rituximab each patient received premedication consisting of paracetamol 15 mg/kg *per* os and diphenhydramine 1 mg/kg in a slow intravenous infusion.

Rituximab was diluted with 0.9% NaCl and was administered for the first time at a speed of 0.5 mg/kg/hour (max. 50 mg) during the first hour, and was subsequently increased to 0.5mg/kg with every following hour.

During following cycles of chemotherapy the infusion started with a dose of 1 mg/kg, and the dose was subsequently increased to 1 mg/kg every following hour.

In case of adverse reactions the infusion was stopped immediately and saline solution was administered. After 30 minutes the rituximab was continued at initial speed.

In the event of more severe reactions or anaphylaxis, the patient received treatment as for other allergic (anaphylactic) reactions. Supportive treatment was administered according to NHL-BFM 95 protocol.

Patients' follow up

Complete remission (CR) was defined by the absence of tumor mass confirmed by clinical examination and imaging methods, normal FBC, BM and cerebrospinal fluid (CSF) findings. Control MSCT to evaluate tumor regression was performed after the second cycle of chemotherapy; in case of complete remission, defined by presence of less than one third of tumor mass, it was repeated after the sixth cycle. In case more than one third of tumor mass remained (i.e. partial remission or non-response); the MSCT was repeated after the fourth and sixth cycle.

In patients with FAB-L3 ALL the percutaneous sternal (BM) aspiration was performed before second, third and fourth cycle of chemotherapy.

After completing the treatment, all patients underwent monthly blood and metabolic evaluation during the first year, and every three to six months during the second year. A control MSCT scan of neck, thorax, abdomen and pelvis was performed at six, twelve and twenty four months after having received the last cycle of chemotherapy and the last is planned after thirty six months.

In patients with leukemia, BM aspiration was performed twelve and eighteen months after completing the chemotherapy and the last is planned after thirty months.

Results

A total of 7 patients of different sex and age were enrolled between November 2006 and December 2007, as demonstrated in Table 1. The median age was 8 years,

TABLE 1
ELIGIBLE PATIENTS FOR TREATMENT WITH MODIFIED B-NHL-BFM-95 PROTOCOL AND RITUXIMAB

Patient	Age	Diagnosis	Primary site	Blasts in the bone marrow
BV, male	8	DLBCL, st II	nasopharynx	2%
BD, male	6	DLBCL, st II	tonsil	1%
BN, female	15.5	DLBCL, st IV	abdomen, thorax	2.50%
GS, female	7	DLBCL/L3ALL, st IV,t(8;14)	abdomen	83%
MA, male	4	Burkitt-like B-NHL, L3ALL, st IV	abdomen, thorax	96%
VM, male	11	BL, L3ALL, st IV	abdomen	70%
KA, male	15.5	DLBCL, st IV	abdomen, thorax	2%

BL – Burkitt's lymphoma, BLL – Burkitt's-like lymphoma, DLBCL – diffuse large B-cell lymphoma

ranging from 4 years to 15,5 years. All the patients were first diagnosed with B-NHL in our institution, two of them with DLBCL stage II, two with DLBCL stage IV, one with DLBCL/L3ALL stage IV, one with BLL/L3ALL stage IV and one with BL/L3ALL stage IV disease. Two patients with grade II disease had LDH level higher than 500U/L which is more than two fold the institutional upper value and therefore met all the required criteria for rituximab treatment.

None of the patients had malignant cells in the cerebrospinal fluid.

Cytogenetic analysis was performed in all patients and showed translocation t(8;14) in one patient with DLBCL/L3ALL stage IV disease. Other patients had normal cytogenetics.

Radical surgery was performed in two patients with disease stage II localized in tonsils and epipharynx respectively, whereas all other patients underwent surgery to obtain a tumor sample for histological analysis.

Chemotherapy was initiated directly after recovering from surgery, usually within one week after admittance to our institution.

Two patients with stage II disease received 4 cycles of rituximab and 4 cycles of chemotherapy, respectively. Five patients with stage IV disease received 6 cycles of rituximab and 6 cycles of chemotherapy.

Complete remission was achieved in all 7 patients. Six patients achieved remission before completing the second cycle of chemotherapy and only one patient with DLBCL grade IV achieved remission just before the fourth cycle of chemotherapy. Six patients are still in complete remission (19, 22, 23, 25, 26 and 27 months after completing the treatment).

One patient with DLBCL/L3ALL stage IV suffered a meningeal relapse two months after having completed the sixth cycle of chemotherapy; at that time the PCR analysis of bone marrow aspirate showed clonal rearrangement of the IgH gene. The patient was treated according to NHL-BFM 1995 protocol and received rituximab on day –5. An Ommaya reservoir was implanted for intraventricular therapy. Following chemotherapy, the radiation of cranium and upper 3 segments of cervical spine were performed with a total of 2400 cGy. Despite the aggressive multi-agent treatment approach, hematological dissemination of disease occurred and the patient died 8 months after the first relapse.

Adverse effects

Mucositis was registered in all patients. Major adverse effects of rituximab therapy were prolonged B-cell depletion and myelosupression that resulted in severe disseminated fungal infections and sepsis. This condition was then treated with amphotericin B or caspofungin and antibiotics, respectively.

All patients developed fungal infection of oral mucosa, ranging from intense oral thrush to wide spread excessive infection of oral and esophageal mucosa, disabling any oral intake, as well as maceration and epidermolysis of perigenital area in the most severe cases.

One patient had a transitory loss of intestinal peristaltics which was treated conservatively. In one patient a transitory increase of blood urea and creatinin levels was noted during the first cycle of chemotherapy, which subsided spontaneously after completing the treatment. All patients required blood products due to severe bone marrow aplasia.

Discussion

The recent REAL and WHO classification suggests four major subtypes of pediatric NHL: small, non-cleaved cell (Burkitt and Burkitt's like), lymphoblastic, B- large cell lymphoma and anaplastic large cell lymphoma. According to clinical presentation, NHL can be roughly classified as low-, intermediate- (indolent) and high-grade (aggressive) tumors.

However the classification includes immunophenotyping and molecular and genetic analysis of tumor tissue, which erases differences between certain entities in NHL but it also allows more accurate definition of origin of tumor cells thus steering approach in treatment, targeting tumor specific antigens by means of a growing number of selected antibodies. More than 90% of children are diagnosed with high grade tumors, whereas low and intermediate grade tumors gain in frequency after adolescence.

In adult population approximately 5% of new cases of invasive cancer diagnosed in the US in 2001 were NHL[2], the indolent type accounting for 25–40%, and the aggressive forms for 60–75%[3,4]. Almost 90% of all lymphomas are of B-cell origin however in contrast to pediatric NHL, the most common forms are follicular B-cell lymphoma, which is very rare in children, and large B- or T-cell lymphoma. In pediatric population 9–10% of all diagnosed cancers are NHL[10], B-cell NHL accounting for up to 60% of all diagnosed NHL. This difference in immunophenotype and histology presumably reflects changes in function and cellular profiling of the immune system of pediatric and adult patients[6].

Although more than 90% of pediatric NHL is of high-grade histology, the outcome of treatment is better than the adult's and after intensive multiagent chemotherapy more than 80% of patients achieve long-term EFS[8,9,11]. In children with B-NHL the overall EFS at 5 years amounts to 86±2%[11]. Boys are affected two to three times more frequently than girls, with incidence in both sexes steadily increasing throughout life[6,10].

During the past 17 years 76 patients were diagnosed with Non Hodgkin lymphoma in this institution. 31 of them (40%) were diagnosed with B-cell Non Hodgkin lymphoma but none with follicular lymphoma. 16 patients were male and 15 were female, with median age 9.3 years (2–16 years), which stands to some extent in discordance with general statistics for childhood B-NHL.

Following the encouraging results reported from treatment of adult NHL with rituximab in combination with

CHOP, seven patients in this institution were chosen for the first time for treatment with NHL-BFM95 protocol and rituximab.

In pivotal trials in adults, rituximab was administered on day 1 of chemotherapy. However our patients received rituximab on day –5, to reduce the putative side-effects and more closely monitor the outcome of therapy, concerning the overall well-being of children, blood and metabolic evaluation. The standard NHL-BFM95 protocol had to be modified, as most children developed excessive fungal infections of oral, esophageal and rectal mucosa, which required prolonged antimycotic treatment and was to that extent never observed in patients treated with standard protocol. Therefore in children receiving rituximab new cycles of chemotherapy were started every 28 days instead of recommended 21 days. It remains to be disputed, whether rituximab is responsible for such severe clinical presentations.

Although the numbers of patients we presented in this study are too small, we believe the results are optimistic. Further trials are necessary to establish the role of rituximab in treatment of children with CD20 positive Non-Hodgkin's lymphoma.

REFERENCES

1. REITER A, KLAPPER W, Br J Haematol, 142 (2008) 329. — 2. CAIRO MS, 2003 Lymphoma Research Foundation. — 3. PATTE C, AUPERIN A, MICHON J, BEHRENDT H, LEVERGER G, FRAPPAZ D, LUTZ P, COZE C, PEREL Y, RAPHAËL M, TERRIER-LACOMBE MJ, Blood, 97 (2001) 3370. — 4. REITER A, SCHRAPPE M, TIEMANN M, LUDWIG WD, YAKISAN E, ZIMMERMANN M, MANN G, CHOTT A, EBELL W, KLINGEBIEL T, GRAF N, KREMENS B, MÜLLER-WEIHRICH S, PLÜSS HJ, ZINTL F, HENZE G, RIEHM H, Blood, 94 (1999) 3294. — 5. PATTE C, AUPERIN A, GERRARD M, MICHON J, PINKERTON R, SPOSTO R, WESTON C, RAPHAEL M, PERKINS SL, MCCARTHY K, CAIRO MS; FAB/LMB96 Blood, 109 (2007) 2773. — 6. CAIRO MS, SPOSTO R, PERKINS SL, MEADOWS AT, HOOVER-REGAN ML, ANDERSON JR, SIEGEL SE, LONES MA, TEDESCHI-BLOK N, KADIN ME, KJELDSBERG CR, WILSON JF, SANGER W, MORRIS E, KRAILO MD, FINLAY JL, Br J Haematol, 120 (2003) 660. — 7. PERKINS SL, LONES MA, DAVENPORT V, CAIRO MS, Clin Adv Hematol Oncol, 1 (2003) 314. — 8. STOLZ C, HESS G, HÄHNEL PS, GRABELLUS F, HOFFARTH S, SCHMID KW, SCHULER M, Blood, 112 (2008) 3312. — 9. COIFFIER B, LEPAGE E, BRIERE J, HERBRECHT R, TILLY H, BOUABDALLAH R, MOREL P, VAN DEN NESTE E, SALLES G, GAULARD P, REYES F, LEDERLIN P, GISSELBRECHT C, N Engl J Med, 346 (2002) 235. — 10. MCLAUGHLIN P, GRILLO-LÓPEZ AJ, LINK BK, LEVY R, CZUCZMAN MS, WILLIAMS ME, HEYMAN MR, BENCE-BRUCKLER I, WHITE CA, CABANILLAS F, JAIN V, HO AD, LISTER J, WEY K, SHEN D, DALLAIRE BK, J Clin Oncol, 16 (1998) 2825. — 11. GREENLEE RT, HILL-HARMON MB, MURRAY T, THUN M, CA Cancer J Clin, 51 (2001) 15. — 12. CHESON BD, N Engl J Med, 346 (2002) 280. — 13. CZUCZMAN MS, FALLON A, MOHR A, STEWART C, BERNSTEIN ZP, MCCARTHY P, SKIPPER M, BROWN K, MILLER K, WENTLING D, KLIPPENSTEIN D, LOUD P, ROCK MK, BENYUNES M, GRILLO-LÓPEZ AJ, BERNSTEIN SH, Semin Oncol, 29 (2002) 36. — 14. SANDLUND JT, DOWNING JR, CRIST WM, N Engl J Med, 334 (1996) 1238. — 15. PILLON M, DI TULLIO MT, GARAVENTA A, CESARO S, PUTTI MC, FAVRE C, LIPPI A, SURICO G, DI CATALDO A, D'AMORE E, ZANESCO L, ROSOLEN A, Cancer, 101 (2004) 385.

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CD20 POZITIVNI B NE-HODGKINOVI LIMFOMI U DJECE (B-NHL): MORFOLOGIJA, IMUNOFENOTIPIZACIJA I NOVIJA TERAPIJSKA DOSTIGNUĆA: ISKUSTVA JEDNOG CENTRA

SAŽETAK

Limfomi su treći najčešći maligni tumor u djece i adolescenata, od čega na B ne-Hodgkinov (B-NHL) limfom otpada oko 60% novodijagnosticiranih slučajeva. Dijagnoza B-NHL-a temelji se na morfološkoj analizi, imunofenotipizaciji, citogenetskoj analizi i na molekularnoj genetici. Bitan napredak u liječenju B-NHL-a u odraslih postignut je dodavanjem rituximaba uz protokol CHOP nakon čega je došlo do poboljšanja preživljenja za oko 19%. Rituximab je kimerično monoklonsko protutijelo koje se veže za CD20, transmembranski receptor koji se nalazi na normalim i malignim B limfocitima i ima citotoksični, proapoptotički i antiproliferativni učinak. Učinak rituximaba u djece još je uvijek nepoznanica. Temeljem analize uzoraka tkiva kod sedmero djece postavljena je dijagnoza B-NHL-a. Svi su liječeni u našoj ustanovi prema protokolu liječenja B-NHL-BFM 95 sa dodatkom rituximaba koji je primjenjen pet dana prije početka kemoterapije. Kompletna remisija je postignuta u svih sedam pacijenata. Šestero pacijenata se još uvijek nalazi u kompletnoj remisiji najmanje 12 mjeseci od prestanka liječenja. Jedan pacijent je dobio relaps dva mjeseca po prestanku terapije i kasnije umro. Glavne neželjene nuspojave terapije bile su produljeni nedostatak B limfocita i produljeno potiskivanje rada koštane srži. Cjelokupno gledajući djeca su dobro podnosila rituximab sa protokolom B-NHL-BFM--95. Broj pacijenata u ovom radu je premali i za konačne zaključke bit će potrebno praćenje veće skupine pacijenata.