6-MP based maintenance therapy of childhood ALL in Slovenia: A retrospective study from 1970 to 2004

Uporaba 6-merkaptopurina v vzdrževalni fazi zdravljenja ALL pri otrocih v Sloveniji: Retrospektivna študija za obdobje od 1970 do 2004

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Ključne besede:
akutna limfoblastna levkemija; 6-merkaptopurin; tiopurini; stranski učinki; relaps

Key words:
acute lymphoblastic leukaemia; 6-mercaptopurine; thiopurines; side effects; relapse

Citirajte kot/Cite as:
Zdrav Vestn 2015; 84: 99–107

Prispelo: 24. dec. 2013,
Sprejeto: 20. avg. 2014

Abstract

Background: Maintenance therapy with 6-mercaptopurine (6-MP) as its major component has been one of the main reasons for the drastic increase in overall survival of paediatric patients with acute lymphoblastic leukaemia (ALL). In our cohort, consisting of patients receiving maintenance therapy for ALL and encompassing time period of three decades, we evaluated dosage, safety and efficiency of 6-MP in the treatment of childhood ALL according to treatment protocol, age and gender.

Methods: Slovenian paediatric ALL patients diagnosed and treated from 1970 to 2004 were identified through the national oncology patients` registry. Of 414 registered paediatric ALL patients, 320 received maintenance therapy for ALL and were included in the final study cohort. Information about age, gender, treatment protocol, 6-MP related toxic events and ALL relapse was extracted from patients` files. Differences in 6-MP dose reduction, 6-MP side effects and relapse according to gender, age and treatment protocol were statistically evaluated.

Results: After adjustment for gender and age, the incidence of 6-MP dose reduction ($p < 0.001$) and bone marrow suppression ($p = 0.019$) was higher in recent treatment protocols, while relapse was more common in older protocols ($p < 0.001$). Younger patients had greater risk for sepsis/infection ($p = 0.002$), while greater age was a risk factor for osteonecrosis ($p = 0.001$). No statistically significant associations were found according to gender.

Conclusions: In the retrospective study, encompassing three decades of maintenance treatment of childhood ALL in Slovenia, recent protocols exhibited greater effectiveness and toxicity than older protocols.

Izvleček

Izhodišča: Vzdrževalno zdravljenje s 6-merkaptopurinom (6-MP) je eden od vodilnih vzrokov za drastično povečanje stopnje preživetja pediatričnih bolnikov z akutno limfoblastno levkemijo (ALL). V študiji smo retrospektivno ovrednotili odmerke 6-MP, varnost in učinkovitost vzdrževalnega zdravljenja pri otrocih z ALL glede na starost, spol in protokol zdravljenja v obdobju tridesetih let.

Metode: Pediatrične bolnike, ki so se med letoma 1970 in 2004 v Sloveniji zdravili za ALL, smo identificirali s pomočjo nacionalnega registra raka. Od 414 registriranih bolnikov jih je 320 prejelo vzdrževalno zdravljenje za ALL in smo jih vključili v končno študijsko populacijo. Iz bolniških kartonov smo pridobili informacije o starosti, spolu, protokolu zdravljenja, stranskih učinkih 6-MP in relapsu bolezni. Statistično smo ovrednotili razlike v zmanjšanju odmerka 6-MP, toksičnih učinkih 6-MP in relapsu ALL glede na spol, starost in protokol zdravljenja.

Rezultati: Ob upoštevanju starosti in spola sta bila zmanjšanje odmerka 6-MP ($p < 0.001$) in

zavora kostnega mozga (p = 0.019) bolj pogosta pri bolnikih, ki so bili zdravljeni z novejšimi protokoli zdravljenja, medtem ko je bilo več relapsov v starejših protokolih (p < 0.001). Mlajši bolniki so bili bolj nagnjeni k sepsi in infekcijam (p = 0.002), medtem ko je bila osteonekroza bolj pogosta pri starejših (p = 0.001). Noben izmed preiskovanih dejavnikov pa se ni statistično značilno razlikoval med spoloma.

Zaključki: V retrospektivni študiji, ki zajema tri desetletja vzdrževalnega zdravljenja otroške ALL v Sloveniji, so se novejši protokoli zdravljenja izkazali za bolj učinkovite, vendar tudi bolj toksične, kot starejši protokoli.

Background

Acute lymphoblastic leukaemia (ALL) can occur in both children and adults, but 60 % of cases are people younger than 20 years, with its incidence peaking between ages 2 and 5. ALL represents 13 % of all diagnosed leukaemias and 25 % of all childhood cancers, thus being the most common childhood malignancy.[1,2] Overall survival in children aged 1 to 10 years approaches 90 %, but is much lower in infants and adults. In addition, high presenting leukocyte count, male gender, Hispanic or black race, T-cell immunophenotype and delayed response to the induction therapy are adverse clinical prognostic factors in childhood ALL.[2]

Multiagent chemotherapy for ALL typically lasts 2–3 years and is divided into four phases: remission induction, consolidation, reinduction and maintenance. Induction therapy consisting of glucocorticoid (prednisone or dexamethasone), vincristine and asparaginase eradicates the initial leukemic cell burden and restores normal haematopoiesis.[2,3] Consolidation therapy eradicates residual leukemic cells and comprises high-dose methotrexate (MTX) and 6-mercaptopurine (6-MP) with or without vincristine and glucocorticoid pulses and asparaginase.[2] This is followed by the reinduction phase in which similar drugs as in the induction therapy are administered in addition to intermediate or high dose MTX.[3] Maintenance therapy is the longest phase, lasting 2 or more years and comprises mainly daily 6-MP and weekly MTX with or without pulses of vincristine and dexamethasone.[2,3] Although 97–99 % of childhood ALL patients achieve a complete remission of the disease, only 75–80 % are cured.[4] Most of the deaths occur due to the relapse of the disease. The maintenance therapy is the most important component of ALL treatment in terms of relapse prevention as demonstrated by several studies on adults,[5] adolescents[6] and children.[7] A long- term report of five ALL-BFM trials indicates that the shortening of the maintenance therapy for only 6 months resulted in a significant increase of relapse rate.[7]

Thiopurine drug 6-MP is the backbone of the maintenance therapy, as it is applied daily for 2–3 years. It has been synthesised by Elion and Hutchins by substitution of oxygen with sulphur at carbon 6 of hypoxanthine and shown to be active against rodent tumours.[8] In 1953 it was proved to be active in children with ALL: in one of the first double-blind randomized trials ALL children induced into remission with steroids were found to have prolonged remission if maintained on 6-MP compared to placebo.[9] 6-MP is found to be the crucial part of ALL therapy, both in terms of its efficiency (relapse risk) and safety (toxic side effects). A recent study indicated that lower adherence to oral 6-MP significantly increases risk of relapse.[10] The most common toxic effects of 6-MP include bone marrow suppression, which leads to sepsis, recurrent infection and febrile leukopenia. Furthermore, stomatitis and osteonecrosis were noted at an increased frequency during maintenance therapy. Finally, 6-MP is known to increase risk of secondary malignancies in patients on thiopurine treatment, the most common being AML, brain tumours and skin cancers.[11,12]

In this retrospective study encompassing three decades of childhood ALL treatment in Slovenia, we investigated the incidence of 6-MP dose reduction, 6-MP related side effects and relapse, and their dependence of age, gender and treatment protocol.

Methods

Subjects

Slovenian paediatric ALL patients diagnosed and treated from 1970 to 2004 were identified through the national oncology patient registry for the purpose of several other retrospective pharmacogenetic studies. Ethical approval was obtained from the Medical Ethics Committee of Slovenia. Of 414 registered paediatric ALL patients, 64 medical files were missing or inadequate, 9 patients with acute myeloid leukaemia and 3 with non-Hodgkin lymphoma were misclassified as ALL, 3 had bone marrow transplantation before maintenance therapy and in 15 patients 6-MP was not administered due to death or relapse before the beginning of the maintenance therapy. The final study group thus consisted of 320 patients, of which 47.5 % were female and 52.5 % male, their median age at diagnosis being 4.4 years (range: 2 months to 17 years). Different treatment protocols were applied in the study periods from 1970 to 2004 (Table 1), which was extensively reviewed by Jazbec et al.[4]

The toxic event (bone marrow suppression, stomatitis, sepsis/infection) was defined as an event causing discontinuation of the therapy for longer than 1 week, a reduction of over 10 % of 6-MP dose for a duration longer than 3 months, or the hospitalization of the patient. Febrile neutropenia was defined as concurrent absolute neutrophil count of less than 1000/mm^3 and body temperature higher than 38.3 °C. Hepatotoxicity was defined as a persistent elevation of transaminase activities that led to replacement of 6-MP with 6-TG, discontinuation of therapy for longer than 1 week, or a reduction of over 10 % of 6-MP dose for a duration longer than 1 month. Only symptomatic cases of osteonecrosis causing pain and impaired mobility were included in the analysis. The follow-up time for secondary neoplasms was 5 years or more for all patients.

Statistical analysis

Differences in 6-MP dose reduction, 6-MP side effects and relapse according to gender and treatment protocol were evaluated using Fisher exact test. Differences in the

Table 1: Characteristics of paediatric ALL patients (N = 320), treated with 6-MP in Slovenia from 1970 to 2004.

Gender	female: 152 (47.5 %) male: 168 (52.5 %)
Age at diagnosis (years)	median: 4.4 min: 0.2 max: 17.0
Treatment protocol	POG: 99 (30.9 %) BFM 83: 37 (11.6 %) BFM 86: 56 (17.5 %) BFM 90: 60 (18.8 %) BFM 95: 55 (17.2 %) BFM IC 2002: 13 (4.1 %)
6-MP dose reduction	63 (19.7 %)
Bone marrow suppression	44 (13.8 %)
Stomatitis	14 (4.4 %)
Sepsis and/or infection	67 (20.9 %)
Febrile neutropenia	24 (7.5 %)
Hepatotoxicity	10 (3.1 %)
Osteonecrosis	9 (2.8 %)
Secondary neoplasms[1]	14 (4.4 %)
ALL relapse	108 (33.8 %)

[1] *AML (3/13), meningioma (2/13), glioblastoma (2/13), oligodendroglioma (1/13), astrocytoma (1/13), Hodgkin disease (1/13), non-Hodgkin lymphoma (2/13), osteosarcoma (1/13), basal cell carcinoma (1/13).*

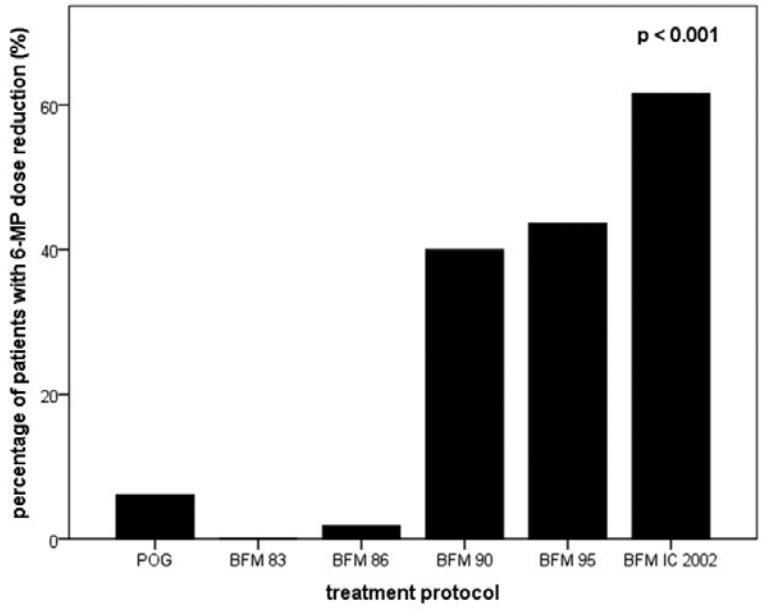

Figure 1: 6-MP dose reduction in ALL patients according to treatment protocol. 6-MP dose reduction was more frequent in recent protocols (BFM 90, 95 in IC 2002). 6-MP dose reduction greater than 10 % that lasted longer than 3 months was considered significant.

abovementioned variables according to age were calculated my means of Mann-Whitney U test. Logistic regression analysis was used for the adjustment for other covariates in the model. All statistical tests were performed using SPSS 21 for Windows. The p value of less than 0.05 was considered statistically significant.

Results

Incidence of 6-MP dose reduction, bone marrow suppression, stomatitis, sepsis/infection, febrile neutropenia, hepatotoxicity, osteonecrosis, secondary neoplasms and relapse in the cohort of 320 ALL patients are reviewed in Table 1.

Dose of 6-MP was reduced due to toxic effects during the maintenance therapy in 19.7 % of patients. Most dose reductions occurred in recent protocols (BFM 90, 95 and IC 2002), where 40–60 % of patients had 6-MP dose reduction (Figure 1). This association remained statistically significant after adjustment for gender and age. Age and gender had no effect on dose reduction.

Bone marrow suppression was experienced by 13.8 % of patients in the cohort. Its incidence was higher in recent protocols (Figure 2A), and after adjustment for age and gender, patients treated with BFM IC 2002 were 5.4 (CI 95 % 1.3–22.2) times more likely to develop bone marrow suppression than patients treated with BFM 90 (p = 0.019).

Stomatitis during maintenance phase occurred in 4.4 % of patients and its incidence did not correlate with age, gender or treatment protocol (Figure 2).

Sepsis or infection was present in 20.9 % of patients during the maintenance therapy. Younger patients had an increased risk for developing sepsis/infection (Figure 2B). After adjustment for gender and protocol, the odds ratio was 1.14 (CI 95 % 1.05–1.23), which is probably not clinically significant, although it reached the statistical significance in the logistic regression model (p = 0.002).

Febrile neutropenia was developed by 7.5 % of patients, and it correlated with treatment protocol and age (Figure 2). It was more common in recent protocols (Figure 2A) and in younger patients (Figure 2D). However, these associations disappeared after adjustment in the logistic regression model.

Hepatotoxicity was found in 3.1 % of patients during the maintenance treatment. Its occurrence was higher in recent protocols (Figure 2A), but after adjustment for age and gender the significance disappeared.

Osteonecrosis was the rarest side effect, occurring in only 2.8 % of patients. In all patients with osteonecrosis unilateral or bilateral aseptic necrosis of the hip was present. It was more common in recent protocols (Figure 2A) and in older patients (Figure 2D). After adjustment, the association with protocol disappeared, conversely, older patients were 1.4 (CI 95 % 1.2–1.7) times more likely to develop osteonecrosis (p = 0.001).

A secondary neoplasm, most commonly being brain tumor (6 patients) and AML (3 patients) occurred in 4.4 % of patients (Table 1). Median time to neoplasm development from the end of the primary maintenance therapy was 6.0 years (range: 0.0–17.3 years), with younger patients having shorter time to neoplasm occurrence (Spearman`s rho = -0.615, p = 0.019).

Overall, 33.8 % of patients relapsed, but relapse rate differed significantly between protocols (also after adjustment for gender and age), with almost 60 % in POG and 0 % in BFM IC 2002 (Figure 3B). In simple stati-

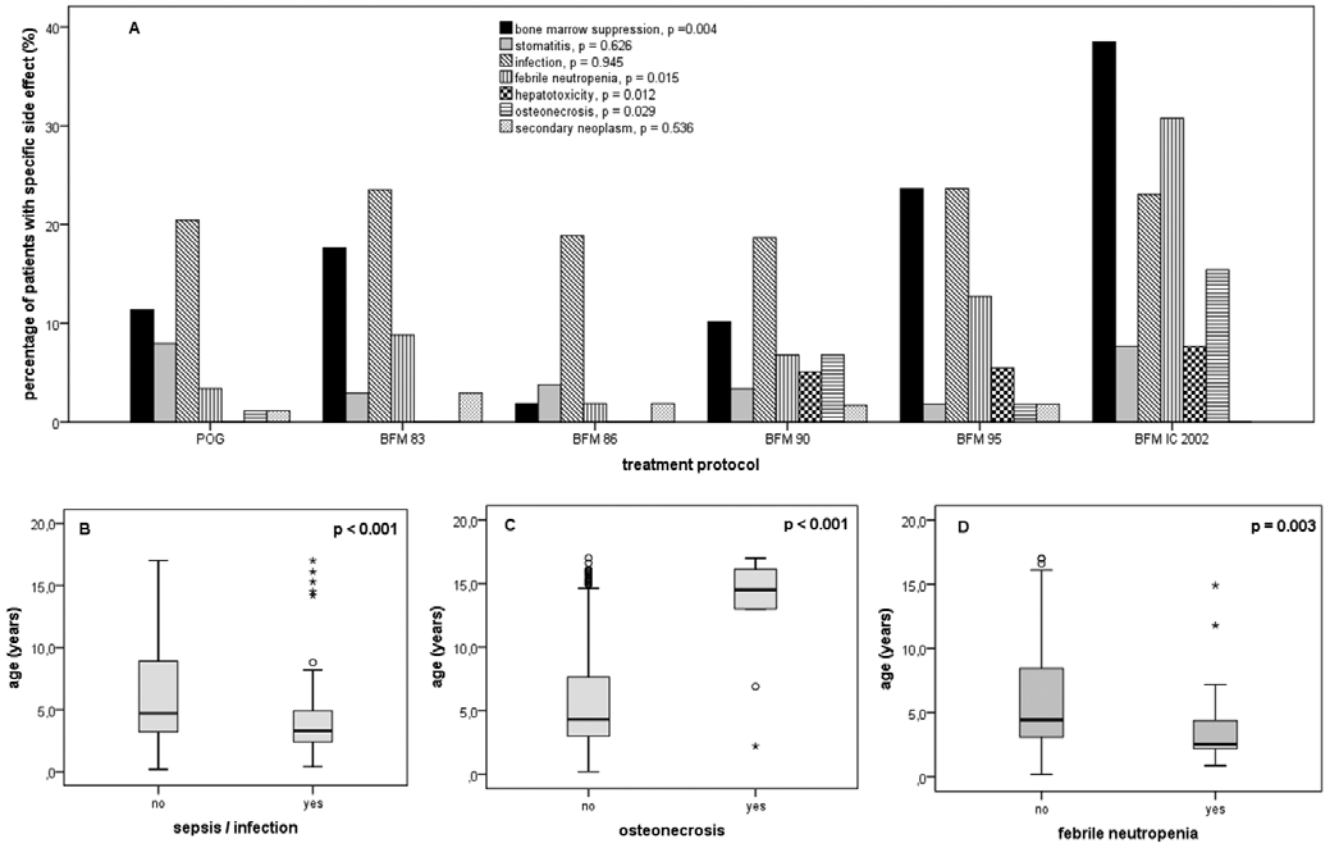

Figure 2: Toxic side effects of 6-MP during the maintenance therapy in ALL patients according to treatment protocol (A) and age (B, C, D). The incidence of bone marrow suppression, hepatotoxicity, osteonecrosis and febrile neutropenia was higher in recent protocols (A). Risk of developing sepsis / infection and febrile neutropenia was higher in younger children (B, D), while osteonecrosis was more common in adolescents (C).

stical analysis, the association between relapse and gender did not reach statistical significance (Figure 3A), but after adjustment for age and protocol, boys were found to relapse 1.7 (CI 95 % 1.0–2.9) times more likely than girls (p = 0.039). More detailed analysis revealed that difference in relapse rate among males and females was present only in POG protocols (p = 0.004), where 72 % of boys relapsed compared to only 41 % of girls. Median time to relapse from diagnosis was 2.2 years (range: 4 months – 8.25 years). Time to relapse was not associated with age, gender or treatment protocol.

Classification of relapses according to the site is shown in Table 2. The relapse site was not associated with age, treatment protocol and gender (except for testicular relapse). Note that some patients had several different relapse sites.

Discussion

In this retrospective study we aimed to evaluate maintenance treatment of childhood ALL according to 6-MP dose reduction, side effects and relapse rate. In contrast, the most recent study in Slovenian paediatric ALL patients was evaluating only cumulative survival according to year of diagnosis, age at diagnosis and treatment protocol,[4] and two earlier studies encompassed the shorter time period.[13,14]

The idea of adjuvant chemotherapy was first introduced in 1960, when results of the first double blind randomized study on the efficiency of 6-MP in the treatment of childhood ALL showed that patients benefited more from 6-MP given in remission than if it were given in the active phase of disease.[9] Maintenance therapy with 6-MP as its major component has been one of the main reasons for the drastic increase in overall survival of paediatric ALL patients. In our cohort,

consisting of patients receiving maintenance therapy for ALL and encompassing time period of three decades, we evaluated dosage, safety and efficiency of 6-MP in the treatment of childhood ALL.

The first important finding was that the majority of 6-MP dose reductions occurred in three most recent protocols (BFM 90, 95 and IC 2002), whereas in earlier protocols (POG, BFM 83 and 86) dose reductions were very rare, with no dose reductions in BFM 83. This cannot be explained by 6-MP and MTX dosage, as the dose of both drugs was the same across all the protocols: 50 and 20 mg/m^2/day, respectively. Furthermore, study of relevant thiopurine pharmacogenetic markers in the same cohort revealed no difference in their distribution between protocols.[15] Therefore, we speculate that these results are the consequence of different approach to treatment in different time periods. In earlier protocols physicians were more reluctant to lower the dose even when leukocyte count was low, probably because the relapse prevention was seen to outweigh possible side effects. However, when the consequences of aggressive chemotherapy begun to emerge in long-term childhood ALL survivors,[16] the effort was taken to improve not only the efficiency but also the safety of the treatment[7], resulting in more dose reductions. Higher incidence of 6-MP reduction in BFM IC 2002 (60 %) might also be explained by pulses of reinduction therapy during the maintenance phase.

Due to the fact that data used in this study were extracted from the patients` files for the purpose of the thiopurine pharamacogenetic study, only doses of 6-MP were recorded. However, MTX, in addition to 6-MP, also plays an important role in the maintenance therapy of childhood ALL. MTX increases the bioavailability of 6-MP in two ways: (i) by inhibition of the 6-MP deactivating enzyme xanthine oxidase (XO) and (ii) by the cell depletion of activated folates and consequently S-adenosyl-methionine (SAM), which reduces the methylation (and deactivation) of 6-MP by thiopurine-S-methyl transferase (TPMT). MTX also inhibits de novo purine synthesis, thus increasing the levels of phosphoribosyl pyrophosphate (PPRP) and consequently increasing production of active cytotoxic forms of 6-MP thioguanine nucleotides (TGNs). Because of the synergism between 6-MP and MTX, it is recommended to lower the doses of both drugs rather than withhold one drug and continue with the other in the case of toxic side effects.[17] Thus it can be assumed that the incidence of MTX dose reduction in our cohort was similar to that of 6-MP.

In terms of side effects, the most important finding was that the incidence of bone marrow suppression was higher in recent protocols (BFM 95 and IC 2002), while it was very low in BFM 86. This might be explained by the shortening of the maintenance therapy in BFM 86: from 144 weeks in protocols POG and BFM 83 to 56 weeks in BFM 86. On the other hand, boys receiving longer maintenance compared to girls (125 vs. 74 weeks) in BFM 95 and pulses of reinduction therapy during the maintenance phase in BFM IC 2002 might explain higher incidence of bone marrow toxicity.

The only other toxic effect association that remained significant after adjustment in the logistic regression model was the greater age in patients with osteonecrosis (median 15 years) compared to those who did not develop it (median 5 years) ($p < 0.001$). This is in accordance with numerous studies.[18,19] Adolescents are at highest risk for ALL therapy induced osteonecrosis due to their grater vulnerability or rapid bone growth in this period.[20] Among drugs used for childhood ALL treatment glucocorticoids were identified as a main risk factor for osteonecro-

Table 2: Relapse classification according to the site in pediatric ALL patients (N = 108).

ALL relapse site	Percentage of patients according to relapse site
Bone marrow	73 (67.6 %)
Combined[1]	28 (25.9 %)
Isolated CNS	27 (25.0 %)
Isolated Testis	15 (13.9 %)
Isolated Other[2]	6 (5.6 %)

[1] *Bone marrow and CNS / Testis / Other*
[2] *spinal channel, liver, iris, mesenterium, neck lymph nodes, labia major*

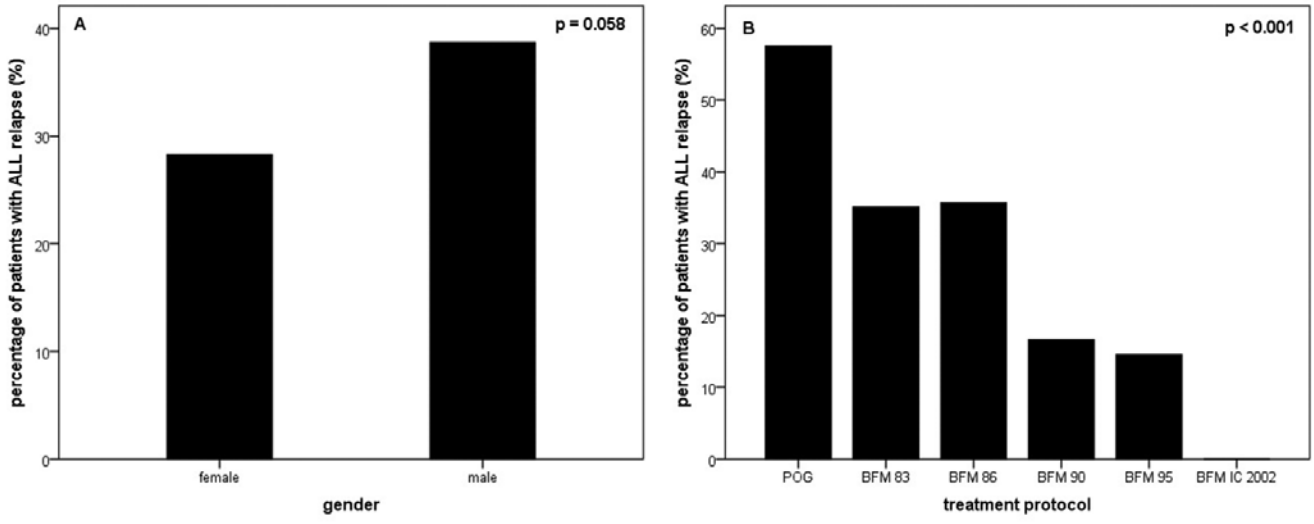

Figure 3: Incidence of ALL relapse according to gender (A) and treatment protocol (B, C, D). The incidence of relapse was higher in boys than in girls (A), but did not reach statistical significance. Relapse was more common in older protocols (B).

sis,[18] although other therapeutic agents such as MTX[18] and 6-MP[19] have been associated with bone morbidity during ALL treatment.

We obtained the expected results considering the association of relapse rate with treatment protocol. Relapse incidence was highest in POG (almost 60 %), and lowest in recent protocols BFM 90, 95 and IC 2002, with the relapse incidence less than 20 %. It is of interest that none of the patients treated with BFM IC 2002 relapsed in the 5 year follow-up period, which might be at least in part due to the small number of patients treated with this protocol included in the study (N = 13). 5-year survival in the same cohort increased from 50 % in POG to almost 80 % in BFM IC 2002.[4] The results are in concordance with the long term results of five BFM trials (N = 6609), where relapse incidence dropped from 34 % in BFM 83 to 17 % in BFM 95.[7] Interestingly, the relapse rates for different protocols reported in the study were very similar to those observed by us. Although 6-MP is a crucial part of maintenance phase of ALL treatment, the observed decrease in relapse rates over the years is not due to the maintenance therapy and 6-MP, since cumulative dose of 6-MP did not differ between protocols. The only exception is protocol BFM 86, where cumulative dose of 6-MP was lower due to shortening of the maintenance therapy. However, since this resulted in an increased relapse rate, the abovementioned protocol was discontinued prematurely. Also, it is of interest that after the steep increase in survival rate seen after the year 1975, only 3 new drugs were registered for the treatment of childhood ALL (Teniposide in 1975, Etoposide in 1983 and Imatinib in 1998), all the other drugs were registered before 1965. All three drugs registered after 1965 are used only in high-risk patients and thus cannot account for the improved outcomes in low and intermediate risk patients. Thus, the increased survival rate in childhood ALL can be attributed mainly to the risk-adapted treatment strategy that adjusts the induction, consolidation and re-induction phases of the treatment according to each patient`s risk assessment (i.e. early treatment response and cytogenetic markers). Major advances in paediatric oncology were made possible due to the: (i) multidisciplinary approach to the treatment, (ii) better knowledge of cytogenetics and behavior of tumors, (iii) collaborative international movement (BFM, POG, CCG, Dana-Farber, MSKCC, NOPHO, St. Jude, AEIOP, UKALL, A-NZ and others in Asia and Latin America) and (iv) improvements in supportive therapy.

Another interesting observation related to relapse was that boys had greater relapse incidence than girls, but this was true only for patients treated with POG protocols. This might be due to the presence of frequent relapse site in boys (testes). Closer inspection of the results revealed that in boys there was a significant decrease in not only testicular, but also medullar and CNS relapse incidence in BFM compared to POG protocols. Thus, in addition to the presence

of testes, other metabolic differences between males and females might be the reason for better response of girls to POG treatment. One such example is TPMT activity, which is frequently reported to be higher in males than females,[21] thus decreasing the levels of active TGN in males. Our observation is in line with older ALL treatment reports that boys better tolerate 6-MP but have more relapses than girls.[22] Compared to POG, later BFM protocols included more aggressive chemotherapy (including asparaginase, cyclophosphamide and ARA-C) and this probably rendered 6-MP related metabolic differences between males and females irrelevant for the overall success of therapy.

Conclusions

1. Most 6-MP dose reductions occurred in recent protocols (BFM 90, 95 and IC 2002).
2. An increased incidence of bone marrow suppression during maintenance therapy was observed in recent protocols (BFM 95 and IC 2002). Conversely, BFM 86 was the protocol with the lowest 6-MP toxicity.
3. Risk of osteonecrosis during ALL treatment is higher in older children and adolescents, with median age 15 years. This finding needs to be further investigated on a larger number of osteonecrosis cases and in a prospective study using more objective bone density evaluation methods.
4. In early POG protocols, boys had significantly increased risk of relapse compared to girls. However, this effect was not observed in BFM protocols.
5. The incidence of relapse continuously decreased with years, from almost 60 % in POG to less than 20 % in most recent BFM protocols (90, 95 and IC 2002).

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