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ORIGINAL ARTICLE

Physicians compliance during maintenance therapy in children with Down syndrome and acute lymphoblastic leukemia

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Children with Down syndrome (DS) and acute lymphoblastic leukemia (ALL) have an inferior prognosis compared with non-DS ALL patients. We reviewed methotrexate (MTX)/mercaptopurine (6MP) maintenance therapy data for children with DS treated according to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL92 or the NOPHO ALL2000 protocols between 1992 and 2007. The 5-year event-free survival probability ($pEFS_{5\,yr}$) for the 66 DS patients was inferior to the 2602 non-DS patients (0.50 ± 0.07 vs 0.77 ± 0.01 ($P < 0.001$)). The 48 DS patients in first remission at the beginning of maintenance therapy had $pEFS_{10\,yr}$ below that of the 522 non-DS control patients ($pEFS_{10\,yr}$: 0.58 (95% confidence interval (CI) 0.43–0.77) vs 0.83 (95% CI 0.80–0.86), respectively ($P < 0.0001$)). The DS patients received lower median doses of MTX (median: 11.8 vs 15.4 ($P < 0.0001$)) and 6MP (median: 43.6 vs 59.4 ($P < 0.0001$)). In Cox regression analysis, male gender, presence of DS and high median maintenance therapy white blood cell levels (mWBC) were associated with increased risk for relapse. DS-ALL patients with mWBC above or below 3.5×10^9/l (protocol target) had $pEFS_{10\,yr}$ of 0.31 and 0.72 ($P = 0.02$), and the mWBC hazard ratio for DS-ALL patients was 2.0 ($P < 0.0005$). We conclude that insufficient treatment intensity during maintenance therapy of DS-ALL patients may contribute to their poor prognosis.

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Keywords: acute lymphoblastic leukemia; Down syndrome; compliance; dose intensity; methotrexate/6-mercaptopurine; maintenance therapy

INTRODUCTION

Children with Down syndrome (DS) have a 20–40 times increased risk of developing acute lymphoblastic leukemia (ALL),[1,2] and 1.5–3.2% of all children with ALL also have DS.[3–5] Compared with the background population of children with ALL, most collaborative groups have reported inferior outcomes for DS-ALL.[4,6–13] These results contrast three biological features that would otherwise suggest a favorable prognosis. First, ALL in DS is nearly always of B-cell lineage and higher risk translocations, including MLL or t(9;22)[BCR/ABL], are rare.[14] Second, malignant cells in DS seem more susceptible to undergo apoptosis.[1,15,16] Finally, children with DS should have an increased sensitivity to methotrexate (MTX) and cytarabine due to the gene copy number effect of the reduced folate carrier (*SLC19A1*) and the *cystathionine-β-syntethase* genes on chromosome 21,[16] and the latter may in part account for the favorable prognosis of DS children with acute myeloblastic leukemia.[5,16] On the other hand, the reported inferior prognosis of DS-ALL could be linked to at least four characteristics. First, the most common and favorable cytogenetic aberrations in childhood ALL, that is, high hyperdiploidy and t(12;21)[*ETV6/RUNX1*], are significantly less common in DS-ALL.[9,14,17] Second, the DS-ALL clones frequently harbor *JAK2* mutations and have enhanced CRLF2 expression, which may promote tumor growth.[18,19] Third, increased MTX and cytarabine sensitivity could increase toxicity and lead to treatment interruptions.[20] Finally, an increased relapse rate could reflect decreased physicians compliance to protocol guidelines or parental and patient adherence to MTX/6-mercaptopurine (6MP) dose adjustments during maintenance therapy in these already psychosocially burdened families. Accordingly, we recently reported that five of the six DS-ALL that entered the randomized Nordic Society of Pediatric Haematology and Oncology (NOPHO) ALL92 maintenance therapy study[21] had significantly higher average white blood cell (WBC) and absolute neutrophil counts (ANCs) than non-DS children, and these five children all relapsed.[22] To explore this issue further, we have reviewed MTX and 6MP dosages, WBC and ANC during MTX/6MP maintenance therapy of the 69 children with DS-ALL diagnosed in the Nordic countries January 1992 through January 2007.

MATERIALS AND METHODS

Patients

The data were collected from the NOPHO leukemia registry and from patient files. We retrieved data on all DS children ($n = 69$), 1.0–17.5 years of age, diagnosed between January 1992 and January 2007 and treated according to the NOPHO ALL92 protocol (1992–2001) or the NOPHO ALL2000 protocol (2002–2007). Three DS-ALL patients were excluded from further analysis, as they did not receive any therapy because of a

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post-mortem diagnosis of ALL ($n=1$) or in accordance with the parents' wish because of severe comorbidity ($n=2$).

To explore the induction failure rate, risk of relapse and event-free survival, the DS cohort was compared with non-DS ALL patients on the NOPHO ALL92 and ALL2000 protocols. For detailed analyses of MTX/6MP maintenance therapy, the DS population was compared with the 522 non-T-cell non-DS patients participating in the randomized NOPHO ALL92 maintenance therapy study that included more than 97% of all patients entering MTX/6MP maintenance therapy.[21]

Risk grouping

The risk group stratifying criteria of NOPHO ALL92 and ALL2000 have been reported in detail previously.[23,24] The presence of DS did not influence risk grouping of antileukemic therapy.

Treatment

The 4-week induction therapy consisted of prednisolone, vincristine, doxorubicin and intrathecal MTX, followed by asparaginase for the subsequent 2 weeks (ALL92: Erwinia asparaginase; ALL2000: *Escherichia coli* asparaginase). Induction therapy, early intensification, consolidation and delayed intensification of both NOPHO ALL protocols have been described in detail previously.[23,24] In the NOPHO ALL92 protocol, oral maintenance therapy with starting doses of 6MP and MTX of 75 mg/m^2 per day and 20 mg/m^2 per week, respectively, was initiated at weeks 13 (standard risk (SR)), 32 (intermediate risk (IR)) or 63 (high risk (HR)), and continued until 2.5 years (SR) or 2 years (IR and HR) after diagnosis. NOPHO ALL2000 protocol had 6MP/MTX maintenance treatment from weeks 17 (SR), 30 (IR), 70 (HR) or 61 (very high risk (VHR)), and continued until 2 years (HR and VHR) or 2.5 years (SR and IR) from diagnosis. The first year of maintenance therapy included for patients with SR- or IR-ALL in both protocols alternate pulses at 4-week intervals of (i) VCR and glucocorticosteroids or (ii) high-dose MTX 5 g/m^2/24 h with intrathecal MTX and leucovorin rescue until five courses of high-dose MTX had been given. The target WBC during maintenance therapy was throughout maintenance therapy in both protocols 1.5–3.5 $\times 10^9$/l for all patients including DS-ALL.[24] Starting doses of MTX and 6MP were 20 mg/m^2 per week and 75 mg/m^2 per day. In ALL2000, the 6MP starting doses were reduced to 50 mg/m^2 per day for thiopurine methyltransferase heterozygous patients and 5–10 mg/m^2 per day for deficient patients. None of the DS patients in this study were thiopurine methyltransferase heterozygous or deficient.

Statistical analysis

The mean levels of leukocyte and neutrophil counts, and mean doses of 6MP and MTX during the maintenance therapy, were for a particular time point calculated by the LOCF (last observation carried forward) procedure, that is, as a weighted average based on all previous registrations, each counting until a new registration or a maximum of 8 weeks. The levels (mWCB, mANC, mMTX and m6MP) were obtained by the end of therapy. Survival probabilities were calculated by the Kaplan–Meier method. Survival curves were compared by the log-rank test. Patients entered the analyses at the initiation of maintenance therapy (delayed entry). Point-wise confidence intervals were based on the exponential Greenwood formula (log(−log) transformation). In multivariate Cox proportional hazards regression analysis of relapse, the patients who died in remission or developed a second malignancy were censored at the time point of these events. The multivariate model was adjusted for sex, risk group, age at diagnosis, mean doses of 6MP (m6MP) and MTX (mMTX), median maintenance therapy white blood cell levels (mWBC) and mean level of neutrophil counts (mANCs). These continuous risk factors were included linearly in the model as no departures from linearity were found. The proportional hazards assumption was assessed by the score processes using graphical methods[25] and the Lin, Wei and Ying test.[26] The proportional hazards assumption was not violated for any of the variables, but in line with previous analyses of these data,[21] risk group was included as a stratification variable. The Wald test was applied to test for differences in outcome. The patients were followed until 11 January 2010. Two-sided *P*-values <0.05 were considered significant. Calculations were performed using SAS version 9.2 and R version 2.13.1.

RESULTS

Analysis of the total population

In total, 2668 patients have been treated according to the NOPHO ALL92 ($n=1645$) or the ALL2000 ($n=1023$) protocols, out of which a total of 66 patients (2.5%) had DS (Table 1). In all, 21 (1.3%) children on the NOPHO ALL92 protocol had induction failure, out of which four had DS-ALL (19.0%). In the NOPHO ALL2000 protocol, 16 patients (1.6%) had induction failure, out of which 3 (18.8%) occurred in the DS group. None of the DS patients had resistant disease and none developed a second cancer. In all, 67 non-DS (2.6%) and four DS patients (6.1%) had central nervous system involvement, and 34 non-DS (1.3%) and no DS-ALL patients had testicular involvement at the time of diagnosis.[24]

The 5-year event-free survival probability (pEFS$_{5\,yr}$) for the DS patients (0.50 ± 0.07) was significantly below that of non-DS patients in both the ALL92 protocol (0.77 ± 0.01) and ALL2000 protocol (0.79 ± 0.02) ($P<0.001$), with no significant difference in pEFS$_{5\,yr}$ for DS-ALL patients between these two protocol periods (data not shown).

In addition to the seven DS-ALL patients with induction failure, three patients experienced a relapse before the start of maintenance therapy, five DS HR-ALL patients received LSA$_2$L$_2$ maintenance therapy[27] and for three DS patients no data on MTX/6MP maintenance therapy could be retrieved. Thus, 48 patients with DS were eligible for detailed analysis of oral MTX/6MP maintenance therapy and were compared with the 522 non-T, non-DS children included in the NOPHO ALL92 maintenance therapy study.

The pEFS$_{10\,yr}$ of the 48 DS patients who were in first remission at the beginning of MTX/6MP maintenance therapy was below that of the 522 non-DS patients (pEFS$_{10\,yr}$: 0.58 (95% confidence interval (CI) 0.40–0.72) and 0.83 (95% CI 0.79–0.86), respectively ($P<0.0001$) (Figure 1), and this was the case for all risk groups, although it only reached statistical significance in the IR-ALL group (Table 2).

The mWBC and mANC did not differ significantly between the DS and non-DS patients (median WBC: 3.4 $\times 10^9$/l vs 3.3 $\times 10^9$/l ($P=0.60$); median ANC: 2.2 $\times 10^9$/l vs 2.0 $\times 10^9$/l ($P=0.06$)) (Table 2). Overall, the DS-ALL patients received lower average doses of both MTX and 6MP when compared with the non-DS ALL patients (median mMTX: 11.8 vs 15.4 mg/m^2 per week ($P<0.0001$); median m6MP: 43.6 vs 59.4 mg/m^2 per day ($P<0.0001$)).

Table 1. Features and treatment outcome of patients treated in NOPHO ALL92 and NOPHO ALL2000 studies

	NOPHO ALL92	*NOPHO ALL2000*	*No. of DS patients in* ALL92 + ALL2000
No. of patients	1645	1023	66
T-cell ALL	152	115	0
Induction failure	21	16	7
Resistant disease	9	14	0
SMN	21	4	0
Male/female	889/756	569/454	34/32
CNS involvement	37	30	4
Testicular involvement	32	2	0
5-EFS ± s.e.	0.77 ± 0.01	0.79 ± 0.02	0.50 ± 0.07

Abbreviations: ALL, acute lymphoblastic leukemia; CNS, central nervous system; 5-EFS, 5-year event-free survival; NOPHO, Nordic Society of Pediatric Hematology and Oncology; s.e., standard error; SMN, second malignant neoplasm.

The target WBC of 1.5–3.5 $\times 10^9$/l was reached in a similar proportion of DS and non-DS patients ($P = 0.21$). For patients with an mWBC above 3.5 $\times 10^9$/l, that is, above the WBC target requiring upward dose adjustments, the mANC was higher for the DS-ALL patients (median 2.9 vs 2.4 $\times 10^9$/l ($P = 0.047$)), but they still received lower mMTX and m6MP doses (median 12.1 vs 15.8 mg/m^2 per week of MTX ($P = 0.02$) and median 44.3 vs 63.1 mg/m^2 per day of 6MP ($P < 0.0001$)).

In Cox multivariate regression analysis, potential risk factors of relapse included age at the time of diagnosis, sex and the presence of DS, mWBC, mANC, m6MP and mMTX. Only sex (hazard ratio = 1.7 ($P = 0.01$)), the presence of DS (hazard ratio = 3.5 ($P = 0.0003$)) and mWBC (hazard ratio = 1.49 ($P = 0.02$)) showed a significant effect on risk of relapse (Table 3). Both backward and forward elimination procedures resulted in a model containing only these three risk factors, the estimated effects being similar to the effects reported in Table 3. Introducing the interaction between mWBC and DS in the multivariate model, the effect of DS as a main effect was no longer significant ($P = 0.36$) and when removed from the model the interaction was highly significant ($P = 0.0005$), demonstrating a larger effect of mWBC for DS (hazard ratio = 2.0; 95% CI 1.4–2.9) than for non-DS (hazard ratio = 1.4; 95% CI 1.0–2.48). For the DS-ALL patients who completed MTX/6MP maintenance therapy in first remission ($n = 35$), the EFS was lower for the 15 DS patients with mWBC $\geqslant 3.5 \times 10^9$/l than for the 20 patients with mWBC $< 3.5 \times 10^9$/l ($P = 0.02$) (Figure 2).

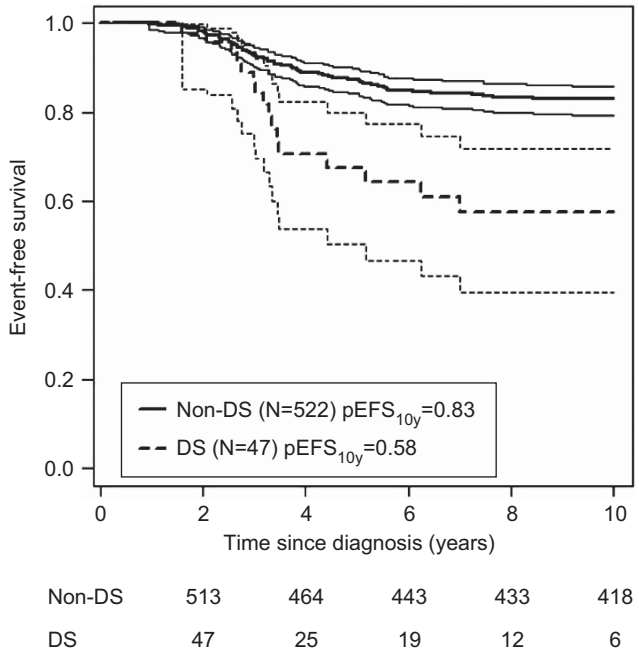

Figure 1. Kaplan–Meier curves for EFS for DS and non-DS children.

DISCUSSION

This study confirms previous reports on the inferior prognosis of DS-ALL, being most pronounced for IR- and HR-ALL, and it adds an important potential explanatory feature underlying their poorer prognosis.

The inferior outcome for DS-ALL is multifactorial and could reflect leukemia biology,[14,18,19] the host's tolerance to therapy,[20] the pharmacology of anticancer drugs[28,29] and treatment compliance and adherence. Acknowledging the wide interindividual variations in bioavailability and pharmacokinetics of MTX and 6MP, all collaborative groups adjust MTX/6MP maintenance therapy doses by a target degree of toxicity, although the groups differ quite widely in their guidelines.[30] Throughout this study period, the target WBC stayed unchanged at 1.5–3.5 $\times 10^9$/l, and the treatment intensity was intended to be the same for DS and non-DS ALL patients. As mWBC and mANC during maintenance did not differ significantly between the DS and non-DS ALL patients, the poorer outcome for the DS patients could reflect less sensitivity of the DS leukemic clones to MTX/6MP maintenance therapy. Another option is that a given WBC may reflect less intensive drug exposure in a DS patient than a similar WBC level in a non-DS ALL patient. In support of the latter, the degree of myelosuppression during maintenance therapy is actually the difference between the individual patient's normal

Table 2. Characteristics and outcome of NOPHO DS maintenance study

	DS patients	*Non-DS-ALL92 cohort*	P-*value*
Study cohort	48	522	
Median age at diagnosis	6	4	0.0015
Sex male/female	23/25	276/246	0.51
WBC at diagnosis ($\times 10^9$/l)	15.0 (47[a])	7.00	0.04
CNS involvement	3	2	0.005
Testicular involvement	0	2	1.00
Risk group, SR/IR/HR	18/21/9	241/231/50	
Protocol 92/2000	24/24	522/0	
Median mWBC ($\times 10^9$/l)[b]	3.44 (39[a])	3.33 (520[a])	0.56
Median mANC ($\times 10^9$/l)[b]	2.17 (38[a])	1.96 (513[a])	0.06
Median mMTX (mg/m^2)[b]	11.80 (41[a])	15.40 (519[a])	<0.0001
Median m6MP (mg/m^2)[b]	43.60 (42[a])	59.43 (519[a])	<0.0001
Number of relapses	16	84	
EFS 10 years (95% CI)	0.58 (0.43–0.77)	0.83 (0.80–0.86)	<0.0001
EFS 10 years SR (95% CI)	0.75 (0.57–1.00)	0.85 (0.80–0.89)	0.13
EFS 10 years IR (95% CI)	0.45 (0.26–0.79)	0.82 (0.78–0.87)	0.0015
EFS 10 years HR (95% CI)	0.48 (0.19–1.00)	0.78 (0.67–0.89)	0.10

Abbreviations: ALL, acute lymphoblastic leukemia; 95% CI, 95% confidence interval; CNS, central nervous system; DS, Down syndrome; EFS, event-free survival; mANC, mean doses of absolute neutrophil counts; m6MP, mean doses of mercaptopurine; mMTX, mean doses of methotrexate; mWBC, high median maintenance therapy white blood cell; NOPHO, Nordic Society of Pediatric Hematology and Oncology; HR, high risk; IR, intermediate risk; SR, standard risk; WBC, white blood cell. [a]Number of patients included in the calculations. [b]'m' indicates the mean levels for each patient at the end of maintenance therapy. For a particular time point, the mean level for each of these variables was calculated by the LOCF (last observation carried forward) procedure, that is, as a weighted average based on all previous registrations, each registration counting until a new registration or a maximum of 8 weeks had passed.

Table 3. Cox regression analysis

Parameters	*Unadjusted hazard ratio*	*95% CI*	P-*value*	*Adjusted hazard ratio*	*95% CI*	P-*value*
mWBC[a]	1.43	1.13–1.81	0.003	1.49	1.06–2.11	0.02
Male	1.80	1.18–2.75	0.006	1.71	1.11–2.62	0.01
m6MP[a]	1.00	0.99–1.01	0.92	1.00	0.98–1.02	0.89
mMTX[a]	1.04	1.00–1.09	0.04	1.04	0.98–1.10	0.17
mANC	1.33	1.12–1.58	0.001	0.87	0.62–1.29	0.43
Age at time of diagnosis	1.05	0.99–1.11	0.09	1.03	0.97–1.09	0.29
Presence of DS	3.60	2.03–6.40	<0.0001	3.54	1.79–6.99	0.0003

Abbreviations: 95% CI, 95% confidence interval; DS, Down syndrome; mANC, mean doses of absolute neutrophil counts; m6MP, mean doses of mercaptopurine; mMTX, mean doses of methotrexate; mWBC, high median maintenance therapy white blood cell. [a]'m' indicates the mean levels for each patient. For a particular time point, the mean level for each of these variables was calculated by the LOCF (Last Observation Carried Forward) procedure, that is, as a weighted average based on all previous registrations, each registration counting until a new registration or a maximum of 8 weeks had passed.

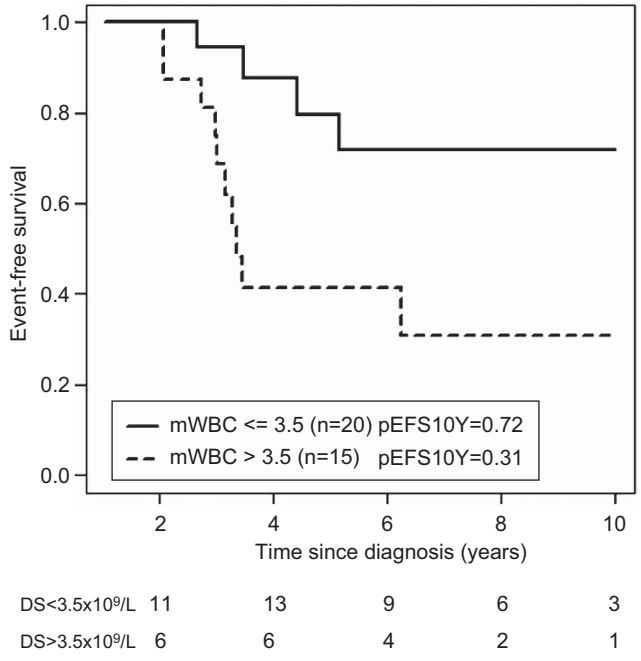

Figure 2. Kaplan–Meier curves for EFS for DS with mWBC above or below 3.5×10^9/l during maintenance therapy (entered analysis at the end of therapy, DS patients with events during maintenance therapy were excluded).

WBC levels and that obtained during therapy,[31,32] and patients with DS seems to have lower normal WBC and ANC values compared with healthy individuals.[2,33,34] Thus, a given mWBC will reflect less myelosuppression in a DS patient and thus lower treatment intensity. However, studies mapping in detail the normal blood counts in non-leukemic DS children are lacking.

During the first year of maintenance therapy, the non-HR patients all received five courses of HD-MTX with leucovorin rescue. Owing to a gene dosage effect of the reduced folate carrier (*SLC19A1*), this may have led to higher intracellular levels of reduced folates and thereby resistance[35] to subsequent low-dose oral MTX in maintenance therapy. In addition, the increased dosage of chromosome 21 genes involved in purine *de novo* synthesis[36–38] may also have skewed the balance between the intracellular levels of 6-thioguanine and endogenous purines, leading to less DNA-6-thioguanine incorporation.[39]

Finally, this study indicates that physicians titrates MTX/6MP less vigorously in DS-ALL compared with non-DS ALL patients. Although the target WBC of $1.5–3.5 \times 10^9$/l was reached in a similar proportion of DS and non-DS patients, the physicians prescribed MTX and 6MP doses far below the recommended standard protocol doses and also below those doses prescribed to non-DS patients. This could be defendable for the patients who actually have WBC levels within the target range, but the significantly lower dosing was also seen for the patients with WBC levels above the target range. These data strongly indicate that physicians were less willing to increase the doses in patients with DS-ALL. This is especially critical as the DS patients may have lower normal levels of WBC and ANC, and therefore may need even lower mWBC levels to obtain the same treatment intensity as non-DS patients, and this study emphasizes that not least for DS-ALL patients the degree of myelosuppression measured by the WBC is strongly associated with the likelihood of staying in remission.

Still, the reasons for the differences in MTX/6MP dose intensity for DS-ALL patients compared with non-DS ALL patients remain uncertain. Extended, intensive treatment with MTX/6MP may cause numerous side effects, including reduced B-cell numbers and function,[40] as well as increased risks of infections,[12] liver dysfunction,[41] gastrointestinal toxicity[20] and low growth hormone levels.[42] DS-ALL patients may be more prone to some of these side effects, although this remains to be explored, and not least their underlying functional impairment of B cells, T cells and phagocytic cells[43] may have led to more infections during ALL treatment, causing the physicians to be less willing to increase drug doses and the parents to be reluctant to implement otherwise indicated dose increments. However, studies that in detail map the treatment burden (somatic side effects and quality of life) for DS patients are lacking.

In conclusion, insufficient treatment intensity during maintenance therapy of DS-ALL patients may contribute to their poor prognosis and there is a need for mapping MTX/6MP maintenance therapy pharmacokinetics and the burden of therapy with respect to both quality of life and somatic side effects for this group of patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

CB designed the study, collected data, analyzed data and drafted the paper; ML drafted the paper; SR analyzed data; BZ collected data; MT collected data;

SH collected data; HB collected data; MH collected data; and KS designed the study, collected data, analyzed data and drafted the paper. All authors approved the final manuscript.

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