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The effect of leucovorin rescue therapy on methotrexate-induced oral mucositis in the treatment of paediatric ALL: A systematic review

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ABSTRACT

Introduction: This study aimed to determine the efficacy of different Leucovorin regimens to reduce oral mucositis in children with acute lymphoblastic leukemia after high-dose Methotrexate (HD-MTX).
Methods: Twelve articles were included in a systematic literature review. Articles were categorized into low/medium/high risk of bias.
Results: As no randomized controlled trial assessing the effect of Leucovorin has been performed, the efficacy of Leucovorin to reduce oral mucositis remains unknown. Leucovorin was initiated at 24, 36 or 42 h after HD-MTX at a dose of 15 or 30 mg/m^2. No meta-analysis could be performed as treatment regimens differed. When comparing studies with similar HD-MTX doses, we observed lower oral mucositis rates in regimens with higher cumulative doses of Leucovorin and early initiation of Leucovorin after MTX.
Conclusion: Even though future studies are necessary, higher cumulative Leucovorin doses and early initiation of Leucovorin after start of MTX seem to reduce oral mucositis.

1. Introduction

Over the past decades, five-year survival rates of children with acute lymphoblastic leukemia (ALL) have reached 90% in the developed countries (Hunger et al., 2012; Pui et al., 2011; Kamps et al., 2010). However, patients often suffer from toxicity of chemotherapeutic drugs such as Methotrexate (MTX) (den Hoed et al., 2015; Pieters et al., 2016).

MTX is an antifolate drug that depletes intracellular reduced folate levels by inhibiting the enzymes Thymidylate Synthase and Dihydrofolate Reductase. This depletion of intracellular reduced folate levels impairs DNA- and RNA-synthesis leading to cell death in cells with a high cell turnover, such as in the bone marrow and in the gastrointestinal tract including the oral mucosa (Funk et al., 2014; Kao et al., 2013; Bostrom et al., 2003). In addition, MTX was shown to alter the diversity and principal components of oral and gut microbiota, thereby affecting the inflammatory response associated with damage to the mucosal epithelium (Zhang et al., 2015; Zhou et al., 2018). These changes in the composition of microbiota in response to MTX were associated with developing MTX-induced mucositis in mice studies and in rheumathoid arthritis patients (Zhang et al., 2015; Zhou et al., 2018). After HD-MTX infusions, folinic acid rescue therapy (Leucovorin – LV) is administered to reduce toxic side effects (Wennerstrand et al., 2013; Cohen and Wolff, 2014). LV is a reduced folate that bypasses the block of DHFR by MTX (Relling et al., 1994; Wolfrom et al., 1993; Goldie et al., 1975). The administration of LV is therefore thought to decrease toxic side effects by "rescuing" cells from MTX-induced cell death (Relling et al., 1994; Wolfrom et al., 1993; Goldie et al., 1975). We previously showed that severe (grade III/IV) MTX-induced oral mucositis occurs in 20% of patients in a prospective study of children with ALL despite adequate LV rescue therapy (den Hoed et al., 2015). Severe MTX-induced oral mucositis causes an impairment of the quality of life of children and can lead to treatment delays (den Hoed et al., 2015; Shimasaki et al., 2006; Liu et al., 2011).

HD-MTX and additional LV rescue therapy protocols differ between countries. The introduction of LV rescue therapy has been established entirely empirically throughout the past decades and, even today, the basis for the "effectivity" to reduce oral mucositis rates and the

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"selectivity" of this regimen is not widely appreciated nor fully understood (Relling et al., 1994; Borsi et al., 1991). Furthermore, it has been suggested that plasma and intracellular folate status before the start of HD-MTX and LV therapy may affect oral mucositis rates (den Hoed et al., 2015; Robien, 2005).

The present review was conducted to determine the effectivity of various Leucovorin dosing- and timing regimens to reduce oral mucositis and to determine the role of pre-treatment plasma- and intracellular folate levels in the development of MTX-induced oral mucositis.

2. Methods

2.1. Literature search strategy

A literature search was performed on June 18th 2018 in Pubmed, Embase and Cochrane. A systematic search was developed with the following main search terms: Leucovorin / Folate rescue therapy AND Methotrexate AND Acute Lymphoblastic Leukemia (Supplemental Table 1). As 'oral mucositis' was often not mentioned in the title / abstract, we first included all articles in which it seemed that data on toxicity would be available in the full-text and only identified articles with data on 'oral mucositis' after full-text screening.

2.2. Inclusion and exclusion criteria

To be eligible for inclusion, a study had to include (Hunger et al., 2012) children aged ≤21 years with acute lymphoblastic leukemia or lymphoma (including studies on children with Down Syndrome) (Pui et al., 2011), high-dose methotrexate treatment (≥1 g/m^2) (Kamps et al., 2010), data on LV or folinic acid rescue therapy including dosing and / or timing (den Hoed et al., 2015), incidence/prevalence data on oral mucositis. Furthermore, studies that contained data on (Pieters et al., 2016) folate plasma- or erythrocyte folate levels in relation to the development of oral mucositis were included. Animal or cell-line studies, case reports, expert opinions, reviews, conference abstracts, articles in languages other than English or Dutch and studies without a full text available were excluded.

2.3. Data extraction and analysis

After removal of duplicates, 659 studies remained (Fig. 1). After title/abstract selection by two independent reviewers (JNvdB, NO), the remaining 85 articles were assessed full text. In total, 12 studies were identified to be relevant based on the defined aims and inclusion criteria.

2.4. Data search

The following data were extracted from the included studies: author and year of publication; country; study design; number of patients including median age; protocol; methotrexate dosing and/or timing; leucovorin dosing and / or timing; folate (plasma / intracellular) levels; system used for toxicity screening; prevalence and/or severity of mucositis, focused mainly on 'clinically relevant' mucositis. Oral mucositis grade ≥3 according to either the National Cancer Institute Common Toxicity Criteria (NCI-CTC) or the World Health Organization (WHO) toxicity grading scale was defined as clinically relevant mucositis in this study. In the NCI-CTC scale oral mucositis grade 3 was defined as "severe pain, interfering with oral intake" (CTCAE, 2006; WHO, 2003). In the WHO toxicity grading scale oral mucositis grade 3 was defined as "painful and the inability to swallow solids" (WHO, 2003).

2.5. Quality of evidence assessment

The quality of the included studies was determined by assessing the level of bias using the Quality in Prognosis Studies (QUIPS) tool (Hayden et al., 2013). The studies were reviewed for the six domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding and statistical analysis / reporting. The quality of evidence was categorized into 3 levels, being 'high risk of bias', 'moderate risk of bias' and 'low risk of bias'. According to the recommendation of Hayden et al., we chose *a priori* domains out of the six domains most relevant for this review to determine a sum score (Hayden et al., 2013). Study participation, prognostic factor measurement and outcome measurement, were used as *a priori* domains.

3. Results

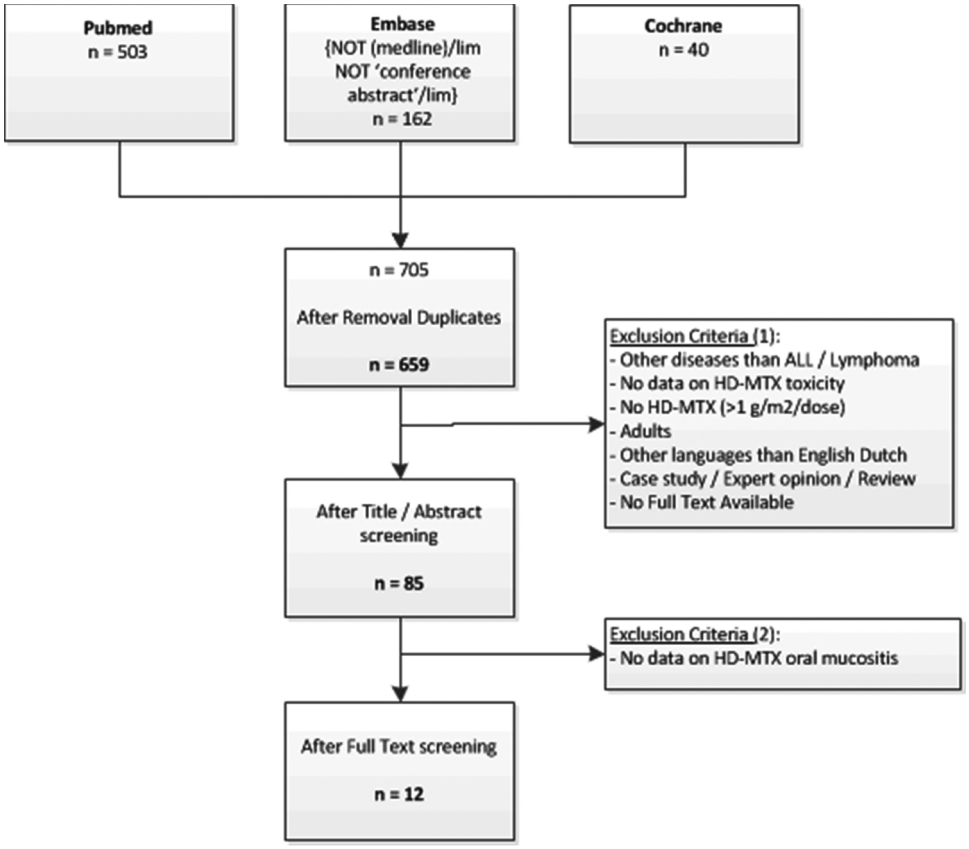
Studies were published between 1983 and 2018 and included between 12 and 485 study participants. In total, 4 studies were retrospective cohort studies, 6 were prospective cohort studies and 2 were randomized controlled trials that compared two doses of HD-MTX. The included studies described patients receiving HD-MTX doses ranging between 1 g/m^2/dose and 12 g/m^2/dose, in which 1 g/m^2/dose (2 studies), 3 g/m^2/dose (3 studies) and 5 g/m^2/dose (3 studies) were the most common doses. The prevalence of oral mucositis NCI-CTC/WHO grade ≥3, ranged between 0% and 41%. Albertioni et al reported a percentage of 44%, with a cut-off value of WHO grade ≥1 (Albertioni et al., 1997). None of these studies compared the outcome of patients with and without LV rescue therapy in a randomized setting and we could therefore not determine the effect of LV rescue therapy on reducing the oral mucositis rate.

3.1. The effect of leucovorin dosing on HD-MTX induced oral mucositis

Twelve studies on the effect of dosing of LV rescue therapy were included in our study (Table 1), of which 4 had a 'low risk of bias' and 8 had a 'medium risk of bias' (Supplemental Table 2) (den Hoed et al., 2015; Relling et al., 1994; Wolfrom et al., 1993; Albertioni et al., 1997; Zhang et al., 2014; Pauley et al., 2013; Vaishnavi et al., 2018; Gemma et al., 2017; Moulik et al., 2016; Kapoor et al., 2012; Xu et al., 2007; Frankel et al., 1983). The starting doses of LV rescue therapy ranged between 10 mg/m^2/dose and 30 mg/m^2/dose, of which 15 mg/m^2 and 30 mg/m^2 were used most commonly. The cumulative doses of LV rescue therapy ranged between 30 mg/m^2 and 90 mg/m^2. Out of twelve included studies, two studies described the use of locally administered topical doses of LV to the oral mucosa during HD-MTX in addition to the LV administered orally or intravenously. It was not possible to determine the effect of locally administered topical doses of LV to the oral mucosa on the oral mucositis rate as these two studies did not randomize patients into patients with and without the topical administered doses (Albertioni et al., 1997; Xu et al., 2007).

We were able to compare data on the cumulative doses of LV administered in relation to developing MTX-induced oral mucositis in five out of twelve included studies (Fig. 2) (den Hoed et al., 2015; Wolfrom et al., 1993; Moulik et al., 2016; Kapoor et al., 2012; Frankel et al., 1983). Seven studies were excluded from this analysis as they either used individualized MTX-dosing, lacked a specified LV rescue therapy regimen or MTX regimen in which the cumulative dose per therapy group could not be calculated, lacked a grading system for toxicity or had a cut-off for toxicity other than grade ≥3 (Relling et al., 1994; Albertioni et al., 1997; Zhang et al., 2014; Pauley et al., 2013; Vaishnavi et al., 2018; Gemma et al., 2017; Xu et al., 2007).

Two out of five studies administered 1 g/m^2/dose MTX in either 36 h followed by a cumulative LV dose of 30 mg/m^2 or in 1 h followed by a cumulative LV dose of 45 mg/m^2, resulting in an oral mucositis rate of 41% and 12% respectively (Fig. 2) (Wolfrom et al., 1993; Frankel et al., 1983). In these studies, we observed a lower rate of oral mucositis when HD-MTX was infused in a short period with higher cumulative LV doses (Frankel et al., 1983), than when HD-MTX was



Flowchart of literature search and number of included studies.

Fig. 1. Flowchart literature study.
Flowchart of literature search and number of included studies.

infused in a longer period with lower cumulative LV doses (Wolfrom et al., 1993).

One out of five studies administered 3 g/m^2/dose MTX in an unknown infusion period followed by a cumulative LV dose of 90 mg/m^2, resulting in an oral mucositis rate of 29% (Fig. 2) (Moulik et al., 2016).

Two out of five studies administered 5 g/m^2/dose MTX in 24 h followed by either a cumulative LV dose of 45 mg/m^2 or 90 mg/m^2, resulting in an oral mucositis rate of 20% and 5% respectively (Fig. 2) (den Hoed et al., 2015; Kapoor et al., 2012). In these two studies, we observed a decreased rate of oral mucositis when higher LV doses were administered (Kapoor et al., 2012).

This Figure shows the percentage of oral mucositis per HD-MTX dose and cumulative LV dose per study

3.2. The effect of leucovorin timing on HD-MTX induced oral mucositis

Twelve studies on the timing of LV rescue therapy after start of HD-MTX were included in our study (Table 1), of which 4 had a 'low risk of bias', 7 had a 'medium risk of bias' and 1 had a 'high risk of bias' (Supplemental Table 2) (den Hoed et al., 2015; Relling et al., 1994; Wolfrom et al., 1993; Albertioni et al., 1997; Zhang et al., 2014; Pauley et al., 2013; Vaishnavi et al., 2018; Gemma et al., 2017; Moulik et al., 2016; Kapoor et al., 2012; Xu et al., 2007; Frankel et al., 1983). Most studies started their LV rescue therapy at 24 h, 36 h or 42 h after initiation of HD-MTX (den Hoed et al., 2015; Relling et al., 1994; Wolfrom et al., 1993; Albertioni et al., 1997; Zhang et al., 2014; Pauley et al., 2013; Vaishnavi et al., 2018; Gemma et al., 2017; Moulik et al., 2016; Kapoor et al., 2012; Xu et al., 2007). We were able to compare data on the timing of LV in relation to developing MTX-induced oral mucositis in six out of twelve included studies as previously mentioned (Fig. 3) (den Hoed et al., 2015; Wolfrom et al., 1993; Moulik et al., 2016; Frankel et al., 1983).

Two out of six studies administered 1 g/m^2/dose MTX in either 36 h followed by LV starting at 24 h or in 1 h followed by LV dose starting at 6 h, resulting in an oral mucositis rate of 41% and 12% respectively (Wolfrom et al., 1993; Frankel et al., 1983). Another two studies administered 5 g/m^2/dose MTX in 24 h followed by LV starting at 42 h, resulting in an oral mucositis rate of 20% and 5% respectively (den Hoed et al., 2015; Kapoor et al., 2012). One study randomized patients to receiving 5 g/m^2/dose MTX in either 7 or 24 h followed by LV starting at 36 h, resulting in an oral mucositis rate of 0% (Xu et al., 2007). Overall, we observed an increase of the oral mucositis rate when the timing of LV administration after initiation of MTX was delayed in the 1 g/m^2/dose- and 5 g/m^2/dose MTX-studies (Fig. 3).

In the two studies, in which 3 g/m^2/dose MTX followed by LV starting at either 24 h or 36 h was administered, the opposite effect was observed showing lower mucositis rates when LV initiation was delayed (Fig. 3) (Moulik et al., 2016; Xu et al., 2007). One factor that might explain that the opposite effect was observed in the studies of Moulik et al. and Xu et al. could be that in addition to normal LV administration also topical LV was administered to the oral mucosa in the study of Xu et al. to reduce oral mucositis (Moulik et al., 2016; Xu et al., 2007).

This Figure shows the percentage of oral mucositis per HD-MTX dose and LV timing (number of hours after start of HD-MTX infusion) per study.

3.3. Plasma- and erythrocyte folate levels

Two out of twelve included studies provided data on the association between baseline plasma- and intracellular folate and the development

Table 1
Overview studies literature review – effect LV.

Author, Year	Country	Study design	Number of patients (n)	Median age, years (range / ± SD)	Protocol	Dose + frequency MTX	Dose + frequency LV	System toxicity screening	Prevalence/Severity Mucositis, n (%)	Risk of Bias
Den Hoed et al, 2015	Netherlands	P	134	5.3 (1.4 - 18.1)	DCOG-ALL-10	5 g/m^2 iv in 24 h 4 courses / 2 weeks	15 mg/m^2 after 42 h every 6 h (minimum of 3 doses)	NCI-CTC v.3.0 grade ≥ 3	Total: 26/134 (20%) First course: 18 (15%) Remaining three courses: 10 (8%)	low risk of bias
Zhang et al., 2014	China	P	136	TT: 5.3 (± 2.8) TC/ CC: 4.9 (± 2.3)	ALL-BFM 2000	3-5 g/m^2 iv in 24 h 3 courses / 2 weeks	15 mg/m^2 at 36 h, 42 h and 48 h	WHO grade ≥ 3	8 (5.9%)	low risk of bias
Pauley et al., 2013[a]	USA	RCT	LR: 233 SR/HR: 252	LR: 4.0 (1.0-18.5) SR/HR: 8.3 (1.0-18.9)	St. Jude Total Therapy Study XV	2,8 g/m^2 (0.9-5.3 g/m^2) 4 courses / every other week	LR: 10 mg/m^2 iv after 42 h, every 6 h 5 times SR/HR: 15 mg/m^2 iv after 42 h, every 6 h 5 times	NCI-CTC v. 2.0 grade ≥ 3	Gastrointestinal: LR individualized: 52 (6.9%) LR not individualized: 14 (8.7%) S/HR individualized: 50 (8.0%) S/HR not individualized: 44 (13.0%)	low risk of bias
Relling et al., 1994	USA	R	C: 67 T: 67	5.1 (0.3 – 18.5)	Total Therapy Study XII	C: 1.5 g/m^2 iv in 24 h T: adjusted MTX doses (1.1 – 3.6 g/m^2) iv in 24 h 3 courses (1-5)	30 mg/m^2 iv at 36 h, 30 mg/m^2 orally at 42 h, 15 mg/m^2 at 48 h, 5 mg/m^2 orally at 54 h, 68 h and 78h	NS	123/481 courses (26.2%)	low risk of bias
Vaishnavi et al., 2018	India	P	53 100 cycles	6.8 (± 3.2)	ICiCLe protocol	HR B-ALL: 3 g/m^2 iv in 24 h T-ALL / T-NHL: 5 g/m^2 iv in 24 h Relapsed ALL: 6 g/m^2 iv in 24 h	15 mg/m^2 iv at 42 h, 48 h, 54 h, 60 h, 66 h, 72 h (3 extra doses in relapsed ALL)	NCI-CTC. V. 4.0	Grade 1 – 2: 29/100 (29%) Grade 3 – 4: 3/100 (3%)	medium risk of bias
Gemma et al., 2017	Japan	R	DS: 4 NDS: 16	DS: 7.4 (4.0 – 8.4) NDS: 5.1 (0.9 – 14.5)	TCCSG L99-15 / LO4-16	3 g/m^2 iv 3 times weekly in 24 h (LO4-16: in 12 h during 2nd and 3rd dose when no toxicity)	Dose: NS; at 36 h, 42 h, 48 h, 54 h, 60 h, 66 h	NCI-CTC v.2.0 grade ≥ 3	DS: 4 (100%) NDS: 0 (0%)	medium risk of bias
Moulik et al., 2016	India	P	21	8.4 (± 3.2)	NS	3 g/m^2 iv 4 courses / 2 weeks	30 mg/m^2 iv at 24 h + 4 x 15 mg/m^2 at 12 h intervals	NCI-CTC. V. 4.0	6/21 (28.6%)	medium risk of bias
Kapoor et al., 2012	India	R	41	6.0 (1.0 – 15.0)	ALL protocol (2004)	5 g/m^2 iv in 24 h 4 courses (1-4)	30 mg/m^2 iv 42 h after iv MTX + 15 mg/m^2 orally at 48 h, 54 h, 60 h, 66 h	NCI-CTC v.3.0 grade ≥ 3	Grade 1-2: 50 (33.6%) Grade 3-4: 8 (5.4%) Total: 58/149 cycles (38.93%)	medium risk of bias
Xu et al., 2007	China	P	121	10.3 (1.6-19.0)	NPCAC97	SR: 3 g/m^2 iv HR: 5 g/m^2 iv Randomization MTX: 7 h infusion vs. 24 h infusion	SR: 15 mg/m^2 at 36 h HR: 30 mg/m^2 at 36 h SR + HR: 15 mg/m^2 4-7x every 6 h *CF (3 mg/d) spread to oral mucosa*	WHO grade ≥ 3	SR – 7 h infusion: 2 (4.0%) SR – 24 h infusion: 4 (8.2%) HR – 7 h infusion: 0 (0%) HR – 24 h infusion: 0 (0%)	medium risk of bias
Albertioni et al., 1997	Norway	R	12	NS	NOPHO-ALL (1992)	5-8 g/m^2 iv in 24 h 36 courses; 3 / child	15 mg/m^2 iv at 36 h, 42 h, 48 h and 54 h + *Topical doses (3 mg/d) on oral mucosa in 72.7% of the infusions*	WHO grade ≥ 1	16/36 courses (44.4%)	medium risk of bias
Wolfrom et al., 1993[b]	Germany	RCT	60	7.1 (1.0 – 21.0)	BFM / COALL / Münchener protocols / Modified Memphis or CCSG protocols	HDM: 12 g/m^2 iv in 4 h IDM: 1 g/m^2 iv in 36 h	HDM: 15 mg/m^2 orally at 24 h, every 6 h for 12 doses IDM: 15 mg/m^2 orally at 48 h and 54 h	NS	Stomatitis / Ulcerations[c] HDM: 9/21 (43%) IDM: 18/22 (82%) Deep mucosal ulcerations: HDM: 0/21 (0%) IDM: 9/22 (41%)	medium risk of bias
Frankel et al., 1983	USA	P	17	Median NS (1.9 – 15.0)	POG 7420 (ALinC 11)	1.0 g/m^2 iv in 1 h	15 mg/m^2 orally at 6 h, 12 h and 18 h	NS	2/17 (11.8%)	medium risk of bias

SD – Standard Deviation; LV – Leucovorin; R – Retrospective; P – Prospective; iv – intravenous; h – hours; NCI-CTC – National Cancer Institute-Common Toxicity Criteria; v – version; DS – Down Syndrome; NDS – Non Down Syndrome; NS – Not specified; WHO – World Health Organization; C – control group; T – treatment group; WHO – World Health Organization Criteria; LR – Low Risk Group; SR – Standard Risk Group; HR – High Risk Group; HDM – High-dose Metothrexate; IDM – Intermediate-dose methotrexate.

[a]RCT with n = 67 patients randomized to receive 5 fixed doses of MTX (1.5 g/m^2 iv in 24 h) and the other n = 67 patients randomized to adjusted MTX doses based on each individual's clearance, to achieve a target level of systemic exposure or area under the plasma concentration time curve (AUC) of 640–900 μmol/L*h.

[b] Study in patients with ALL and an early bone marrow relapse.

[c] Data on toxicity were available in 22 patients in the IDM group and in 21 patients in the HDM group.

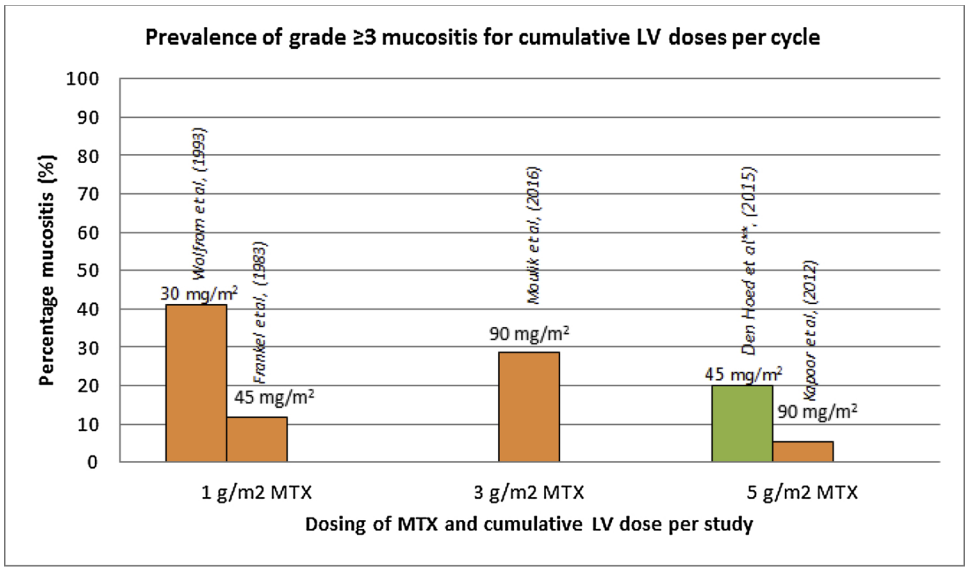


Fig. 2. Prevalence of mucositis grade ≥3 for cumulative LV doses per cycle per HD-MTX dose.
● = High risk of bias; ● = Moderate risk of bias; ● = Low risk of bias.

● = High risk of bias; ● = Moderate risk of bias; ● = Low risk of bias

of MTX-induced oral mucositis (Table 2), of which 1 had a 'low risk of bias' and 1 had a 'medium risk of bias' (Appendix 2) (den Hoed et al., 2015; Moulik et al., 2016). We previously showed that patients with oral mucositis had a significantly higher level of baseline erythrocyte folate compared to patients without oral mucositis, whereas plasma folate was not associated to oral mucositis. We showed that when the erythrocyte folate concentration increased with 1 µmol/l, the odds of developing oral mucositis were 1.23 (95% confidence interval (CI) 1.04–1.45) (den Hoed et al., 2015). Moulik et al. examined a small group of patients in whom a plasma folate deficient state was not associated with oral mucositis (Moulik et al., 2016). Erythrocyte folate levels were not measured in this study (Moulik et al., 2016).

3.4. Other findings on the prevalence of HD-MTX induced oral mucositis

First, when comparing the oral mucositis rate between studies using different HD-MTX doses, the overall rate of oral mucositis seemed to be lower in the studies using 5 g/m^2/dose HD-MTX compared to the studies using lower HD-MTX doses. This result was also reported in two randomized clinical trials comparing 3 versus 5 mg/m^2/dose MTX and 1 versus 12 mg/m^2/dose MTX respectively (Wolfrom et al., 1993; Xu et al., 2007). However, in the study of Wolfrom et al. this difference could be explained by a longer infusion period of 36 h in the 1 g/m^2 MTX versus only 4 h in the 12 g/m^2 MTX and a more intensive LV rescue therapy regimen in the 12 g/m^2 MTX dose, which included both higher cumulative doses of LV and a more early timing of LV (Wolfrom et al., 1993). In the study of Xu et al. this difference could be explained by more intensive pre-hydration measures and higher cumulative LV doses in the 5 g/m^2 MTX group versus the 3 g/m^2 MTX group. This finding of a lower oral mucositis rate in patients receiving higher doses of MTX could thus be explained by either a longer MTX infusion period or a more intensive LV regimen (Xu et al., 2007).

Second, when comparing the oral mucositis rate in recent studies compared to older studies, we observed less oral mucositis in the more

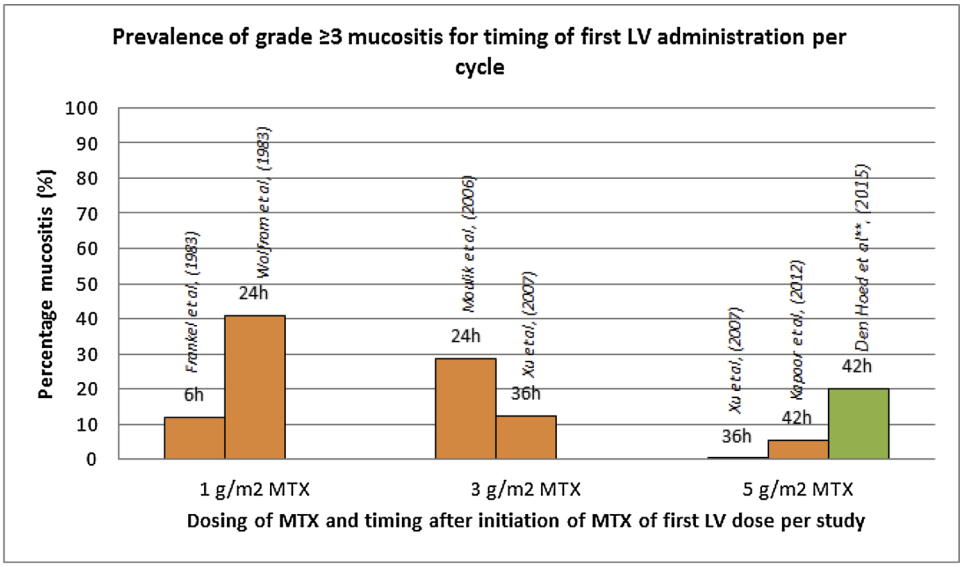


Fig. 3. Prevalence of mucositis grade ≥3 for timing of first administration of LV after initiation of MTX per cycle per HD-MTX dose.
● = High risk of bias; ● = Moderate risk of bias; ● = Low risk of bias.

● = High risk of bias; ● = Moderate risk of bias; ● = Low risk of bias

Table 2
Overview included studies literature review – pretreatment folate.

Author, Year	Country	Number of patients (n)	Median age, years (range / ± SD)	Protocol	Dose + frequency MTX	Dose + frequency LV	System toxicity screening + cut-off	Incidence/Severity Mucositis	Conclusion	Risk of Bias
Den Hoed et al, 2015	Netherlands	134	5.3 (1.4 - 18.1)	DCOG-ALL-10	5 g/m^2 iv in 24 h every 2 weeks	15 mg/m^2 42 h after iv MTX, every 6 h (minimum of 3 doses)	NCI-CTC v.3.0 grade ≥ 3	26/134 (20%)	Higher levels of baseline erythrocyte folate were found in patients with mucositis (P = 0.012). For every increase in 1 μmol/L erythrocyte folate, the odds of developing mucositis was 1.10 (95%CI 0.97-1.25)	low risk of bias
Moulik et al., 2016	India	21	8.4 (± 3.2)	NS	3 g/m^2 iv every 14 days	30 mg/m^2 iv 24 h after iv MTX + 4 x 15 mg/m^2 at 12 h intervals	NCI-CTC v.4.0	**Folate status:** Deficient: 4/10 (40%) Not deficient: 2/11 (18%) **MTHFR C667T:** CC: 2/12 (17%) CT + TT: 4/9 (44%) **MTHFR A1298C:** AA: 3/14 (21%) *AC + CC:* 3/7 (43%)	Folate deficiency[a] was associated with more toxic effects during HD-MTX therapy.	medium risk of bias

SD – Standard Deviation; LV – Leucovorin; NCI-CTC - NCI-CTC – National Cancer Institute-Common Toxicity Criteria; MTX – Methotrexate; NS – Not specified.
[a] No clear cut-off value or used diagnostics for 'folate deficiency' are specified in the article.

recent studies, published since 2007 (range 0–29%), compared to the older studies (range 12–41%) (Table 1).

Third, two studies described the use of individualized MTX and LV dosing based on MTX plasma levels and pharmacokinetic models (Relling et al., 1994; Pauley et al., 2013). Pauley et al. compared individualized MTX and LV dosing compared to standardized MTX and LV treatment and observed lower oral mucositis rates (low risk therapy group: 7% - standard/high risk group: 8%) in the individualized therapy group compared to the rates in the standardized therapy group (low risk therapy group: 9% - high risk therapy group: 13%) (Pauley et al., 2013).

Finally, two studies reported that the rate of MTX-induced oral mucositis was higher after the first cycle of MTX (den Hoed et al., 2015; Kapoor et al., 2012). Den Hoed et al. showed a prevalence of NCI-CTC (v.3.0) grade ≥3 mucositis of 15% in the first cycle compared to a prevalence of 8% in the remaining three HD-MTX courses (den Hoed et al., 2015). Kapoor et al. showed a prevalence of NCI-CTC (v.3.0) grade ≥3 mucositis of 12% in the first cycle compared to a prevalence of 3%, 6% and 0% in cycle 2, 3 and 4 respectively (Kapoor et al., 2012).

4. Discussion

The present systematic review was conducted to determine the effect of LV rescue therapy on developing oral mucositis during HD-MTX treatment in pediatric ALL. This is the first systematic review to summarize knowledge on different LV regimens in relation to the oral mucositis rate. We could not perform a meta-analysis of results as treatment regimens differ throughout the world. However, when comparing studies with similar HD-MTX doses, we observed lower mucositis rates in therapy regimens with higher cumulative doses of LV and early initiation of LV after MTX.

Overall, the percentage of MTX-induced oral mucositis seemed to decrease when the LV dose increased. This could be explained by the hypothesis that LV (folinic acid) bypasses Dihydrofolate Reductase and restores the cellular folate pool after HD-MTX therapy and thereby decreases toxic side effects in a dose – response relation (Relling et al., 1994; Wolfrom et al., 1993; Goldie et al., 1975). By initiating LV earlier after HD-MTX therapy, it is thought that the sooner the cellular folate pool is restored after HD-MTX in normal cells, the less toxicity occurs. These results held even when MTX doses were higher and when the MTX infusion period was shorter.

Several studies reported a higher prevalence of oral mucositis in the first cycle compared to the following cycles of HD-MTX. One explanation might lie in the previously proposed 'folate overrescue' concept (Cohen and Wolff, 2014; Borsi et al., 1991; Zhang et al., 2014). This concept is based on the fact that LV and MTX are structural analogues and might compete for the same cellular transport mechanisms and / or act antagonistically inside the cell during subsequent HD-MTX and LV courses (den Hoed et al., 2015; Skarby et al., 2006). This would lead to a reduction in toxicity rates during subsequent HD-MTX and LV courses. However, this raises the important question if rescue therapy with LV may compromise the effect of MTX by 'rescuing' malignant cells from the effect of HD-MTX and may induce more relapses. Studies on this 'folate overrescue' concept show inconsistent and contradictory results (Cohen and Wolff, 2014; Skarby et al., 2006; Sterba et al., 2005; Cohen, 2004; Takacs and Rodriguez, 2005; Valik et al., 2005). Another explanation for reduced toxicity over time during consecutive MTX doses might be that in pre-clinical studies a downregulation in the Reduced Folate Carrier 1 has been shown in response to an initial dose of MTX (Ma et al., 2000). It is clinically important that these two perspectives are taken into account in future studies on how LV doses and timing should be optimized to reduce the oral mucositis rate, as the anti-leukemic effectivity of HD-MTX therapy should not be compromised.

In this review we included two studies on the effect of baseline plasma and intracellular erythrocyte folate levels in relation to the development of MTX-induced oral mucositis (den Hoed et al., 2015;

Moulik et al., 2016). In literature, two hypotheses exist on plasma and intracellular folate levels in relation to developing HD-MTX toxicity. First, studies have suggested that baseline intracellular folate levels are a reflection of the cell's capacity to transport and retain cellular folates / MTX. This would suggest that the higher the intracellular level of folate at baseline, the higher subsequent intracellular MTX levels and the higher the oral mucositis rate (de Rotte et al., 2013; Kim et al., 1998). This is supported by our earlier study, even though the effect size of this study was small (den Hoed et al., 2015). Furthermore, several rheumatoid arthritis studies support this hypothesis (den Hoed et al., 2015; Dervieux et al., 2005). In contrast, studies have suggested that a low level of intracellular folate at baseline predisposes to increased HD-MTX toxicity rates as more MTX is able to enter the cell due to low competition for the same transport mechanisms (Robien, 2005; Moulik et al., 2016; Lopez-Lopez et al., 2013). This poses the question whether pre-treatment folate supplementation would reduce oral mucositis rates in HD-MTX therapy (Cohen and Wolff, 2014). More research to elucidate the exact role of baseline folate levels in relation to developing oral mucositis is necessary. Future studies could then focus on personalizing LV to the individual need of a patient.

Out of the twelve studies in our systematic review on the effect of LV rescue therapy on MTX-induced oral mucositis, the majority of studies were classified as having a 'moderate risk of bias'. No randomized controlled trial in which patients with and without LV rescue therapy were compared has been performed. Therefore, the exact effect of LV rescue therapy on reducing the oral mucositis rate remains unknown. As dosing and timing of LV were never studied separately and none of the studies was designed specifically to answer whether LV dosing or timing affected oral mucositis rates, we cannot fully distinguish between the effect of dosing and of timing of LV on reducing oral mucositis and results should be interpreted as such. In addition, many studies did not specify other relevant supportive care measures that might affect toxicity rates, such as hyperhydration or urine alkalinisation. Finally, the oral mucositis rate was often based on a retrospective study of toxicities and different types of grading systems, which might influence comparison of numbers between studies. We aimed to decrease the interindividual differences in reporting by only including severe grade $\geq$ 3 oral mucositis, which requires medical intervention and is thought to be less underreported. Nevertheless, these important factors should be taken into account in future studies into the dosing or timing of LV to reduce toxicity.

In conclusion, when comparing studies with similar treatment characteristics we observed less oral mucositis in HD-MTX and LV regimens in which higher cumulative doses of LV were administered and when LV was initiated early after HD-MTX, but more studies designed specifically to determine the most optimal dosing and timing of LV are necessary to confirm this. The effect of baseline folate levels on the development of MTX-induced oral mucositis remains unclear.

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Authorship contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. JNvdB and NO performed the literature review, the extraction of data, the initial interpretation of data and the drafting of the manuscript. RP, RdJ, MMvdHE and SGH were involved in the design of this study. All authors (JNvdB, NO, RP, RdJ, MMvdHE, SGH) were involved in the final interpretation of data and subsequent revisions of the manuscript. MMvdHE and SGH supervised the study.

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Appendix A. Supplementary data

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