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Original Research

Hypersensitivity to Pegylated *E.coli* asparaginase as first-line treatment in contemporary paediatric acute lymphoblastic leukaemia protocols: a meta-analysis of the Ponte di Legno Toxicity working group

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KEYWORDS
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Abstract ***Background:*** Hypersensitivity reactions to asparaginase challenge its use and occur frequently (30–75%) after native *Escherichia Coli (E.coli)* asparaginase. Comparison of incidence of allergic reactions to pegylated *E.coli asparaginase* (PEGasparaginase) across contemporary paediatric acute lymphoblastic leukaemia (ALL) protocols is lacking.
Method and patients: Questionnaires were sent to all members of the international ALL Ponte

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Hypersensitivity;
Risk factors

di Legno Toxicity Working Group. Meta-analyses were conducted to estimate the incidence of three types of hypersensitivity (allergy, allergic-like reaction and silent inactivation). Information on protocol level regarding PEGasparaginase dosing regimen, administration route and use of therapeutic drug monitoring was collected for risk analysis.

Results: Newly diagnosed patients with ALL (n = 5880), aged 1–24 years old, were enrolled in seven different upfront ALL protocols using PEGasparaginase as first-line treatment. The incidence of allergic reactions (sum of allergies and allergic-like reactions) [95% confidence interval] was 2% [1%; 3%] during induction and 8% [5%; 11%] during postinduction. Route of administration, number of doses, dosage and number of PEGasparaginase-free weeks did not significantly influence risk of hypersensitivity. Multivariate meta-regression analysis suggests that initiation of PEGasparaginase in postinduction and higher number of PEGasparaginase-free intervals increased the risk for allergic reactions. 9–16% and 23–29% of all hypersensitivities were allergic-like reactions and silent inactivation, respectively.

Conclusion: The incidence of allergic reactions is lower in protocols using PEGasparaginase as first-line treatment compared with that reported for *E.coli* asparaginase or PEGasparaginase after *E.coli* asparaginase.

Postinduction phase, a higher number of PEGasparaginase-free intervals, and initiation of PEGasparaginase in postinduction phase are risk factors for allergic reactions. These results are important for planning of PEGasparaginase administrations in future frontline therapy.

1. Introduction

L-asparaginase is one of the key therapeutics for childhood acute lymphoblastic leukaemia (ALL). It specially targets the ALL cells by catalysing the breakdown of extracellular L-asparagine into L-aspartic acid and ammonia [1,2]. Current asparaginase preparations are derived from *Escherichia Coli (E.coli)* or *Erwinia chrysanthemi* (*Erwinia*) [3,4].

Asparaginase can provoke hypersensitivity reactions by inducing antidrug antibodies. The international ALL Ponte di Legno Toxicity Working Group (PTWG) classifies hypersensitivity to asparaginase as (i) allergy in case of symptoms of allergy (always associated with undetectable asparaginase activity levels), (ii) allergic-like reactions in case of symptoms without inactivation and (iii) silent inactivation (SI) with inactivation of asparaginase activity but without hypersensitivity symptoms [5]. Allergic-like reactions and SI can only be diagnosed with monitoring of asparaginase activity levels. Distinction between allergy and allergic-like reactions is critical but difficult to determine if intravenous (IV) administration is truncated after little asparaginase has been infused. In a continuous dosing schedule at 14-day interval, inactivation of PEGasparaginase can be determined by a trough level measurement taken just before the dose leading to hypersensitivity [5,6]. Allergic-like reactions are not antibody-mediated, symptoms might be explained by a rapid increase of ammonia levels after PEGasparaginase administration [7,8].

Discontinuation of asparaginase treatment owing to hypersensitivity is one of the major drawbacks of asparaginase and leads to poor outcomes [9–11]. Most contemporary paediatric ALL protocols in high-income countries use pegylated *E.coli* asparaginase (PEGasparaginase) because the attachment of polyethylene glycol to native *E.coli* asparaginase increases the half-life and decreases the immunogenicity of asparaginase [6]. Hypersensitivity rates to native *E.coli* and to PEGasparaginase after native *E.coli* asparaginase are well known with reported incidences rates of 30–75% [10,12–14,16,17]. However, fewer trials reported the incidence of hypersensitivity to PEGasparaginase in contemporary ALL protocols using PEGasparaginase as the first-line of treatment [19,20,34].

A meta-analysis was performed based on data from the international PTWG to estimate the incidence of hypersensitivity and risk factors for hypersensitivity to asparaginase in ALL protocols using PEGasparaginase as the first line of treatment.

2. Patients and methods

Data were collected with a PEGasparaginase hypersensitivity questionnaire that was sent to all study groups represented in the PTWG. Requested information included the PEGasparaginase dosage, dose regimen (e.g. dosing frequency, total number of doses and PEGasparaginase-free interval), route of administration, total induction and postinduction hypersensitivity rates per protocol and per risk group, use of standard

premedication and concomitant steroids, hypersensitivity guideline, number of patients switching to *Erwinia* asparaginase and subsequent allergy rate and use of therapeutic drug monitoring (TDM). Protocols also using native *E.coli* asparaginase as first-line of treatment were excluded.

2.1. Hypersensitivity and therapeutic drug monitoring

The questionnaire focused on three types of hypersensitivity defined in the PTWG consensus definitions [5]: (i) allergy; (ii) allergic-like reactions and (iii) SI.

Definitions of hypersensitivity to asparaginase as per the PTWG consensus definitions [5]

i. Allergy:
An adverse local or general response from exposure to asparaginase characterised by flushing, rash, urticaria, drug fever, dyspnoea, symptomatic bronchospasm, oedema/angio-oedema, hypotension and/or anaphylaxis (accompanied by inactivation of asparaginase activity).

ii. Allergic-like reaction:
An intolerance with, for example, vomiting, stomach ache, rash and so on. These patients have normal activity levels if the infusion is continued. Real allergies often occur at the first drops, whereas allergic-like reactions occur later during infusion.

iii. SI:
Patients without clinical allergy but with asparaginase activity levels, preferably measured in two independent samples, of

PEGasparaginase (biweekly schedule):	Day 7 < 100 and/or day 14 < LLQ
Erwinia asparaginase (3x/week dosing schedule):	Day 2 < LLQ
General definition:	(trough) asparaginase activity level < LLQ

Definitions used in meta-analysis:
Hypersensitivity reactions = i + ii + iii
Allergic reaction = i + ii

To interpret the hypersensitivity data of the various protocols, we collected information on whether TDM was performed and if the three types of hypersensitivity reactions could be distinguished. To facilitate comparison between protocols with and without TDM, we defined allergic reactions as the sum of allergies and allergic-like reactions. SI was analysed separately.

In addition, information on the method of asparaginase activity measurement and the assessment of antidrug antibodies were collected. The severity of allergic reactions, both allergies and allergic-like reactions, were graded as per the Common Terminology Criteria for Adverse Events (CTCAE) [21,22]. Major differences between versions 3.0 and 4.03 are the indication of intervention and the separate categorisation of anaphylaxis in v4.03.

2.2. Risk analysis

Protocol characteristics such as route of administration, risk group stratification, dosage, the total number of doses, use of concomitant medication (e.g. steroids), the number of PEGasparaginase-free intervals, the duration of the PEGasparaginase-free interval (defined as the number of weeks between the last induction dose and first postinduction dose) and treatment phase were analysed as possible risk factors for hypersensitivity. Treatment phase was analysed in two ways, namely, comparisons were performed (i) between the incidence of hypersensitivity reactions in induction and postinduction and (ii) between protocols starting PEGasparaginase treatment in induction or in postinduction (i.e. Cooperative Study Group for childhood acute lymphoblastic leukaemia [CoALL] and Nordic Pediatric Haematology and Oncology Study Group [NOPHO]). A PEGasparaginase-free interval was defined as ≥4 weeks without PEGasparaginase.

2.3. Statistical analysis

A random effects model was used to pool protocol-specific incidence to estimate an overall incidence and its associated 95% confidence intervals (95%CI). Inverse variance method, which gives more weight to larger studies, was used to pool outcomes for different studies. The sizes of the square boxes in the forest plots are proportional to the total number of patients in each protocol. An overall test on heterogeneity between protocols was performed (value I^2). To estimate the between-study variance which is represented as 'tau' in the forest plots, DerSimonian-Laird's method was used [23]. To calculate CIs for each individual protocol results normal approximation interval based on summary measure was used [24]. Linear univariate and multivariate meta-regression was used to study the impact of moderator variables such as dosage, number and duration of PEGasparaginase-free intervals and number of PEGasparaginase doses on study effect size using regression-based techniques [25]. All statistical analyses were performed using the library metagen and metafor in the R software environment [26].

3. Results

A retrospective analysis of data from seven study groups in Europe and North America was conducted (Table 1;

Table 1
Characteristics of included study populations.

Protocol (years of recruitment)	Study group(s)	Total patients at start of PEG asparaginase treatment	
		n	%
CoALL 08–09 (2010–2019)	CoALL	712	12.1
DCOG ALL-11 (2012–2019)	DCOG	587	10.0
DFCI 05-001 (2005–2011)	DFCI	794[a]	13.5
EORTC 58081 (2013–2019)	BSPHO	392	6.7
NOPHO ALL2008 (2008–2016)	NOPHO	1401	23.8
LAL/SEHOP-PETHEMA 2013 (2016–2019)	SEHOP-PETHEMA	92	1.6
UKALL 2011 (2012–2019)	UKALL	1902	32.3
	TOTAL	5880	100

Abbreviations: ALL or LAL, acute lymphoblastic leukaemia; BSPHO, Belgian Society Pediatric Haematology Oncology; CoALL, Cooperative study group for childhood acute lymphoblastic leukaemia; DCOG, Dutch Children's Oncology Group; DFCI, Dana-Farber Cancer Institute; EORTC, European Organisation for Research and Treatment for Cancer; NOPHO, Nordic Pediatric Hematology and Oncology Study Group; PETHEMA, Program for the Study of Therapeutics in Malignant Haemopathy; SEHOP, Spanish Society of Pediatric Hematology and Oncology; UKALL, United Kingdom National Randomised Trial For Children and Young Adults with Acute Lymphoblastic Leukaemia and Lymphoma 2011.
[a] The total number of postinduction patients is 232.

Table S1). Several PTWG members were unable to participate owing to unavailable PEGasparaginase data (e.g. still blinded randomisation or not using PEGasparaginase as the first-line of treatment). A total of 5880 patients aged 1–24 years old with newly diagnosed ALL and treated as per seven different upfront ALL protocols with PEGasparaginase as first-line treatment were enrolled in this study.

3.1. Treatment

Detailed information on PEGasparaginase treatment per protocol is summarised in Table 2. All groups used PEGasparaginase at a dose of 1500 or 2500 IU/m^2 IV in 1–2 h or 1000 IU/m^2 intramuscularly (IM). The Dutch Children's Oncology Group (DCOG) group used a TDM-based individualised dosing schedule after the first three fixed doses. PEGasparaginase dosing frequency was once every two weeks in all protocols. Five of seven protocols had a PEGasparaginase-free interval ranging from 4 to 18 weeks between the last induction dose and the first postinduction dose, whereas the other two protocols, the NOPHO ALL2008 and CoALL 08–09, started PEGasparaginase treatment after induction. Additional PEGasparaginase-free intervals in postinduction were most common in high-risk (HR) regimens.

3.2. Therapeutic drug monitoring

Asparaginase activity measurement was an integral part of PEGasparaginase treatment in five of seven protocols. TDM was not performed in the UKALL United Kingdom National Randomised Trial For Children and Young Adults with Acute Lympoblastic Leukaemia and Lymphoma (UKALL) 2011 and the Acute Lymphoblastic Leukaemia (LAL)/Spanish Society of Pediatric Hematology and Oncology(SEHOP)-Program for the Study of Therapeutics in Malignant Haemopathy(PETHEMA) 2013 protocols.

Depending on the asparaginase sampling schedule of each protocol, study groups measured asparaginase activity in real-time, batchwise or a combination of both. The Belgian Society of Pediatric Haematology Oncology (BSPHO) and DCOG performed real–time asparaginase measurements to detect SI. The DCOG also used the activity levels to detect allergic-like reactions and to individualise the dose. The NOPHO was able to report allergic-like reactions and SI, retrospectively. CoALL and Dana-Farber Cancer Institute (DFCI) measured batchwise and were not able to share data of SI nor allergic-like reactions.

The BSPHO, CoALL, and DCOG measured asparaginase activity with an L-aspartic acid β-hydroxamate (AHA) assay [27]. The DFCI and NOPHO used a validated assay [28] and Nessler's reagent method [29], respectively. Anti-*E.coli* asparaginase [30], anti-*Erwinia* [33], anti-PEG [31,32] or anti-linker [33] antibody levels were determined by three of seven study groups for research purposes only.

3.3. Hypersensitivity to PEGasparaginase

For each protocol, the incidence of allergic reactions (allergy and allergic-like reactions taken together) was calculated and pooled together in the meta-analysis. The single-study incidence together with the estimated overall effect of allergic reactions is shown in Fig. 1. The overall incidence of allergic reactions along with 95% CI was 9% [6%; 13%], 2% [1%; 3%] and 8% [0.05%; 11%] in the overall protocol, induction and postinduction, respectively. The incidence of allergic reactions per protocol risk group is shown in Table 3.

Hypersensitivity per treatment phase or dose was reported by four study groups. The UKALL 2011 regimen C protocol did observe allergic reactions in the consolidation, interim maintenance and delayed intensification phases. In addition, the BSPHO observed that patients with VHR had allergic reactions in the first and second consolidation. In the DCOG ALL-11 protocol, PEGasparaginase in postinduction

Table 2
PEGasparaginase treatment per protocol.

Protocol	Dose (IU/m^2)	RoA	No. of PEGasparaginase doses in:		Weeks between last induction and first postinduction dose	Concomitant Steroids *with* PEGasparaginase dose:
CoALL 08–09	2500	IV	Induction	0	–	NA
			SR-reduced	4[c]	NA	4th
			SR-standard	7[c]	NA	4th
			SR-intensified	9[c]	NA	5–6th
			HR-reduced	5[c]	NA	5th
			HR-standard	9[c]	NA	5–6th
			HR-intensified	10[d]	NA	5–7th
DCOG ALL-11	1500/individualised	IV	Induction	3	–	1–3rd
			SR	1	12	4th
			MR	14	12	NA[d]
			HR[a]	5[d]	5	5th
DFCI 05–001	2500	IV	Induction	1	–	1st
			SR	15	6	NA[e]
			HR	15	6	NA[e]
			VHR	15	10	NA[e]
EORTC 58081 (BSPHO)	2500	IV	Induction	2	–	1–2nd
			SR	1	18	3rd
			AR Arm 1	1	18	3rd
			AR Arm 2	7[c]	18	3rd
			VHR	4[d]	3.5	4–6th
LAL/SEHOP-PETHEMA 2013	1000	IM	Induction	2	–	1–2nd
			SR	1	18	3rd
			MR	11[c]	18	3rd
			HR[a]	9[e]	8	6–11th
NOPHO ALL2008	1000	IM	Induction	0	–	NA
			SR[b]	5	NA	1st
			IR[b]	5	NA	1st
			HR[a]	10[c]	NA	NA[e]
UKALL 2011	1000	IM	Induction	2	–	1–2nd
			Regimen A	1	14	3rd
			Regimen B	1	14	3rd
			Regimen C	6[d]	5	7th

Abbreviations: ALL or LAL, acute lymphoblastic leukaemia; AR, average risk; BSPHO, Belgian Society Pediatric Haematology Oncology; CoALL, Cooperative study group for childhood acute lymphoblastic leukaemia; DCOG, Dutch Children's Oncology Group; DFCI, Dana-Farber Cancer Institute; EORTC, European Organisation for Research and Treatment for Cancer; HR, high risk; IM, intramuscular; IR, intermediate; IV, intravenous; MR, medium risk; NA, not applicable; NOPHO, Nordic Pediatric Hematology and Oncology Study Group; PEG-ASP, pegylated *Escherichia coli* asparaginase; PETHEMA, Program for the Study of Therapeutics in Malignant Haemopathy; RoA, route of administration; SEHOP, Spanish Society of Pediatric Hematology and Oncology; SR, standard risk; UKALL, United Kingdom National Randomised Trial For Children and Young Adults with Acute Lympoblastic Leukaemia and Lymphoma 2011; VHR, very high risk.

[a] Maximum number of doses in HR.

[b] After five standard consolidation doses, patients were randomised into an intermitted or continuous arm and received an extra three or 10 PEGasp doses, respectively. Post randomisation data were excluded from analysis, owing to missing data.

[c] Indicates one extra asparaginase interval of at least four weeks during postinduction PEG-ASP treatment.

[d] Indicates three or more intervals.

[e] Depending on the timing, a few doses coincide with dexamethasone treatment.

was administered without further PEGasparaginase-free intervals, the allergic reactions in the post-induction phase only occurred on the first and second dose. In the NOPHO ALL2008 protocol, starting PEGasparaginase after induction, reactions occurred mainly on the second dose.

Three study groups, DCOG, BSPHO and NOPHO, reported the incidence of allergic-like reactions and/or SI (Table 4). The DCOG and NOPHO observed that 2.0% and 1.6% of the patients had an allergic-like reaction, which was 16% and 9% of their total hypersensitivity reactions, respectively. The BSPHO, DCOG and NOPHO observed that 4.1%, 3.7% and 4.1% of the patients had SI, which was 27%, 29% and 23% of their total hypersensitivity reactions, respectively.

Severity of allergic reactions were described, as per the CTCAE version 3.0 or 4.03, per protocol (Fig. S1). Forty-seven per cent of the reactions were classified as grade 3/4.

3.4. Intravenous versus intramuscular administration of PEGasparaginase

The median incidence of allergic reactions was 6.5% (range, 5.5–14.8%) after IM administration and 8.9% (range, 8.6–10.5%) after IV administration ($P = 0.43$).

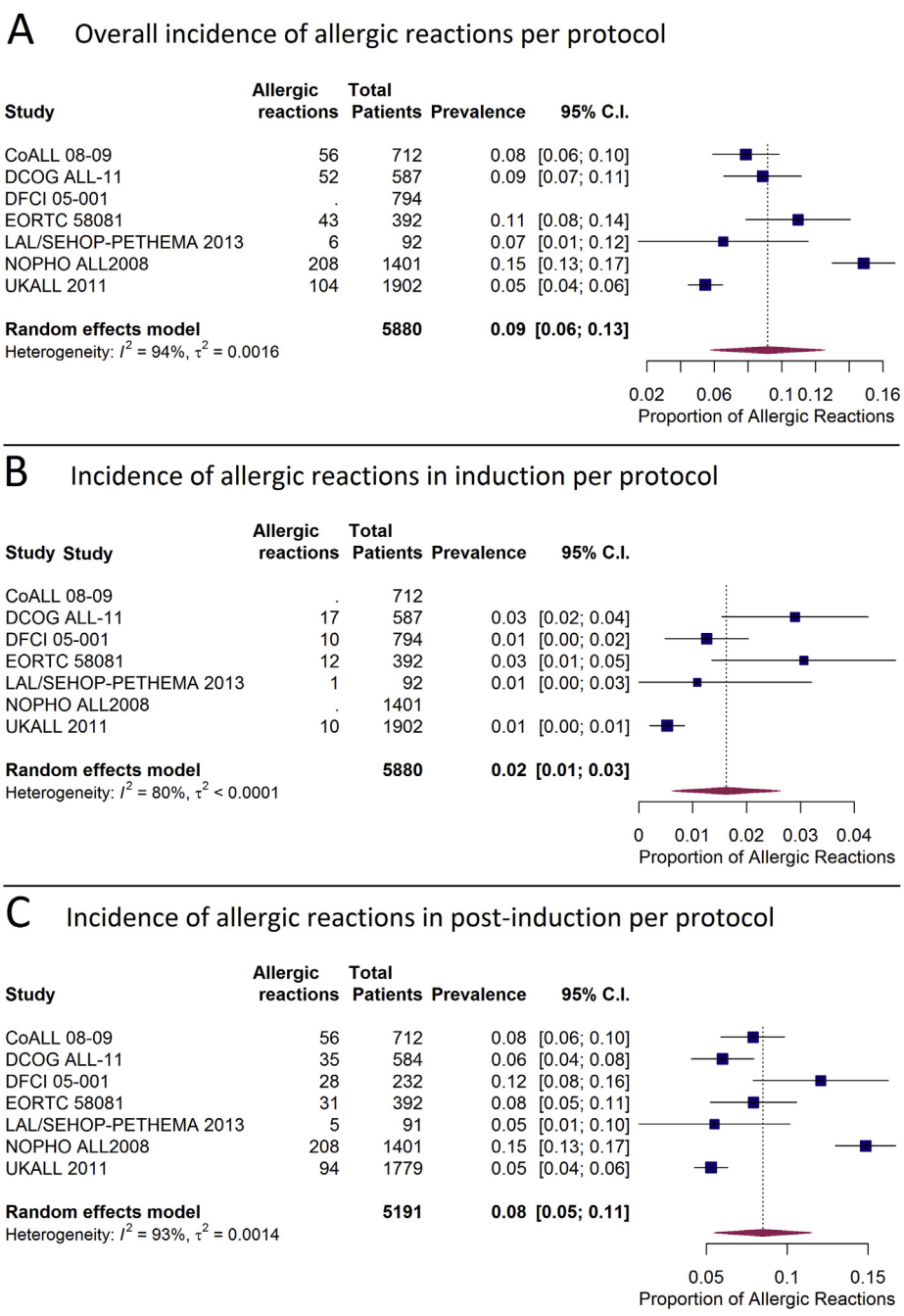

Fig. 1. Overall (**A**), induction (**B**) and postinduction (**C**) incidence of allergic reactions (sum of clinical allergies and allergic-like reactions) to PEGasparaginase of all protocols. Forest plots present the number of allergic reactions (sum of allergic and allergic-like reactions), total patients and the prevalence or incidence, of allergic reactions for each protocol with 95% CI. The summary estimate of the random effects model is represented by a diamond. The DFCI was excluded from the overall incidence analysis (1A) because only 232 of the 794 patients were randomised to receive PEGasparaginase in postinduction. CI, confidence interval; DFCI, Dana-Farber Cancer Institute.

The incidence of allergic-like reactions and SI was not different in the NOPHO protocol with IM administration versus the DCOG and BSPHO protocols using both IV administration (Table 4).

3.5. Hypersensitivity to Erwinia Asparaginase

All protocols prescribed a switch to *Erwinia* asparaginase in case of clinical hypersensitivity and/or SI. One PEGasparaginase dose was substituted by six or seven *Erwinia* asparaginase doses of 20.000 IU/m^2, with exception of the CoALL that used two doses of 45.000 IU/m^2 as substitution for one PEGasparaginase dose. Three-hundred and eight of 348 (89%) of the patients with hypersensitivity to PEGasparaginase received *Erwinia* asparaginase. Nineteen of these 308 (6%) exposed patients had an allergic reaction to *Erwinia* asparaginase, of which 7 of 19 (37%) were grade 3/4.

Table 3
Incidence of allergic reaction per protocol risk group.

Protocol	Postinduction					
	SR		IR		HR	
	Patients (n)	Allergy (%)	Patients (n)	Allergy (%)	Patients (n)	Allergy (%)
CoALL 08–09	287	7.0	–	–	425	8.5
DCOG ALL-11	173	8.1	372	5.1	39	5.1
DFCI 05-001	119	11.8	–	–	113[a]	12.4
EORTC 58081 (BSPHO)	43	7.0	285	6.3	64	15.6
LAL/SEHOP-PETHEMA 2013	6	0.0	70	5.7	15	6.7
NOPHO ALL2008	See IR[b]		1115	14.1	286	17.8
UKALL 2011	See IR[b]		951	0.0	828	11.4
Total:	628		2793		1770	
Median allergy rate (%):		7.0		5.7		14.4

ALL or LAL, acute lymphoblastic leukaemia; BSPHO, Belgian Society Pediatric Haematology Oncology; CoALL, Cooperative study group for childhood acute lymphoblastic leukaemia; DCOG, Dutch Children's Oncology Group; DFCI, Dana-Farber Cancer Institute; HR, high risk; IR, intermediate risk; EORTC, European Organisation for Research and Treatment for Cancer; NOPHO, Nordic Pediatric Hematology and Oncology Study Group; PETHEMA, Program for the Study of Therapeutics in Malignant Haemopathy; SEHOP, Spanish Society of Pediatric Hematology and Oncology. SR, standard risk. United Kingdom National Randomised Trial For Children and Young Adults with Acute Lympoblastic Leukaemia and Lymphoma 2011.
Allergy is the sum of allergies and allergic-like reactions. Weighted univariate linear meta-regression showed that the incidence of allergic reactions was associated with risk group stratification ($P < 0.001$).
[a] Patients with very high risk and high risk combined.
[b] SR and IR data was received aggregated.

3.6. Risk factors

Univariate meta-regression analysis showed a positive association between the incidence of allergic reactions and number of PEGasparaginase-free intervals, see bubble plot in Fig. 2. HR group stratification ($P < 0.001$), postinduction treatment phase ($P < 0.001$) and start of PEGasparaginase treatment in postinduction were also associated with a higher incidence of allergic reactions ($P = 0.006$) (Table 5). The incidence of allergic reactions was not associated with dosage ($P = 0.3$), route of administration ($P = 0.5$), duration of the first PEGasparaginase-free interval ($P = 0.1$) or number of doses ($P = 0.4$).

Multivariate meta-regression analysis showed a positive association between the incidence of allergic reactions and the number of PEGasparaginase-free intervals ($P = 0.006$) and start of PEGasparaginase in the postinduction treatment phase (i.e. CoALL and NOPHO patients) ($P = 0.02$) (Table 5).

CoALL was the only group that administered standard antihistaminic drugs as premedication, but concomitant steroids (e.g. dexamethasone as antileukaemic agents) were administered in all protocols (Table 2). All induction

Table 4
Allergy, allergic-like reaction and silent inactivation in induction and postinduction per protocol.

Protocol	Induction				Post-induction			
	No. of patients	Allergy (%)	Allergic-like reaction (%)	Silent inactivation (%)	No. of patients	Allergy (%)	Allergic-like reaction (%)	Silent inactivation (%)
CoALL 08–09	NA	–	–	–	712	7,9	–	–
DCOG ALL-11	587	1,9	1,0	1,0	570	5,0	1,0	2,7
DFCI 05–001	794	1,3	–	-	232	12,1	–	-
EORTC 58081 (BSPHO)	392	3,1	–	1,8	392	7,9	–	2,3
LAL/SEHOP-PETHEMA 2013	92	1,1	–	–	91	5,5	–	–
NOPHO ALL2008	NA	–	–	–	1401	13,6[a]	1,6[b]	4,1[b]
UKALL 2011	1902	0,5	–	–	1779	5,3	–	–

ALL or LAL, acute lymphoblastic leukaemia; BSPHO, Belgian Society Pediatric Haematology Oncology; CoALL, Cooperative study group for childhood acute lymphoblastic leukaemia; DCOG, Dutch Children's Oncology Group; DFCI, Dana-Farber Cancer Institute; EORTC, European Organisation for Research and Treatment for Cancer; NOPHO, Nordic Pediatric Hematology and Oncology Study Group; PETHEMA, Program for the Study of Therapeutics in Malignant Haemopathy; SEHOP, Spanish Society of Pediatric Hematology and Oncology; United Kingdom National Randomised Trial For Children and Young Adults with Acute Lympoblastic Leukaemia and Lymphoma 2011.
[a] Incidence of allergies was 13,6% in all risk groups during the first five non-randomised PEGasparaginase doses and 12,5% in patients with SR and IR (n = 1115). After randomisation, 13 of 625 (2,1%) patients with SR and IR had an allergy.
[b] Only SR and IR patients.

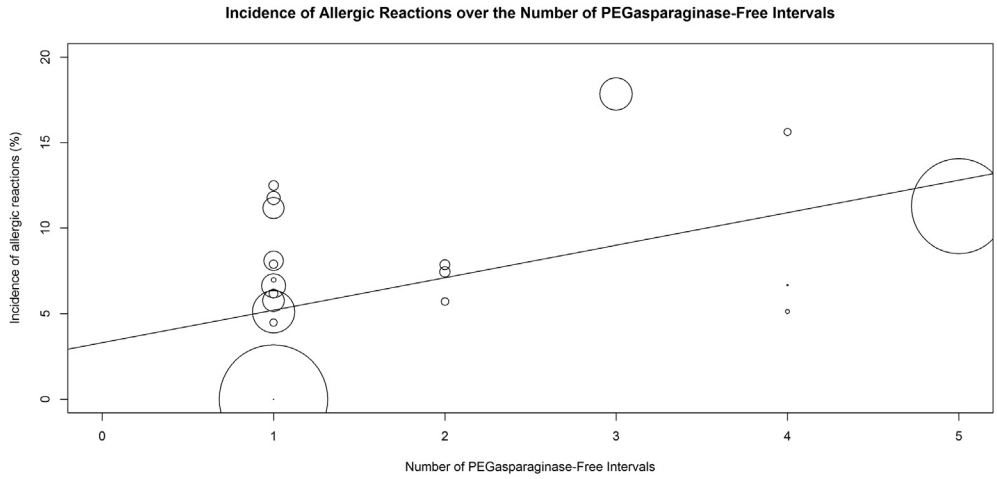

Fig. 2. Bubble plot of the incidence of allergic reactions (sum of allergies and allergic-like reactions) over the number of PEGasparaginase-free intervals. Each bubble presents a study (SR, IR or HR). The size of the bubble indicates the size of the study. Univariate linear regression ($P = 0.005$). HR, high risk; IR, intermediate risk; SR, standard risk.

Table 5
Weighted univariate and multivariate linear meta-regression.

Determinants	Univariate			Multivariate		
	Regression coefficient	SE	P-value	Regression coefficient	SE	P-value
Dosage	−0.0018	0.0016	0.29			
Number of doses	0.27	0.28	0.36			
Number of PEGasparaginase-free intervals	1.9	0.60	0.0048	2.3	0.72	0.0058
Duration of first PEGasparaginase-free interval	−0.34	0.20	0.12			
Risk group stratification[a]						
IR	2.4	0.17	0.16	−2.6	2.5	0.31
HR	9.8	1.5	<0.001	−2.6	3.1	0.41
IV administration	−1.6	2.5	0.52			
Grade 3/4[b]	−10.1	11.1	0.43			
Starting in postinduction	6.3	2.0	0.0056	5.4	2.1	0.019
Treatment phase (induction = 0 and postinduction = 1)	7.4	1.7	<0.001	NA[c]		

HR, high risk; IR, intermediate risk; IV, intravenous; SR, standard risk.
Weighted univariate and multivariate linear meta-regressions conducted with the incidence of allergic reactions as outcome. The incidence of allergic reaction of each study was weighted by study size.
[a] SR was used as reference.
[b] Dependent variable is the incidence of grade 3/4 allergic reactions.
[c] Observations in induction consisted of missing data (e.g. not stratified yet, no PEGasp-free intervals) and were therefore deleted owing to missingness. SE, standard error; NA, not applicable.

doses coincided with steroid treatment, whereas this varied per dose in postinduction. We were not able to test the concurrent use of steroids because hypersensitivity data were aggregated and not described per dose.

4. Discussion

The incidences of hypersensitivity reactions presented here are lower than previously reported for native *E.coli* asparaginase or PEGasparaginase after *E.coli* asparaginase being up to 5% and 75% in induction and postinduction, respectively [12–14,16,17]. In addition, the incidence of SI was lower [10,12,13,17]. Postinduction phase, a higher number of PEGasparaginase-free intervals, and initiation of PEGasparaginase in postinduction phase are risk factors for allergic reactions, whereas the total number of doses, duration of the PEGasparaginase-free interval, the route of administration (IV or IM) and dosage were not.

Results based on meta-regression suggested that route of administration was not a risk factor. This is in contrast to the findings of Burke et al. [34] who found that grade ≥3 hypersensitivity reactions occurred more frequently with intramuscular compared with IV administration. On the other hand, Hasan et al. [35] reported a higher incidence of allergic reactions with IV administration. They did include a smaller number of patients (n = 752) in the meta-analysis and did not specify if PEGasparaginase was used as the first-line of treatment. Using PEGasparaginase as the first- or

second-line of treatment is important for analysis, because the incidence of allergic reactions to PEGasparaginase after native *E.coli* has been shown to be higher [12,17].

IV administration can cause infusion-related reactions, also known as allergic-like reactions in the PTWG consensus definitions, which could lead to a higher total number of allergic events compared with intramuscular administration. However, in the NOPHO ALL2008 protocol, in which PEGasparaginase was administered IM, 1.6% of the patients (standard risk and intermediate risk) had an allergic-like reaction. Hence, although allergic-like reactions have previously been associated with IV administration only, they can occur after both IV or intramuscular administration of PEGasparaginase.

The frequency of allergic reactions and SI overall is low regardless of the protocol. The incidence of allergic reactions of those switching to *Erwinia* asparaginase was also quite low. Hence, most patients should not have to omit courses of asparaginase owing to hypersensitivity reactions, provided *Erwinia* asparaginase is available.

Treatment phase was associated with the risk for hypersensitivity. Univariate meta-regression analysis showed that the incidence of allergic reactions increased in the postinduction treatment phase. This might be owing to the PEGasparaginase-free intervals in most protocols. After exposure to PEGasparaginase, these intervals are a window of opportunity for complement activation and subsequent antibody production [36]. Therefore, more allergies occur after a break of several PEGasparaginase-free weeks or months [37]. This is supported by our finding that PEGasparaginase-free intervals were associated with the incidence of allergic reactions in both univariate and multivariate meta-regression analyses. Our data also showed that the duration of the gap was not associated with the risk of allergic reactions.

The observation that PEGasparaginase in postinduction was associated with an increased risk of an allergic reaction, regardless of whether asparaginase has already been given in induction therapy, is remarkable. It strongly suggests that, in addition to the number of asparaginase-free intervals, the treatment context also plays a decisive role, for instance, the use of concomitant steroids. Steroids are able to suppress the development of antibodies and the clinical symptoms of an allergy [38]. All protocols administered concomitant steroids during induction, but the use of steroids and PEGasparaginase during postinduction varied per protocol and dose. The use of steroids could therefore not be included in our risk analysis. Univariate analysis showed that HR group stratification was also a risk for allergic reactions. It might be that the higher number of PEGasparaginase-free intervals in HR protocols contributed to an increased risk for allergic reactions, because risk group stratification was no longer significantly associated with the incidence of allergic reactions in the multivariate analysis. This is supported by the observations of the BSPHO and UKALL who both observed in their HR protocols that allergic reactions occurred after each PEGasparaginase-free interval.

Allergies to and inactivation of PEGasparaginase usually occur at the first or second dose after an asparaginase-free interval [39,40], so the number of subsequent doses would be less relevant in case that asparaginase is continued without additional breaks. A limitation of our analysis was that we were unable to collect the number of allergies per dose number but only the total hypersensitivity rate during induction and postinduction. In addition, protocols with continuous dosing schedules that started PEGasparaginase treatment in induction were also not included in the analysis. The recently completed randomised study of the DCOG group comparing continuous administration without PEGasparaginase-free intervals from the start of therapy compared with a discontinuous schedule including PEGasparaginase-free intervals may clarify this point further.

In conclusion, our meta-analysis showed that the total incidence of allergic reactions to PEGasparaginase in contemporary paediatric ALL protocols was relatively low compared with those reported for use of native *E.coli* asparaginase and clearly associated with the dosing regimen. These results are important for planning future PEGasparaginase administration in frontline ALL protocols.

Author contributions

Leiah J Brigitha: conceptualisation, methodology, data collection, formal analysis and writing — original Draft. **Marta Fiocco:** methodology, formal analysis and writing — original draft, **Rob Pieters:** investigation and writing — original draft. **Birgitte K Albertsen:** investigation and writing — review and editing. **Gabriele Escherisch:** investigation and writing — review and editing, **Elixabet Lopez–Lopez:** investigation and writing — review and editing. **Veerle Mondelaers:** investigation and writing — review and editing. **Ajay Vora:** investigation and writing — review and editing, **Lynda Vrooman:** investigation and writing — review and editing. **Kjeld Schmiegelow:** writing — review and editing. **Inge M van der Sluis:** conceptualisation, methodology, investigation and writing — original draft.

Conflict of interest statement

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Appendix A. Supplementary data

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