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Prospective, real-time monitoring of pegylated *Escherichia coli* and *Erwinia* asparaginase therapy in childhood acute lymphoblastic leukaemia and non-Hodgkin lymphoma in Belgium

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Summary

Asparaginase (ASNase) is an important anti-leukaemic drug in the treatment of childhood acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL). A substantial proportion of patients develop hypersensitivity reactions with anti-ASNase neutralising antibodies, resulting in allergic reactions or silent inactivation (SI), and characterised by inactivation and rapid clearance of ASNase. We report results of a prospective, real-time therapeutic drug monitoring of pegylated *Escherichia coli* (PEG-)ASNase and *Erwinia* ASNase in children treated for ALL and NHL in Belgium. *Erwinia* ASNase was given as second-line after hypersensitivity to PEG-ASNase. In total, 286 children were enrolled in the PEG-ASNase cohort. Allergy was seen in 11·2% and SI in 5·2% of patients. Of the 42 patients treated with *Erwinia* ASNase, 7·1% experienced allergy and 2·4% SI. The median trough PEG-ASNase activity was high in all patients without hypersensitivity. After *Erwinia* administration significantly more day 3 samples had activities <100 IU/l (62·5% vs. 10% at day 2 (D2)). The median D2 activity was significantly higher for intramuscular (IM; 347 IU/l) than for intravenous *Erwinia* administrations (159 IU/l). This prospective, multicentre study shows that monitoring of ASNase activity during treatment of children with ALL and NHL is feasible and informative. Treatment with *Erwinia* ASNase warrants close monitoring and optimally adherence to a 2-day interval of IM administrations.

Keywords: acute lymphoblastic leukaemia, childhood, asparaginase, allergy, silent inactivation, therapeutic drug monitoring.

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For several decades, asparaginase (ASNase) has proven to be a key element in the multidrug chemotherapy regimen for acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) in children (Pui *et al.*, 2008; Hunger & Mullighan, 2015). The enzyme hydrolyses extracellular asparagine into aspartic acid and ammonia, leading to a depletion of asparagine in the systemic circulation. Lymphoblasts depend on extracellular asparagine for their growth and consequentially undergo rapid cell death in the absence of asparagine (Avramis & Panosyan, 2005; Avramis & Tiwari, 2006).

Currently, three ASNase formulations are used in paediatric ALL and NHL protocols. Native *Escherichia coli* ASNase and pegylated *E. coli* ASNase (PEG-ASNase) are derived from the bacteria *E. coli*, while the third formulation, *Erwinia* ASNase, is produced by the bacteria *Erwinia chrysanthemi*. All three preparations have different pharmacodynamics and immunogenic properties, therefore many contemporary protocols use PEG-ASNase as the preferred first-line ASNase due to its lower immunogenicity and longer half-life. *Erwinia* ASNase is often used in second-line after hypersensitivity reactions (HSR) to diminish the risk of cross-reactivity of antibodies (Pieters *et al.*, 2011; van der Sluis *et al.*, 2016).

A major concern in the treatment with ASNase is failure to achieve sufficient ASNase activity, due to immune responses such as allergic reactions and silent inactivation (SI), the latter referring to the inactivation of the ASNase product by neutralising antibodies in the absence of any clinical allergic reaction. In patients who develop HSR or neutralising antibodies to ASNase, increased ASNase clearance was demonstrated leading to subtherapeutic serum levels of the drug. Importantly, several studies described an inferior outcome in cases of suboptimal efficacy of this potent antileukaemic drug (Amylon *et al.*, 1999; Avramis *et al.*, 2002; Pession *et al.*, 2005; Moghrabi *et al.*, 2007). To overcome inefficient ASNase exposure these patients benefit from switching ASNase preparations, preferably to a formulation from another bacterial source (van der Sluis *et al.*, 2016; Salzer *et al.*, 2018).

Recently, it became clear that SI can only be detected by consecutive monitoring of ASNase levels during therapy. In the present study, we prospectively monitored ASNase in a uniformly treated, large Belgian cohort of newly diagnosed childhood ALL and NHL patients and provide further evidence on the importance of monitoring during treatment.

Patients and methods

Patients and study design

Children (1–18 years) with newly diagnosed ALL and precursor B or T lymphoblastic NHL treated in Belgian departments for paediatric haematology-oncology were enrolled in the real-time ASNase monitoring programme (Belgian registration number: B67020109345).

All patients were treated according to the treatment guidelines of the European Organisation for the Research and Treatment of Cancer Children's Leukaemia Group (EORTC-CLG) 58081 study, based on EORTC-protocol 58951 (De Moerloose *et al.*, 2010; Domenech *et al.*, 2014; Mondelaers *et al.*, 2017), and received PEG-ASNase in frontline (Table I; Data S1).

Patients with allergic reactions of Grade ≥ 2 to PEG-ASNase or SI were switched to *Erwinia* ASNase. One dose of PEG-ASNase 2500 IU/m^2 was replaced by six doses of 20 000 IU/m^2 *Erwinia* ASNase, given every 2–3 days. For average risk 2 (AR2)-patients with allergy or SI during maintenance, PEG-ASNase (1000 IU/m^2) was replaced by two doses of *Erwinia* ASNase (25 000 IU/m^2/dose) with a dose interval of 48 h (Table I). PEG-ASNase was given intravenously (IV), *Erwinia* ASNase IV or intramuscularly (IM) upon preference of the treating physicians.

Serum samples were collected at time points of routine blood examinations. For PEG-ASNase, samples were collected before (nadir) and 1 h after (peak) administration (D0), at day 7 ± 1 (D7) and day 14 ± 1 (D14). Some centres also collected day 3/4 (D3) and day 10/11 (D10) samples. Samples to determine trough level *Erwinia* ASNase activities were measured before and at 2 (D2) to 3 days (D3) after *Erwinia* ASNase (prior to next *Erwinia* ASNase).

Details on activity measurements are described in the Supporting Information.

Definition of allergy and SI

Clinical allergy to ASNase was defined according to Common Terminology Criteria for Adverse Events (CTCAE) version 4·03 criteria (Miller *et al.*, 1981) and graded according to the Ponte di Legno toxicity working group (PTWG) consensus (Schmiegelow *et al.*, 2016).

Inactivation of PEG-ASNase was defined as an activity level of <100 IU/l (at or) before D7 or below lower limit of quantification (<LLQ) (at or) before D14 after administration. Inactivation of *Erwinia* ASNase was present if the level was <LLQ at D2/D3 after *Erwinia* ASNase. SI was defined as inactivation in a patient without symptoms of allergy (Schmiegelow *et al.*, 2016; van der Sluis *et al.*, 2016). HSR included both clinical allergy and SI. Insufficient activity refers to an activity level of <100 IU/l, rapid clearance of PEG-ASNase is used if patients have levels of <100 IU/l at D14, but normal activities in the preceding (D1–D10) and subsequent measurements.

Statistical analysis

The data were analysed with the Statistical Package for the Social Sciences (SPSS®) software, version 20.0.0.1 (SPSS Inc., Chicago, IL, USA) and Sigmaplot version 13.0 for Windows 2013.

Table I. General design of the EORTC-CLG 58081 treatment guidelines.

Risk group	Induction	Consolidation	Interval	Re-induction	Maintenance
VLR	IA reduced *2 PEG-ASNase 2500 IU/m²*	IB	4xHDMTX	IIAreduced+IIB *1 PEG-ASNase 2500 IU/m²*	Oral 6-MP+MTX
AR1	IA *2 PEG-ASNase 2500 IU/m²*	IB	4xHDMTX	IIA+IIB *1 PEG-ASNase 2500 IU/m²*	Oral 6-MP+MTX, IT and pulses
AR2-B	IA augmented *2 PEG-ASNase 2500 IU/m²*	IB	4xHDMTX	IIA+IIB *1 PEG-ASNase 2500 IU/m²*	Oral 6-MP + MTX, HDMTX (*6 PEG-ASNase 1000 IU/m²*) and pulses
AR2-T	IA *2 PEG-ASNase 2500 IU/m²*	IB	4xHDMTX	IIA+IIB *1 PEG-ASNase 2500 IU/m²*	Oral 6-MP+MTX, HDMTX (*6 PEG-ASNase 1000 IU/m²*) without pulses

	Induction	1°Consolidation	2°Consolidation	1°Interval	1°Re-induction	2°Interval	2°Re-induction	Maintenance
VHR	IA+cyclo *2 PEG-ASNase 2500 IU/m²*	IB augmented *1 PEG-ASNase 2500 IU/m²*	VANDA *1 PEG-ASNase 2500 IU/m²*	3xHDMTX	IIA+IIB *1 PEG-ASNase 2500 IU/m²*	3xHDMTX	IIA+IIB *1 PEG-ASNase 2500 IU/m²*	Oral 6-MP+MTX

6-MP, 6-mercaptopurine; MTX, methotrexate, HDMTX, high-dose MTX (5 g/m²); VLR, very low risk; AR1 and AR2, average risk 1 and 2; VHR, very high risk.

Repeated measurements of the PEG-ASNase activity levels were evaluated using mixed models analysis of variance with an unstructured covariance matrix and time point as fixed effect. We assume that missing data are completely at random.

ASNase activity levels are presented as median and ranges, unless otherwise specified. Mean ASNase activity levels are used for the mixed models analysis.

Categorical values were compared using Fisher's exact tests. Differences in activity levels were tested using the Kruskal–Wallis ANOVA on ranks. A two-sided $P < 0{\cdot}05$ was considered statistically significant.

For detailed Methods see Data S1.

Results

Patient characteristics

Between January 2013 and November 2017, 286 consecutive patients (118 girls and 168 boys) with newly diagnosed ALL ($n = 260$) and precursor B or T lymphoblastic NHL ($n = 26$) were enrolled in the prospective real-time ASNase monitoring programme and a total of 3777 samples from the patients were analysed. In the same period, parents of 12 patients declined participation. Demographic characteristics of the PEG-ASNase and *Erwinia* ASNase groups are shown in Table II. Clinical allergic reactions were seen in 32 (11·2%) of the patients treated with PEG-ASNase. The majority were classified as CTCAE 4·03 Grade 2 (28·1%) or 3 (65·6%) and 68·8% was severe according to the PTWG criteria. SI was seen in 15 (5·2%) patients treated with PEG-ASNase (Figs 1 and 2A). Most allergies occurred after the second (28·1%) or third administration (59·4%). SI was seen after the second (33·3%) or the third administration (26·7%) and in maintenance therapy (40%) (Fig 1). Patients were more at risk for HSR after an ASNase-free period. Almost all patients who had a hypersensitivity reaction at the second dose had a delay for the administration of this dose due to clinical problems such as infections. The median interval between the two doses was 22·5 days in the patients with HSR compared to 14 days in patients without HSR ($P < 0{\cdot}001$).

In all, 42 of the hypersensitive patients were switched to *Erwinia* ASNase. Three (7·1%) experienced a clinical allergic reaction, and one (2·4%) a SI on *Erwinia* ASNase (Figs 1 and 2B). All three allergic reactions were CTCAE 4·03 Grade 3 or severe according to the PTWG criteria.

PEG-ASNase activity

After exclusion of trough samples, adequate PEG-ASNase activity of >100 IU/l was obtained in 97·7% of the samples from 286 patients treated with PEG-ASNase 2500 IU/m² and in >90% of the samples from 42 AR2-patients treated with PEG-ASNase 1000 IU/m² during maintenance therapy. The median (range) ASNase activity after the first PEG-ASNase

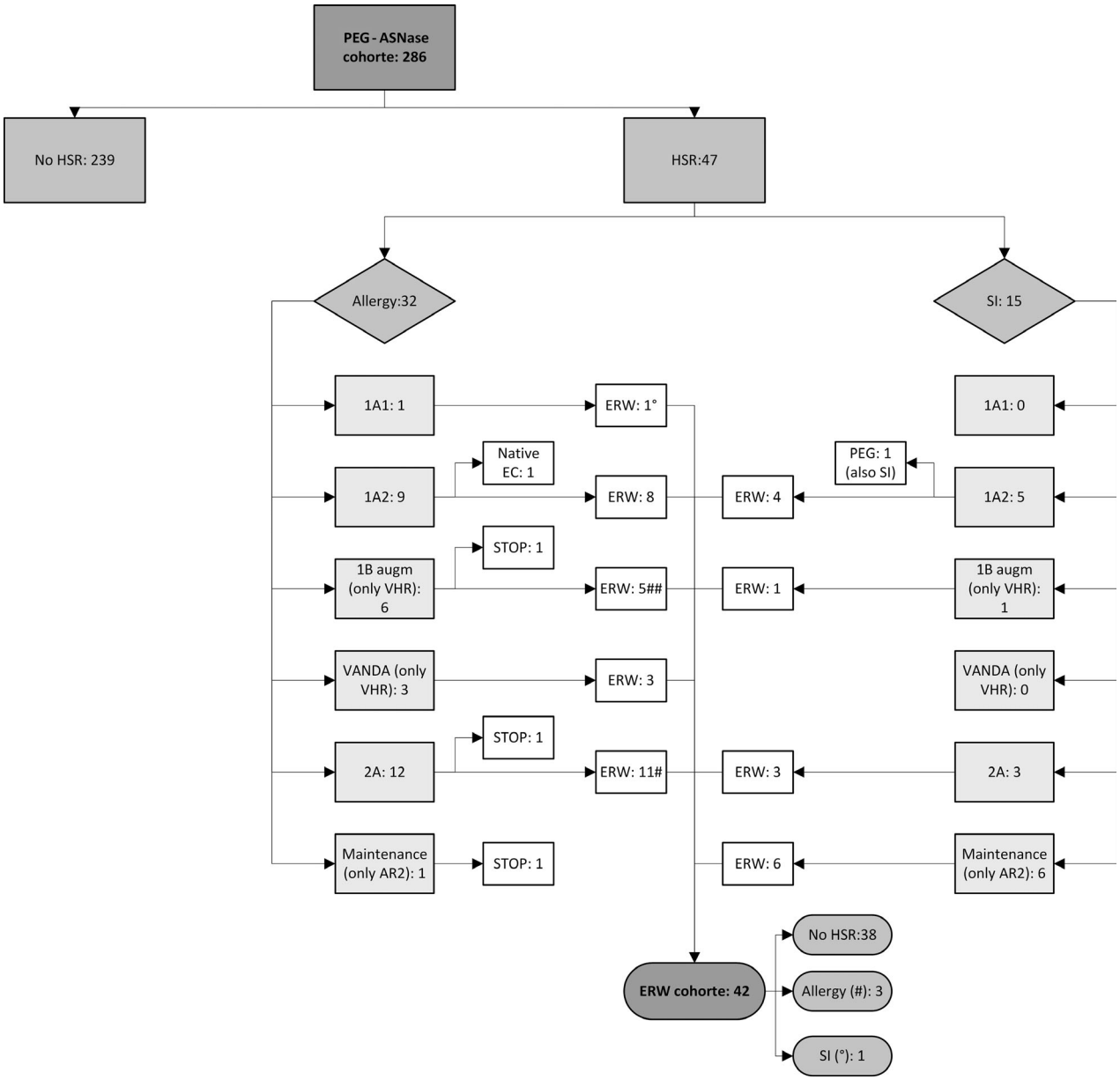

Fig 1. Consort statement for the PEG-ASNase and *Erwinia* ASNase cohort. HSR, hypersensitivity reaction; SI, silent inactivation; PEG, PEG-ASNase; ERW, *Erwinia* ASNase; Native EC, native *E. coli* ASNase; STOP, discontinuation of ASNase therapy; VHR, very high risk; AR2, average risk 2. One patient had an allergic reaction to PEG-ASNase and a SI to *Erwinia* ASNase. [#]Three patients had an allergic reaction to PEG-ASNase and to *Erwinia* ASNase.

administration was 1254 (704–2027) IU/l (peak sample), 921 (147–1727) IU/l at D7 and median trough level at D14 was 574 (<5–1807) IU/l. After the second administration in induction patients ASNase activity levels were higher: median (range) 2091 (<5–5208) IU/l (peak), 1181 (<5–2107) IU/l at D7 and 666 (<5–1151) IU/l at D14. After PEG-ASNase in re-induction, the median (range) ASNase activity was 1916 (<5–13 255) IU/l (peak), 1358 (<5–5621) IU/l at D7 and 802 (<5– 3346) IU/l at D14 (Table III; Table SI). After exclusion of patients with HSR, we measured significantly higher mean trough levels at D14 after the second administration in induction and after the administration in re-induction (Fig 3). In induction no statistically significant different activity levels were observed according to EORTC-risk group or corticosteroid (prednisolone/dexamethasone) used (data not shown).

Evolution of patients with SI or allergy

Table SIII outlines the evolution of ASNase activity during treatment. Target ASNase activity of ≥100 IU/l was achieved in all samples until D10/11 after the first PEG-ASNase in

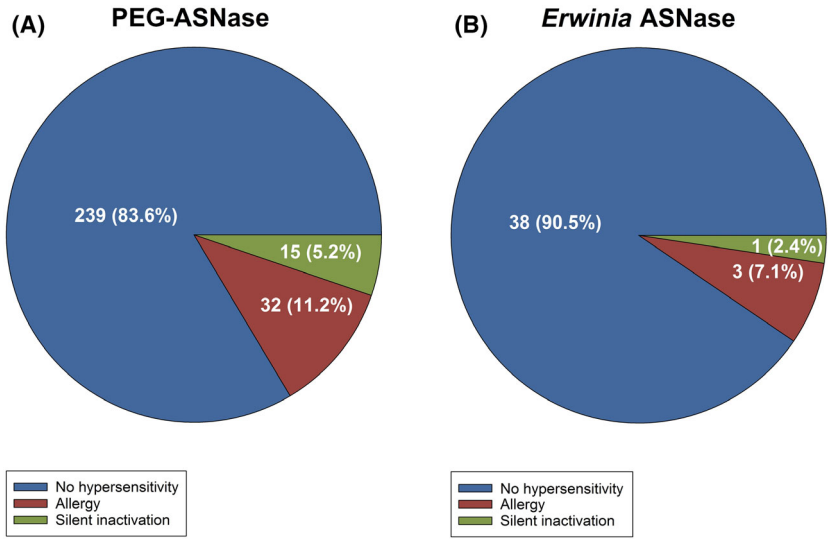

Fig 2. Frequency of hypersensitivity reactions (HSR) after administration of PEG-ASNase (A, $N = 286$) or *Erwinia* ASNase (B, $N = 42$). [Colour figure can be viewed at wileyonlinelibrary.com]

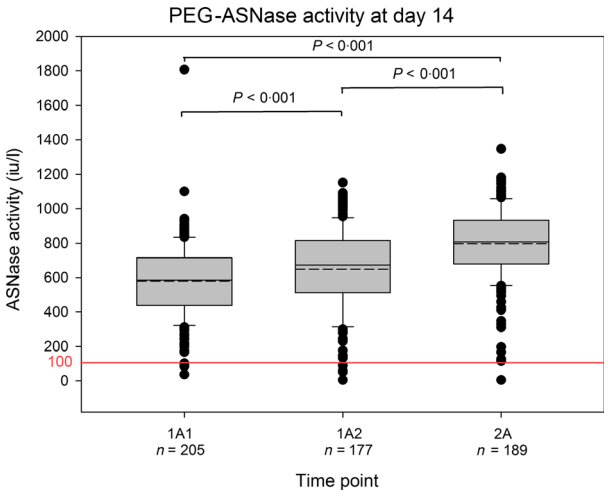

Fig 3. ASNase activity at D14 after PEG-ASNase 2500 IU/m^2 in patients without HSR ($n = 239$). 1A1: first administration in induction, 1A2: second administration in induction; 2A: administration in late intensification; D14: trough ASNase activity 14 days after the administration. [Colour figure can be viewed at wileyonlinelibrary.com]

induction. The activity dropped under the level of sufficient ASNase activity at D14 in six of 243 patients and in two of them under the LLQ, indicating a possible SI. In one of those two patients, SI was confirmed after the second administration in induction, i.e. activity levels <5 IU/l at all time-points. The other patient with SI at D14 experienced an allergic reaction at the second dose, as well as one of the remaining four patients with ASNase activity <100 IU/l at D14. Seven additional patients had an allergic reaction at the second administration, three of which with low activity levels, and four with peak activity levels >100 IU/l. The ASNase activity in one of the latter patients declined rapidly under the LLQ at D7 and D14 after the second administration. One of the allergic patients with adequate peak activity was subsequently treated with native *E. coli* ASNase and had ASNase activity levels >100 IU/l for the remainder of the treatment.

After the second dose in induction, 11 patients had an insufficient ASNase activity at D14, with six patients having activity levels <5 IU/l. Two patients with a D14 sample <LLQ and one with a D14 sample of 58 IU/l were not switched to *Erwinia* and received a third PEG-dose in late intensification and SI was confirmed in each of them. One had a D7 activity of 102 IU/l after the third dose and at D14 was < LLQ. The second one had a high peak (2012 IU/l) and D3 (1546 IU/l) after the third dose, but rapidly declined in the D7 and D14 samples to < LLQ. The third patient with a D14 activity of 58 IU/l after the second dose, had the same pattern after the third administration (peak: 2187 IU/l; D7 and D10 < LLQ).

Another interesting case was an AR2-patient, in whom the second PEG-dose in induction was postponed to the delayed intensification due to a symptomatic sinus sigmoideus thrombosis. This patient had D10 and D14 activities <LLQ after the first administration in delayed intensification, but normal activities after the second (catch up) dose in delayed intensification (D11: 880 IU/l; D14: 698 IU/l); however, he presented SI after the subsequent administration during maintenance (peak: 625 IU/l; D7: 65 IU/l; D14: <5 IU/l).

During maintenance, some of the AR2-patients had normal activities at D7 of all six blocks, but D14 activities were <5 IU/l. Probably the 1000 IU/m^2 dose was too low due to rapid clearance.

Erwinia *ASNase activity*

Erwinia ASNase was given as a second-line after allergic reaction or SI to previous PEG-ASNase administrations. The median (range) activity 2 days after administration was 321 (14–1195) IU/l and significantly lower 3 days after administration, at 76 (<5–529) IU/l ($P < 0·001$) (Fig 4A). In 11/33 patients (33%) or 23/238 samples (10%), D2-ASNase activity was <100 IU/l. None of the samples taken at D2 showed total inactivation of *Erwinia* ASNase. In contrast, 15/

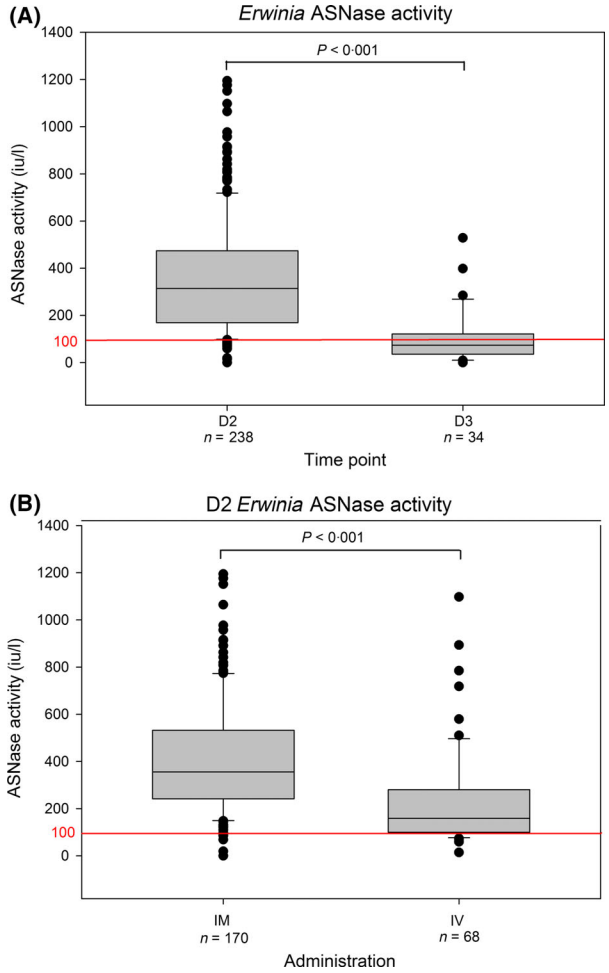

Fig 4. ASNase activity after *Erwinia* ASNase 20 000 IU/m^2 according to time point after administration (A) and according to the route of administration on D2 (B). D2: ASNase activity 2 days after the *Erwinia* administration; D3: ASNase activity 3 days after the *Erwinia* administration. IM, intramuscularly; IV, intravenously. [Colour figure can be viewed at wileyonlinelibrary.com]

20 patients (75%) or 22/32 samples (65%) demonstrated ASNase activity <100 IU/l at D3.

Interestingly, the median (range) D2 activity was significantly higher for IM administrations, at 347 (19–1195) IU/l, than for IV administrations, at 159 (14–1097) IU/l). A larger proportion of samples (17/68) and patients (six of 11) in the IV group did not reach sufficient activity (>100 IU/l) at D2, compared to six of 165 samples and five of 24 patients in the IM group (Fig 4B and Table IV).

More than two-thirds of the IV- and IM-treated patients showed D3 activities of <100 IU/l. One patient in the IM group had activities <LLQ in all samples, so called Tables IV and SII.

In all, 54% of the IV-treated patients had an activity exceeding the threshold of 100 IU/l in at least 75% of all samples, compared to 86% of patients with IM administrations. After limitation to D2 samples, 61·5% of IV patients and 90·5% of IM patients achieved an activity >100 IU/l in ≥75 % of the samples (Table SII).

Discussion

Patients with low ASNase activity during treatment of ALL have been reported to have increased risk of relapse (Silverman *et al*., 2001; Vrooman *et al*., 2013). Hence, it is important to optimise ASNase therapy by real-time monitoring of ASNase activity and to react promptly upon SI and insufficient activities.

We demonstrated that an adequate PEG-ASNase activity was reached in 97·7% of the samples from 286 patients after PEG-ASNase 2500 IU/m^2 and in >90% of the samples after PEG-ASNase 1000 IU/m^2 during maintenance therapy. This is in line with other studies (Appel *et al*., 2008; Henriksen *et al*., 2017). By contrast, studies with native *E. coli* ASNase in induction followed by PEG-ASNase in late intensification or PEG-ASNase in the treatment for relapsed ALL report higher incidences (20–30%) of patients who did not reach levels of >100 IU/l (Schrey *et al*., 2010; Tong *et al*., 2014).

Consistent with other monitoring projects, we found high median trough levels with an increase after subsequent PEG-ASNase administrations. One study described an increase in mean trough levels after the first two administrations, reaching a steady state from week 4 until week 26. Remarkably they observed a significant increase in activity levels after the second last dose ($P = 0·01$), although the other treatment elements did not change (Tong *et al*., 2014). Similarly, in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) 2008 study, ASNase activity initially increased and reached steady state trough levels after the fifth PEG-ASNase (Henriksen *et al*., 2017). More research should be done to investigate the role of concomitant administered drugs and alterations in ASNase clearance throughout the course of the treatment and their impact on ASNase related side-effects.

Interestingly, one might argue that trough levels after PEG-ASNase 2500 IU/m^2 are too high and might require dose reductions. However, it should be considered that PEG-ASNase penetrates poorly in the cerebrospinal fluid (CSF), and thus a dose-dependent reduction of CSF asparagine levels is reached, probably due to homeostasis with plasma asparagine concentrations. Appel *et al*. (2003) demonstrated insufficient depletion of asparagine in the CSF after IV PEG-ASNase 1000 IU/m^2, despite complete depletion of plasma asparagine. This is further illustrated by Avramis *et al*. (2002) who reported higher depletion of CSF asparagine levels after IV PEG-ASNase 2500 IU/m^2 compared to the same dose IM (<0·2 μmol/l vs. <0·6 μmol/l).

During the course of the PEG-ASNase treatment, we observed allergic reactions in 11·2% and SI in 5·2% of the 286 patients. If only allergic reactions of Grade ≥ 3 were considered, the rate was 7·7%. Similar frequencies of allergic reactions were reported by August *et al*. (2013) after IV (12·5%) or IM (11·1%) PEG-ASNase. Pidaparti and Bostrom (2012) described allergy incidences of 9% in patients receiving IM PEG-ASNase and of 36% in a very small group of 11

Table II. Demographic characteristics of the PEG-ASNase cohort and *Erwinia* ASNase cohort.

Characteristic	PEG-ASNase cohort	*Erwinia* ASNase cohort
Total, *n*		
Patients	286	42
Samples	3356	421
Age at diagnosis, years, median (range)	5·1 (1·1–18.3)	4·7 (1·1–16.1)
1 to 2, *n*	17	2
>2 to 6, *n*	150	22
>6 to 12, *n*	69	8
>12 to 18, *n*	50	10
Gender, *n*		
Female	118	12
Male	168	30
Malignancy, *n*		
ALL	260	38
NHL	26	4
Immunophenotype, *n*		
B cell	209	29
T cell	74	12
ALAL/biphenotypic	3	1
EORTC risk group, *n*		
VLR	29	4
AR1	138	13
AR2	69	15
VHR	50	10
Allergy (PTWG criteria), *n*	32	3
Mild	1	0
Severe	31	3
Allergy (CTCAE 4·03 criteria), *n*	32	3
Grade 1	1	0
Grade 2	9	0
Grade 3	21	3
Grade 4	1	0
Silent inactivation, *n*	15	1

ALL, acute lymphoblastic leukaemia; NHL, non-Hodgkin lymphoma; ALAL, acute leukaemia of ambiguous lineage; VLR, very low risk; AR1 and AR2, average risk 1 and 2; VHR, very high risk; PTWG, Ponte di Legno Toxicity Working Group.

IV-treated patients. In contrast, analysis of 54 280 PEG-ASNase doses administered to 16 534 patients in six Children's Oncology Group (COG) leukaemia trials (2003–2015) revealed that Grade $\geq$ 3 HSR to PEG-ASNase 2500 IU/m^2 occurred less frequently with IV infusion than IM injections (3·2% vs. 5·4%, $P < 0·0001$). After limiting the analysis to second and third doses, where, as in our present study, most of the Grade $\geq$ 3 allergic reactions occurred, patients were less likely to have an allergic reaction after IV administrations (5·0% vs. 10·1%, $P < 0·0001$) (Burke *et al.*, 2018). Greater allergy (22%) and SI rates (8%) were seen when patients were treated with PEG-ASNase after an induction with native *E. coli* ASNase (Tong *et al.*, 2014).

Longitudinal monitoring of ASNase activity enables predicting of HSR. In several patients, we found that HSR was preceded by a rapid clearance or inactivation of ASNase after the previous dose. This phenomenon underscores the value of additional sampling allowing a quick detection of inactivity and a prompt switch to an alternative preparation.

Patients with allergic reactions on or SI to PEG-ASNase can be switched successfully to *Erwinia* ASNase (Pieters *et al.*, 2011). In our present study, 42 patients were switched to *Erwinia* ASNase. In contrast to PEG-ASNase lower median trough activity levels were observed after *Erwinia* ASNase administration, namely 321 IU/l at D2 and 76 IU/l at D3, with significantly more D3 samples <100 IU/l (62·5% vs. 10% at D2, $P < 0·001$). This is in agreement with other studies. Tong *et al.* (2014) found median trough activity levels of 183 IU/l at D2 and 93 IU/l at D3, with activity levels of <100 IU/l in 67% of the D3 samples compared to 0% of the D2 samples after *Erwinia* ASNase 20 000 IU/m^2 IV three-times per week. In 33% of the patients asparagine was not completely depleted during *Erwinia* ASNase therapy. In their study with *Erwinia* ASNase 25 000 IU/m^2 IV three-times per week Vrooman *et al.* (2016) detected mean ASNase activity levels of 320 IU/l at D2 and 89 IU/l at D3, and activity levels of <100 IU/l in 27% of patients 48 h post-dose and in 57% 72 h post-dose. In the COG AALL07P2 trial, 2 weeks of *Erwinia* ASNase at a dose of 25 000 IU/m^2 IM three-times weekly led to higher trough activity levels across all treatment cycles of 645 IU/l at 48 h and 248 IU/l at 72 h post-dose, and activity levels of <100 IU/l in 7% of patients at D2 and in 22% at D3 (Salzer *et al.*, 2013). The IM administrations in our present study might be responsible for the higher trough levels compared to the studies of Tong *et al.* (2014) and Vrooman *et al.* (2016), where IV administrations were given. The influence of the route of administrations for *Erwinia* ASNase is not completely clear in literature. Some studies did not find differences in activity after IV or IM administrations (Rizzari *et al.*, 2000; Albertsen *et al.*, 2001), whereas others describe a difference. In the ALL-Berlin-Frankfurt-Münster (BFM) 2000 protocol higher median activity was observed after IM administrations of 10 000 IU/m^2 *Erwinia* ASNase compared to IV, with a median activity of 151 IU/l and 115 IU/l, respectively ($P = 0·3$); and fewer samples <100 IU/l (IM:15% vs. IV:45%) at D2 (Schrey *et al.*, 2010). We confirmed the influence of the route of administration on trough levels. The median D2 activity was significantly higher for IM *Erwinia* administrations (347 IU/l) than for IV administrations (159 IU/l). In all, 62% of IV-treated patients and 90·5% of IM-treated patients achieved an activity of >100 IU/l in ≥75% of the D2 samples. The large inter- and intra-patient variation of *Erwinia* ASNase activity in our present study highlights the importance of therapeutic drug monitoring. Three patients (7·1%) developed allergy, and one (2·4%) SI. Patients with HSR after *Erwinia* ASNase had to stop ASNase therapy due to the fact that there exists no valid switching option for patients already having a history of hypersensitivity to PEG-ASNase.

Table III. ASNase activity after 2500 IU/m^2 PEG-ASNase intravenously in induction and delayed intensification for all patients.

Days after administration	Number of samples/patients	ASNase activity, IU/l, median (range)	ASNase activity ≤100 IU/l		ASNase activity ≤5 IU/l	
			Patients, *n* (%)	Samples, *n* (%)	Patients, *n* (%)	Samples, *n* (%)
1°dose Induction	(VLR, AR1, AR2, VHR)					
0 (peak)	224/224	1469 (525–2722)	0 (0)	0 (0)	0 (0)	0 (0)
3/4	63/63	1254 (704–2027)	0 (0)	0 (0)	0 (0)	0 (0)
7 (6–8)	227/225	921 (147–1727)	0 (0)	0 (0)	0 (0)	0 (0)
10/11	57/57	760 (211–1290)	0 (0)	0 (0)	0 (0)	0 (0)
14 (13–15)	248/243	574 (5–1807)	6 (2·5)	6 (2·4)	2 (0·8)	2 (0·8)
2°dose Induction	(VLR, AR1, AR2, VHR)					
0 (peak)	221/220	2091 (<5–5208)	4 (1·8)	4 (1·8)	2 (0·9)	2 (0·9)
3/4	50/50	1705 (<5–3260)	1 (0·2)	1 (0·2)	1 (0·2)	1 (0·2)
7 (6–8)	194/194	1181 (<5–2107)	2 (1)	2 (1)	2 (1)	2 (1)
10/11	52/52	959 (<5–1416)	2 (1·9)	2 (1·9)	2 (1·9)	2 (1·9)
14 (13–15)	203/201	666 (<5–1151)	11 (5·5)	11 (5·4)	6 (3)	6 (3)
Delayed intensification	(VLR, AR1, AR2, VHR)					
0 (peak)	220/203	1916 (<5–13 255)	8 (3·9)	8 (3·6)	4 (2)	4 (1·8)
3/4	8/8	1598 (1253–2040)	0 (0)	0 (0)	0 (0)	0 (0)
7 (6–8)	142/127	1358 (<5–5621)	4 (3·1)	4 (2·8)	4 (3·1)	4(2·8)
10/11	11/9	462 (<5–1169)	4 (44)	4 (36)	4 (44)	4 (36)
14 (13–15)	215/202	802 (<5–3346)	6 (3)	6 (2·8)	4 (2)	4(1·9)

Table IV. ASNase activity after 20 000 IU/m^2 *Erwinia* ASNase intravenously or intramuscularly.

Days after administration	Route of administration	Number of samples/patients	ASNase activity, IU/l, median (range)	ASNase activity ≤100 IU/l		ASNase activity ≤5 IU/l	
				Patients, *n* (%)	Samples, *n* (%)	Patients, *n* (%)	Samples, *n* (%)
2	IV+IM	238/33	321 (14–1195)	11 (33)	23 (10)	0 (0)	0 (0)
2	IV	68/11	159 (14–1097)	6 (54·5)	17 (25)	0 (0)	0 (0)
2	IM	170/22	347 (19–1195)	5 (23)	8(5)	1 (0·5)	2 (0·01)
3	IV+IM	34/20	76 (<5–529)	15 (75)	22 (65)	1 (5)	2 (6)
3	IV	8/6	88 (56–398)	4 (67)	5 (62·5)	0 (0)	0 (0)
3	IM	26/14	72 (<5–529)	11 (79)	15 (62·5)	1 (8)	2 (8)

In conclusion, our present study shows that a national therapeutic drug monitoring (TDM) programme with real-time measurements of ASNase activity is feasible and essential in ensuring optimal ASNase therapy. ASNase activity monitoring is indispensable to identify patients with SI, patients at risk for subsequent HSR and to clarify doubtful allergic reactions. In the absence of specific complications related to PEG-ASNase toxicity (pancreatitis, thrombosis), it is beneficial to administer the second dose on day 14 to avoid HSR. Our *Erwinia* ASNase activity monitoring programme emphasises the relevance for strict monitoring of activity levels and adherence to a 2-day interval of IM administrations. The success of this programme leads to incorporation of ASNase activity monitoring as a standard of care in our future childhood ALL protocols in Belgium. The purpose of future monitoring projects will be to unravel the influence of high activity levels on toxicity and to investigate the optimal trough levels to achieve sufficient ASNase activity balancing toxicity and efficacy.

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Conflict of interest

Veerle Mondelaershas participated in advisory boards for Jazz Pharmaceuticals and received travel expenses and speaker's fees from Jazz Pharmaceuticals and Shire. Tim Lammens and Barbara De Moerloose received travel expenses from Jazz Pharmaceuticals. The authors declare no competing financial interests.

Author contribution

Veerle Mondelaers, Tim Lammens, Barbara De Moerloose developed and coordinated the study and performed data interpretation; Veerle Mondelaers analysed the data, designed the figures and tables and wrote the manuscript; Charlotte Verbeke and Susanne Schmidt helped with data cleaning and analysis; Katrien Vandemeulebroecke performed the asparaginase activity measurements; Tim Lammens supervised the laboratory technicians; Veerle Mondelaers, Alina Ferster, Anne Uyttebroeck, Bénédicte Brichard, Jutte van der Werff ten Bosch, Koenraad Norga, Nadine Francotte, Caroline Piette, Yves Benoit and Barbara De Moerloose included patients in the trial and provided clinical data. All authors participated in study supervision and critically reviewed the manuscript.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supplementary methods.

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