

Desensitization protocol should not be used in acute lymphoblastic leukemia patients with silent inactivation of PEGasparaginase

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Supplemental file accompanying the paper:

Should we use a desensitization protocol in acute lymphoblastic leukemia patients with silent inactivation of PEGasparaginase?

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Supplemental methods

Patients and DCOG ALL-10 protocol

All patients received eight doses of native *E.coli* asparaginase (5,000 IU/m², every three days) in induction. In the MR group, PEGasparaginase (2,500 IU/m², every other week) was given during the first 30 weeks of the intensification/reinduction course. In case of an allergy to PEGasparaginase, the patient was switched to *Erwinia* asparaginase (20,000 IU/m², per dose) 2-3 times per week to complete 30 weeks of *Erwinia* asparaginase therapy.(1) All asparaginase preparations were administered intravenously.

In the starting phase of our drug monitoring program real-time asparaginase measurements were not available, and patients with silent inactivation were not recognized in time and continued with PEGasparaginase.

Study design and definitions

Serum asparaginase activity trough levels and serum asparaginase antibodies were measured at 15 and 13 different time points for PEGasparaginase and *Erwinia* asparaginase analysis, respectively.

Allergy was graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Silent inactivation was defined as serum PEGasparaginase activity level below 100 U/L at day 7±1 or below 20 U/L at day 14±1 after PEGasparaginase infusion in absence of a clinical allergy. Silent inactivation of *Erwinia*

asparaginase was defined as serum asparaginase activity level below 20 U/L at day 2 after administration of *Erwinia* asparaginase in absence of a clinical allergy.

Asparaginase activity levels and asparaginase antibodies

Serum asparaginase activity levels were processed and assessed as described earlier.(2, 3) Serum antibodies against native *E.coli* asparaginase (Asparaginase medac®) (Coli-AAA), against PEGasparaginase (Oncaspar®) (PEG-AAA), and against *Erwinia* asparaginase (*Erwinia*-AAA) were recently described.(4)

Statistical analysis

The data were analyzed with the software packages SPSS for Windows version 20.0.0.1 (SPSS, Chicago, IL, USA) and GraphPad Prism version 5.01 (GraphPad Prism Inc., San Diego, CA, USA). The Coli-AAA, PEG-AAA, and PEGasparaginase activity levels of five patients with silent inactivation of PEGasparaginase were analyzed with individual curves. The *Erwinia*-AAA and *Erwinia* asparaginase activity levels of all patients without an allergy to *Erwinia* asparaginase were also analyzed with individual curves. Changes over time of PEGasparaginase activity levels were evaluated using mixed models analysis of variance (ANOVA). The mean values were estimated by backtransforming the mean of log-values. PEGasparaginase activity levels are presented as mean \pm SEM.

Supplemental results

Overview of the patients in trial

Eighty-nine patients were enrolled to monitor PEGasparaginase courses. Twenty patients had clinical allergic reactions to PEGasparaginase. Seven patients showed silent inactivation of PEGasparaginase of which five patients continued with PEGasparaginase, because real-time asparaginase measurements were not available at that moment. The others were switched to *Erwinia* asparaginase. The remaining 62 patients had no allergy to and silent inactivation of PEGasparaginase.

In another cohort, fifty-nine patients were enrolled to monitor *Erwinia* asparaginase courses. Only two patients (3%) had an allergy to *Erwinia* asparaginase, none of patients had silent inactivation of *Erwinia* asparaginase.

Coli-AAA and PEG-AAA

For comparison, Supplemental Figure 1 shows the Coli-AAA, PEG-AAA and PEGasparaginase activity levels of patients without an allergy and without silent inactivation. 29% of the patients were positive for Coli-AAA before the start of intensification. Also in this group of patients, the Coli-AAA gradually decreased and after five PEGasparaginase infusions, the Coli-AAA levels were below the cut-off. PEG-AAA were absent at the start, gradually increased but remained below the cut-off level. After four doses of PEGasparaginase, the PEG-AAA levels gradually decreased to absent baseline values during therapy. Both asparaginase

antibodies did not affect the PEGasparaginase activity levels which remained stable after two PEGasparaginase infusions until end of therapy, but it should be noted that patients with silent inactivation were removed from this group as well as the patients with clinical allergy (Supplemental Figure 1-C).

***Erwinia*-AAA**

59 patients were treated to complete 30 weeks of exposure with *Erwinia* asparaginase 2-3 times per week, after having experienced allergy to or silent inactivation of PEGasparaginase. The two allergic patients to *Erwinia* asparaginase were excluded from further analysis, hence 57 remaining patients on *Erwinia* asparaginase were available for analysis. In Supplemental Figure 2, the *Erwinia*-AAA and *Erwinia* asparaginase activity levels of patients without an allergy to *Erwinia* asparaginase are shown as individual curves. None of these patients were positive for *Erwinia*-AAA before the start of *Erwinia* asparaginase therapy. Four patients developed *Erwinia*-AAA in the first two weeks of therapy. In total 50% of the non-allergic patients developed *Erwinia*-AAA and again gradually decreased to absent baseline values for nearly all patients. These were mainly present at days 37, 65 and 92 of therapy. The *Erwinia*-AAA did not affect *Erwinia* asparaginase activity levels until end of therapy, but again it should be noted that the two patients with a clinical allergy to *Erwinia* asparaginase were removed from this group (Supplemental Figure 2-B).

The two patients with silent inactivation of PEGasparaginase who were switched to *Erwinia* asparaginase based upon real-time asparaginase measurements are shown in Supplemental Figure 2-C1 and 2-C2. They had therapeutic asparaginase trough levels immediately after the switch and the *Erwinia*-AAA remained below the cut-off of *Erwinia*-AAA positivity.

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Legends to Supplemental Figures

Supplemental Figure 1: Coli-AAA levels (A), PEG-AAA levels (B), and PEGasparaginase activity levels (C) of children without an allergy and without silent inactivation of PEGasparaginase. (n=62)

Panels A and B; mean \pm SEM. Dotted horizontal lines; above the cut-offs: Coli-AAA and PEG-AAA positive.

Panel C; mean \pm SEM.

Supplemental Figure 2: individual *Erwinia*-AAA levels (A) and individual *Erwinia* asparaginase activity levels (B) of children without an allergy and without silent inactivation of *Erwinia* asparaginase. (n=57)

(C1-2) individual *Erwinia*-AAA levels and individual *Erwinia* asparaginase activity levels of the two patients with silent inactivation of PEGasparaginase who were switched to *Erwinia* asparaginase based upon real-time asparaginase measurements.

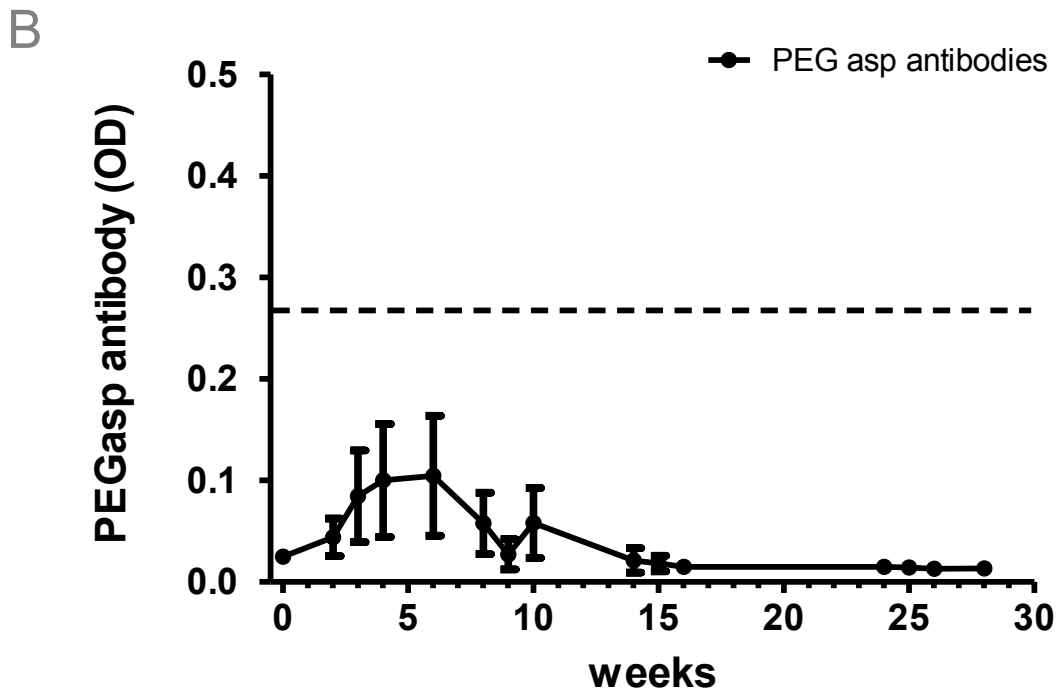
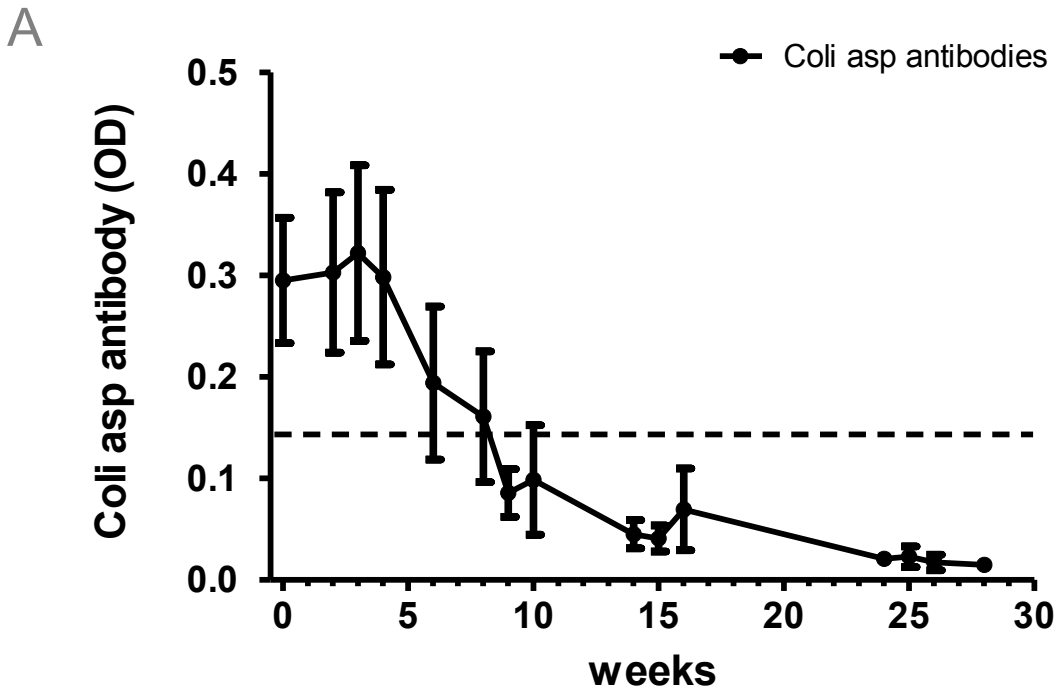
Panel A; dotted horizontal line; above the cut-off: *Erwinia*-AAA positive. The lowest line indicated by an arrow and labelled '20x' represents 20 different *Erwinia* asparaginase patients without *Erwinia*-AAA.

Panel B; mean \pm SEM.

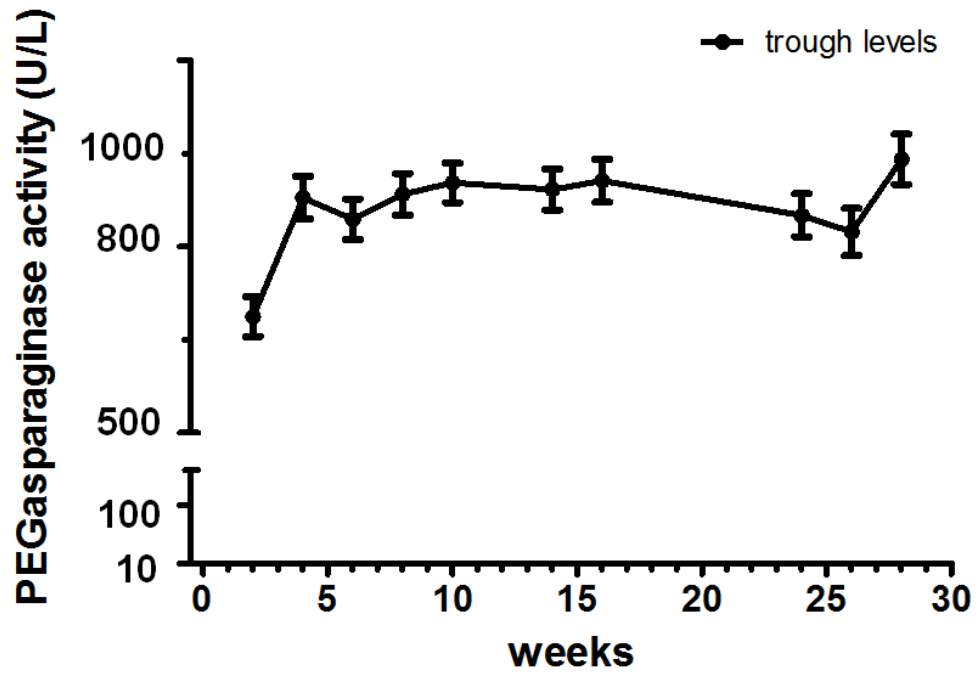
Panels C1 and C2; Upper horizontal dotted line; *Erwinia* asparaginase activity level of 100 U/L which is associated with complete asparagine depletion (lower level of quantification of 0.2 μ M).

Lower horizontal dotted line; above the cut-off: *Erwinia*-AAA positive.

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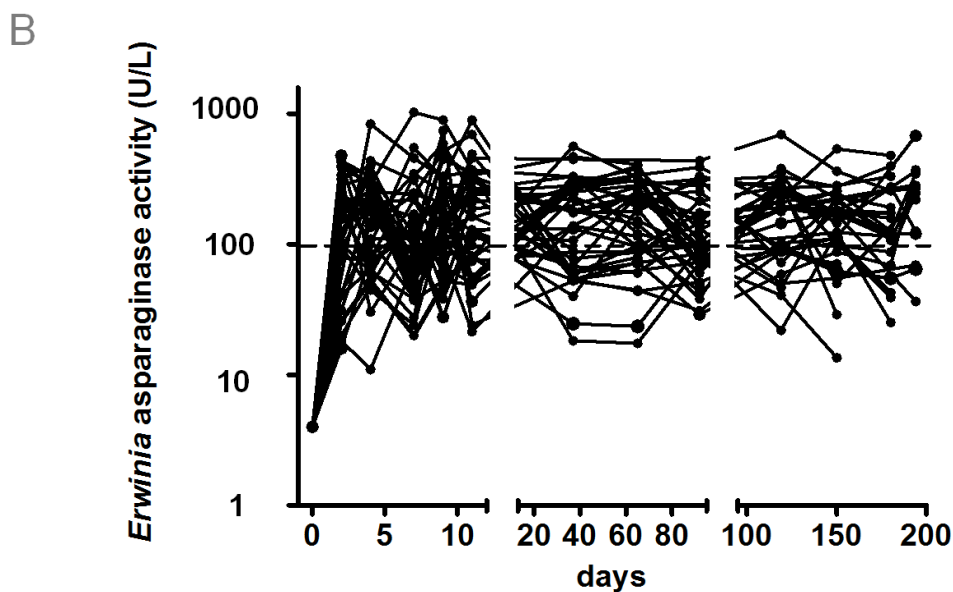
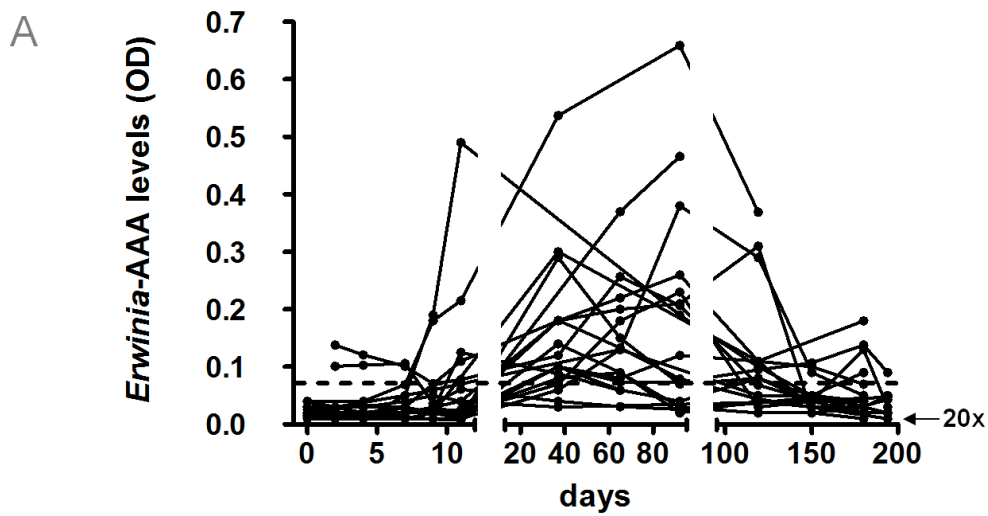


C

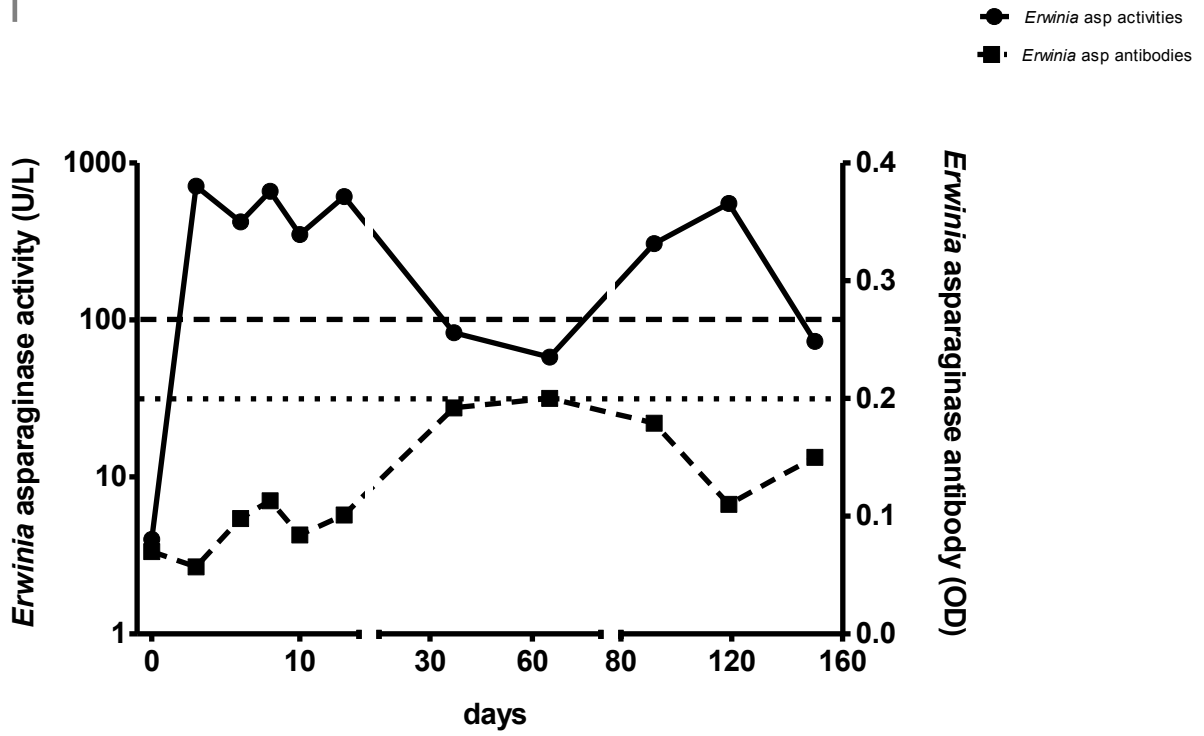


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(C1-2) individual *Erwinia*-AAA levels and individual *Erwinia* asparaginase activity levels of the two patients with silent inactivation of PEGasparaginase who were switched to *Erwinia* asparaginase based upon real-time asparaginase measurements.



C1



C2

