Pathol. Oncol. Res. (2018) 24:345–352
DOI 10.1007/s12253-017-0236-0

ORIGINAL ARTICLE

Expression of Coagulation Factor XIII Subunit A Correlates with Outcome in Childhood Acute Lymphoblastic Leukemia

Bettina Kárai[1] · Zsuzsanna Hevessy[1] · Eszter Szánthó[1] · László Csáthy[1] · Anikó Ujfalusi[1] · Katalin Gyurina[2] · István Szegedi[2] · János Kappelmayer[1] · Csongor Kiss[2]

Received: 15 August 2016 / Accepted: 23 April 2017 / Published online: 18 May 2017

Abstract Previously we identified B-cell lineage leukemic lymphoblasts as a new expression site for subunit A of blood coagulation factor XIII (FXIII-A). On the basis of FXIII-A expression, various subgroups of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) can be identified. Fifty-five children with BCP-ALL were included in the study. Bone marrow samples were obtained by aspiration and the presence of FXIII-A was detected by flow cytometry. G-banding and fluorescent in situ hybridization was performed according to standard procedures. The 10-year event-free survival (EFS) and overall survival (OS) rate of FXIII-A-positive and FXIII-A-negative patients showed significant differences (EFS: 84% vs. 61%, respectively; $p = 0.031$; OS: 89% vs. 61%; $p = 0.008$). Of all the parameters examined, there was correlation only between FXIII-A expression and 'B-other' genetic subgroup. Further multivariate Cox regression analysis of FXIII-subtype and genetic group or 'B-other' subgroup identified the FXIII-A negative characteristic as an independent factor associated with poor outcome in BCP-ALL. We found an excellent correlation between long-term survival and the FXIII-A-positive phenotype of BCP lymphoblasts at presentation. The results presented seem to be convincing enough to suggest a possible role for FXIII-A expression in the prognostic grouping of childhood BCP-ALL patients.

Keywords Precursor B-cell acute lymphoblastic leukemia · Immunophenotype · Factor XIII-A · 'B-other' ALL

Bettina Kárai and Zsuzsanna Hevessy contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s12253-017-0236-0) contains supplementary material, which is available to authorized users.

✉ Csongor Kiss
kisscs@med.unideb.hu

[1] Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Nagyerdei krt 98, Debrecen 4032, Hungary

[2] Department of Pediatric Hematology and Oncology, Institute of Pediatrics, Faculty of Medicine, University of Debrecen, Nagyerdei krt 98, Debrecen H-4032, Hungary

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignant childhood disease, afflicting approximately 50–60 children in Hungary annually. Due to some successful clinical trials for childhood B-cell precursor ALL (BCP-ALL), in which Hungary also participated, disease outcome has improved significantly in the last 20 years [1–3]. These clinical trials introduced risk-tailored treatment intensity. The classification of patients is based not only on such conventional risk factors as age and initial white blood cell (WBC) count but also on genetic alterations and early responses to individual treatment. The International Berlin-Frankfurt-Münster Study Group (I-BFM-SG) incorporated two genetic alterations associated with poor outcomes (t(9;22)/*BCR-ABL1* and *MLL* gene rearrangement) into the BFM ALL-IC 2002 protocol (Table 1). A few years later several recurrent genetic alterations which determine distinct BCP-ALL subgroups were included in the World Health Organization (WHO 2008) classification (t(12;21)/*TEL-AML1*, hyperdiploidy, hypodiploidy, t(5;14)/*IL3-IGH* and t(1;19)/*TCF3-PBX1*) [4]. With the growing number of primary and secondary genetic alterations identified recently as prognostic factors, WHO introduced two provisional entities in 2016: BCP-ALL with intrachromosomal amplification of chromosome 21 and B-ALL with translocations involving tyrosine kinases or cytokine receptors ('*BCR-ABL1*-like ALL') [5–7].

Table 1 Risk stratification of the BFM ALL-IC 2002 protocol [3]

Risk group	Basis for stratification
Standard Risk (SR)	• Early treatment response: prednisone-good responders (less than 1×10^9/L blast in peripheral blood on day 8) • Age: 1–6 years • WBC: initial WBC less than 20×10^9/L • BM status: M1 (less than 5% blasts) or M2 (5% - 25% blasts) marrow on day 15 M1 marrow on day 33 *all criteria must be fulfilled*
Intermediate Risk (IR)	• Early treatment response: prednisone-good responders • Age: younger than 1 year or age 6 years or older • WBC: initial WBC more than 20×10^9/L • BM status: M1 or M2 marrow on day 15 M1 marrow on day 33 SR criteria but M3 (more than 25% blasts) marrow on day 15 and M1 marrow on day 33
High Risk (HR)	• Early treatment response: prednisone-poor responders (more than 1×10^9/L blast in peripheral blood on day 8) • IR and M3 marrow on day 15, M2 or M3 marrow on day 33 •t(9;22) *(BCR-ABL),* or t(4;11) *(MLL-AF4)* *at least one criteria must be fulfilled*

Since minimal residual disease (MRD) during induction therapy proved to be a powerful independent predictor of outcome, MRD has come to play a role in risk-group stratification [8–10]. In the BFM ALL-IC 2002 protocol, the assessment of MRD was based on morphological examination (Table 1) [3], but subsequently several study groups, including the BFM ALL-IC working group, preferred the flow cytometric (FC) method instead [11–13]. The determination of MRD by FC method could become part of international trials due to the intensive standardization efforts [14, 15]. The sensitivity of the FC-MRD method depends on the presence of leukemia-associated immunophenotypes (LAIPs). Certain LAIPs may endow lymphoblasts with special characteristics influencing disease outcome [16]. Participating in the pilot FC-MRD estimation project of BFM ALL-IC 2002, our center determined MRD level in day-15 bone marrow samples of all patients diagnosed with ALL in our laboratory [13].

Using flow cytometry, we confirmed that the expression of subunit A of blood coagulation factor XIII (FXIII-A) was a sensitive intracellular marker for the classification of acute myeloid leukemias (AML) [17–19]. Moreover, the lack of expression of FXIII-A in acute promyelocytic leukemia was associated with an unfavorable outcome [20]. Previously we identified B-cell lineage leukemic lymphoblasts as a new expression site for FXIII-A [21]. A subset of BCP-ALL patients was characterized by the intracellular expression of FXIII-A, whereas normal peripheral blood B lymphocytes as well as normal B lymphoid precursors in the bone marrow were devoid of FXIII-A labeling [19]. The exact role of FXIII-A in malignant cells is still unclear, but this aberrant expression is a characteristic LAIP in BCP-ALL, and it may suggest the existence of a prognostically different subgroup of BCP-ALL patients. The present study aimed to investigate retrospectively FXIII-A expression as a possible prognostic marker in childhood BCP-ALL among patients treated in two centers according to the BFM ALL-IC 2002 protocol.

Patients and Methods

Patients

We retrospectively analyzed the results of 48 consecutive BCP-ALL cases of children aged between 1 and 19 and diagnosed from April, 2003 to March, 2011 at the University of Debrecen as well as 7 test samples of similar patients from the Borsod-Abaúj-Zemplén County Hospital and University Hospital with excess diagnostic bone marrow. The patients were treated according to BFM ALL-IC 2002 protocol [3]. The bone marrow samples were obtained by aspiration in the course of routine diagnostic interventions and were anticoagulated with EDTA. Diagnostic bone marrow was analyzed in the case of all 55 patients, while day-15 bone marrow was examined in the case of 42 children as part of the follow-up.

We conducted the study adhering to the Declaration of Helsinki. Written informed consent was obtained from parents/legal guardians to include these children in the BFM ALL-IC 2002 trial, which encompassed immunophenotype analyses by FC and genetic investigations.

Flow Cytometry Studies

Flow cytometric measurements were performed on a FACSCalibur flow cytometer (Becton Dickinson, San

Jose, CA), using four-color labeling. Surface and cytoplasmic staining was performed according to standard protocols with the following monoclonal antibodies: CD10-FITC, CD19-APC, CD20-FITC, CD34-FITC, CD34-PerCP, CD38-PE, CD45 PerCP, κ-FITC, λ-PE (Becton Dickinson, San Jose, CA); CD58-FITC, CD66c-PE (Beckman Coulter, Brea, CA); CD10-PE, CD79α-PE, TdT-FITC, IgM-PE (DAKO, Glostrup, Denmark). Generation and labeling of mouse monoclonal antibodies against FXIII-A was carried out as previously described, utilizing a fluorescent isothiocyanate (FITC) labeling kit (Sigma, St. Louis MO) [22]. The "positivity" of the blasts for a certain immunophenotype marker was declared based on the 20% cut-off limit of labeling. MRD detection was a pilot project ("Mini-mini project") of the BFM ALL-IC 2002 clinical trial, 300,000 events were acquired for this purpose [13]. Data were obtained and analyzed by CellQuest 3.2 and FACS Diva software, both procured from Becton Dickinson, San Jose, CA.

Chromosome Analysis and FISH

G-banding was performed according to standard procedures. Karyotypes were described according to the International System of Human Cytogenetic Nomenclature [23]. Fluorescence in situ hybridization was carried out on cell suspension samples used for chromosome preparations according to the manufacturer's instructions, using LSI *MLL* DC, BA; LSI *BCR/ABL* DC, DF and *TEL/AML1* DC, SF, ES translocation probes (all from Abbot/Vysis, Downers Grove, IL). Cells were counterstained with DAPI (4,6-diamidino-2-phenylindole). In general, 200 interphase cells were counted in each case. The images were captured by a Zeiss Axioplan2 (Carl Zeiss, Zaventem, Brussels) fluorescence microscope and analyzed by ISIS software (Metasystems, Altlussheim, Germany). Patients were assigned to genetic risk categories according to WHO recommendations [4]. The low-risk group included patients with t(12;21)/*TEL-AML1* and high hyperdiploidy. The intermediate-risk group included the 'B-other' group (patients without recurrent genetic abnormalities, such as t(9;22)/*BCR-ABL1*, *KMT2A* (*MLL*) rearrangements, t(12;21)/*TEL-AML1*, t(1;19)/*TCF3-PBX1* or high hyperdiploidy) and patients with t(1;19)/*TCF3-PBX1*. Finally, the high-risk group consisted of patients with t(9;22)/*BCR-ABL1* or *KMT2A* (*MLL*) rearrangements. In our study there were no patients with t(5;14)/*IL3-IGH* or hypodiploidy.

Statistical Analysis

Data distribution was evaluated by the Shapiro-Wilk test. To compare two groups, we used Student's t-test for parametric and Mann-Whitney U test for non-parametric data. A statistically significant difference was defined as a p-value <0.05. Pearson's Chi square test was applied to assess association between two categorical variables and logistic regression to analyze association between multiple variables.

We compared the disease outcome for patients with FXIII-A-positive and FXIII-negative B-lymphoblasts upon diagnosis. The duration of event-free survival (EFS) was measured from the time of diagnosis of ALL until the date of first relapse, death from any cause, or the date of last contact for all event-free survivors. Overall survival (OS) was calculated retrospectively from the date of diagnosis to the date of the last follow-up or death from any cause. Survival analyses were done using the Kaplan-Meier survival estimator, survival curves were compared by log-rank test. Hazard ratio (HR) and 95% CI were estimated by Cox proportional hazard model analysis. Statistical analysis was performed with SPSS 20.0 statistical program.

Results

Of the 55 common ALL patients enrolled in the study, 18 exhibited FXIII-A-negative lymphoblasts and 37 showed FXIII-A positivity. There were different patterns of FXIII-A expression (Fig. 1). The FXIII-A positivity of the blasts was declared based on the 20% cut-off limit of labeling. The correlation between FXIII-A content of lymphoblasts and clinicobiological parameters are shown in Table 2. Importantly, the classical prognostic markers (age and initial WBC count) did not show significant differences between the FXIII-A-positive and FXIII-A-negative patient groups. The ratio of poor prednisone response, however, was slightly higher in the FXIII-A-negative group than in the FXIII-A-positive one (29% vs. 5.7%), although this difference was statistically not significant.

We examined the relationships between FXIII-A expression and the other categorical variables: age (younger/older than six years), initial WBC count (higher/lower than 20×10^9/L), prednisone response, FC and genetic risk categories, including the 'B-other' genetic group. Among the categorical variables, the distribution of the 'B-other' genetic group differed significantly in the FXIII-A-positive and -negative groups (OR: 7.1; 95% CI: 1.7–29.1; $p = 0.006$) (Fig. 2). The multivariate logistic regression analysis confirmed the association between the FXIII-A characteristics and the 'B-other' group (OR: 7.1; 95% CI: 1.7–29.1; $p = 0.006$). This association persisted even after adjusting for initial parameters such as age and WBC count (OR: 7.8; 95% CI: 1.8–34.7; $p = 0.007$) (Table 3a).

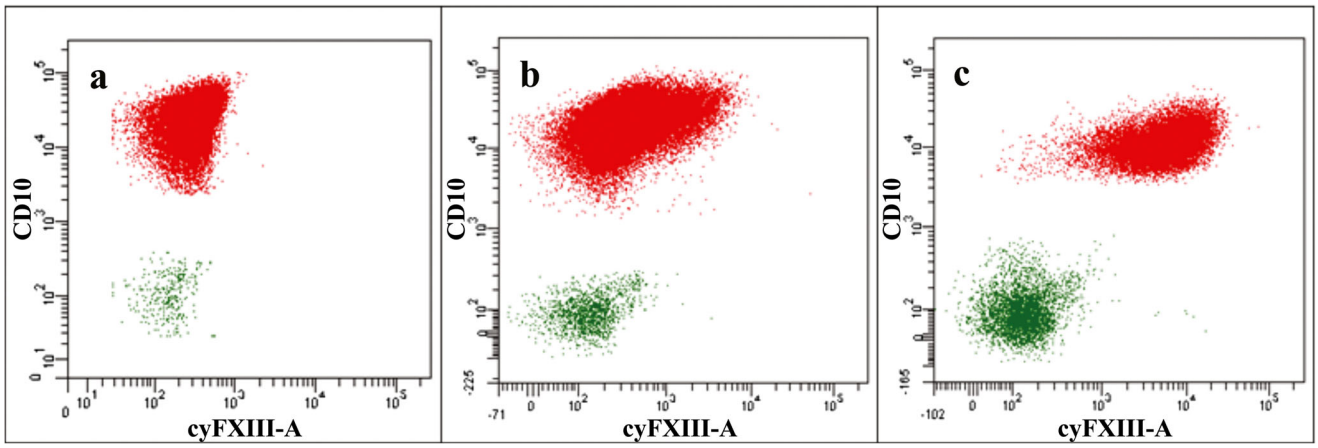

Fig. 1 Patterns of FXIII-A expression in leukemic lymphoblasts. Negative pattern (**a**) was defined below 20%, intermediate positive expression (**b**) between 20% and 80% and strong positive (**c**) expression of FXIII-A over 80% positivity of leukemic lymphoblasts. Red dots: B-lymphoblasts; green dots: normal B cells

The manifestation of FXIII-A in leukemic lymphoblasts correlated with better EFS and OS of children with BCP-ALL (Fig. 3, $p = 0.031$ and $p = 0.008$, respectively). The ratio of 10-year EFS and OS were significantly higher in the FXIII-A-positive patient group (84%, 95% CI: 67.4–92.4 and 89%, 95% CI: 73.7–95.8, respectively) than in the FXIII-A-negative patient group (61%, 95% CI: 35.3–79.2 and 61%, 95% CI: 35.3–79.2). Since we found an association between FXIII-A expression and the 'B-other' group and both EFS and OS showed differences not only for FXIII-A positivity but also for 'B-other' characteristics (EFS $p = 0.058$, OS $p = 0.021$) (Online Resource 1), we performed multivariate Cox regression analysis to evaluate the role of FXIII-A expression in the prediction of survival. In the Cox model, including either parameter and adjusting initial prognostic parameters (age and WBC), the FXIII-A character had the most potent effect on OS (HR: 4.8; 95% CI: 1.2–19.2; $p = 0.025$) (Online Resource 2). This result was supported by another uni- and multivariate analysis where the multivariate model included FXIII-A expression, genetic risk groups, and initial parameters, and both EFS and OS

Table 2 Clinical variables of children with precursor B-cell ALL. On the basis of FXIII-A expression of lymphoblasts, we assigned the patients to FXIII-A-positive and FXIII-A-negative groups (cut-off at 20%). Patients were classified according to the status of minimal residual disease detected by flow cytometry (FC) at day 15. FLR: flow-low-risk (blasts < 0.1%), FMR: flow-medium-risk (blasts 0.1%–10%), FHR: flow-high-risk (blasts >10%), NA: not assessed. Genetic risk categories, 'B-other'cytogenetic group, and risk groups were defined following international guidelines and ALL IC-BFM 2002 (see text). SR: standard risk, IR: itermediate risk, HR: high risk

	All	FXIII-A-positive	FXIII-A-negative
Number of patients	55	37	18
Age (years)	4.7 (1.3–17.9)	4.8 (1.3–17.9)	4.7(1.7–17.1)
Sex (female/male)	32/23	21/16	11/7
WBC ($\times 10^9$/L)	8.5 (0.8–637.8)	8.9 (0.8–637.8)	8.1 (1.3–186.2)
Poor prednisone response	6 (12%)	2 (5.7%)	4 (29%)
Risk group as defined BFM ALL IC 2002 (SR/IR/HR/NA)	18/24/9/4	14/16/3/4	4/8/6/–
Genetic risk categories (low/intermediate/poor)	34/18/3	27/8/2	7/9/1
Recurrent genetic categories			
t(12;21)/*TEL-AML1*	16 (30%)	13 (36%)	3 (18%)
high hyperdiploidy	17 (32%)	13 (36%)	4 (24%)
t(1; 19)/*TCF3-PBX1*	5 (9%)	4 (11%)	1 (6%)
MLL translocation	2 (4%)	1 (3%)	1 (6%)
t(9;22)/*BCR-ABL1*	1 (2%)	1 (3%)	0 (0%)
'B-other'	12 (23%)	4 (11%)	8 (47%)
FC risk categories (FLR/FMR/FHR/NA)	17/19/6/13	12/16/2/7	5/3/4/6

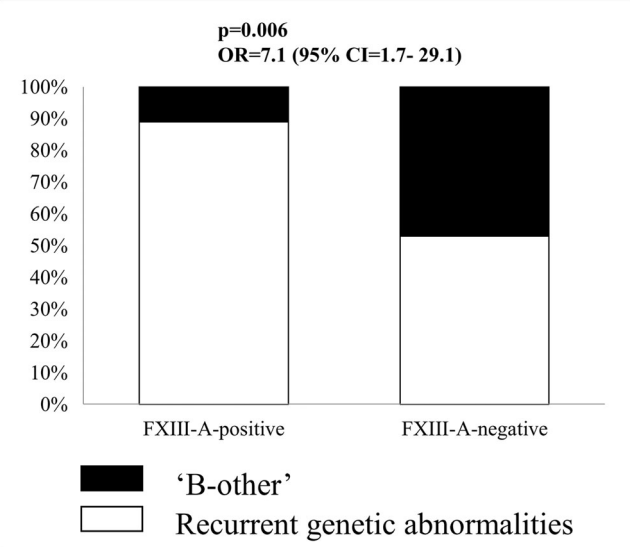

Fig. 2 The relationship between FXIII-A expression profile and genetic classification. The distribution of patients in the various genetic groups differed significantly in terms of FXIII-A profile using Chi square test. Recurrent genetic abnormalities: *BCR-ABL1*, *MLL* gene rearrangements, *ETV6-RUNX1* (*TEL-AML1*), *E2A/PBX1* and high hyperdiploidy

showed significant differences only for FXIII-A expression (HR: 3.6, 95% Cl: 1.2–11.3, $p = 0.027$; HR: 5.6, 95% Cl: 1.5–20.12, $p = 0.009$ respectively) (Table 3b). These results suggest that FXIII-A expression is an independent prognostic factor, and the poor outcome associated with FXIII-A negativity was not merely the result of its association with the 'B-other' characteristic, whose unfavourable effect on survival is well-known.

As expected, the assessment of EFS and OS in the prognostic groups based on day-15 FC-MRD classification indicated significantly better EFS and OS in the low (MRD < 0.1%, flow low risk, FLR) and the intermediate FC-MRD risk group (MRD 0.1–10%, flow medium risk, FMR) compared to the high FC-MRD risk group (MRD > 10%, flow high risk, FHR): $p = 0.01$ for both EFS and OS between FLR and FHR; $p = 0.01$ for EFS and $p = 0.004$ for OS between FMR and FHR (Online Resource 3).

Discussion

The intracellular A subunit of FXIII is normally present in monocytes, megakaryocytes and platelets [24]. We detected the intracellular expression of FXIII-A in a subset of BCP-ALL patients with flow cytometry, immunoblotting, and confocal laser scanning microscopy, whereas the normal counterparts of these pathological cells in healthy bone marrow were devoid of FXIII-A labeling [19, 21]. The aberrant FXIII-A expression in lymphoblasts may suggest the existence of a new, distinct subpopulation of BCP-ALL cases. In this study, both EFS and OS differed significantly according to FXIII-A expression character: FXIII-A positivity was associated with favorable outcome. Although the role of FXIII in blood coagulation and wound healing is well-known [24–27], the function of the intracellular enzyme has not yet been fully understood. According to the results of recent studies on the function of intracellular FXIII-A in monocytes/macrophages and megakaryocytes/platelets, FXIII-A may have a role in connecting lymphoblasts and bone marrow stromal cells [28–32]. It might be hypothesized that, in the absence of FXIII-A expression, the migration of lymphoblast from the osteoblastic niche – located on the inner

Table 3 Analysis of FXIII-A and multiple predictor variables by multivariate logistic regression (a) and Cox-regression (b) The association between FXIII-A expression and 'B-other' characteristics was analysed by logistic regression. Adjusting this model for confounders (age and white blood cell count (WBC) at the diagnosis) resulted in significant difference ($p = 0.007$) and higher Odds ratio (7.8) (A). We examined the effect of unadjusted and adjusted (age and WBC at the diagnosis) FXIII-A and genetic group models for survival using Cox regression (B)

a

	P value	Odds ratio	95°A) Confidence interval
FXIII-A and 'B-other' model	0.006	7.1	1.7–29.1
adjusted FXIII-A and 'B-other' model	0.007	7.8	1.8–34.7

b

	EFS			OS		
	P value	Hazard ratio	95% Confidence interval	*P* value	Hazard ratio	95% Confidence interval
Univariate analysis						
FXIII-A	0.041	3.12	1.05–9.32	0.016	4.5	1.3–15.5
genetic risk group	0.063	2.1	0.96–4.4	0.057	2.2	0.98–5.04
Multivariate analysis model (FXIII-A and genetic risk group, age, WBC)						
FXIII-A	0.027	3.6	1.2–11.3	0.009	5.6	1.5–20.12
genetic risk group	0.24	1.7	0.7–4.1	0.23	1.8	0.7–4.9

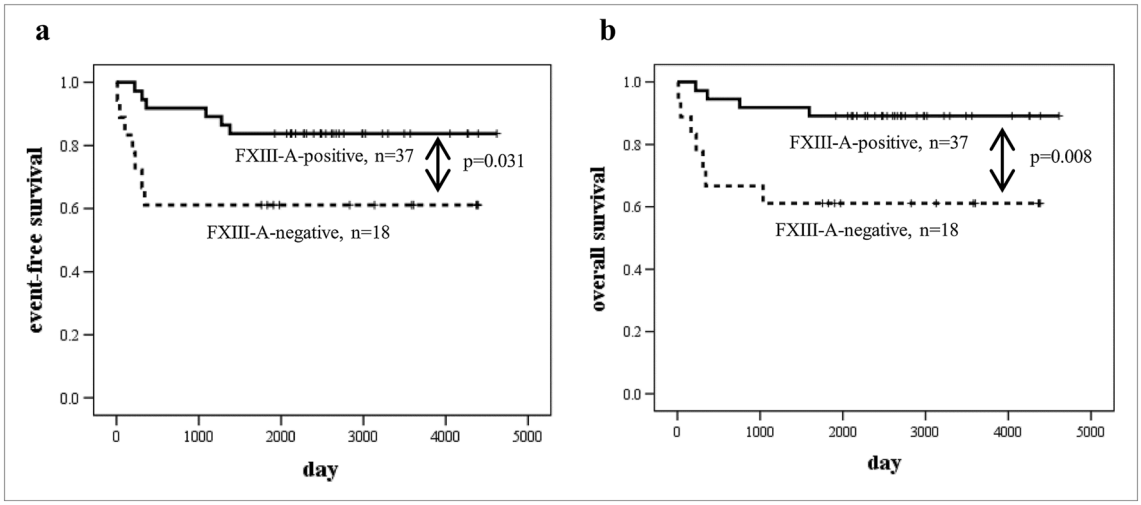

Fig. 3 The prognostic value of FXIII-A expression of lymphoblasts in children with B-cell precursor ALL. Kaplan-Meier plots of event-free (**a**) and overall survival (**b**) showed significant difference between the FXIII-A-positive and FXIII-A-negative groups ($p = 0.031$ and $p = 0.008$)

surface of the bone cavity – to the vascular niche – consisting of sinusoidal endothelial cell lining of blood vessels – may be hindered [33]. This way, FXIII-A-negative lymphoblasts may be retained in the osteoblastic niche and protected from immune- and/or chemotherapeutic drug-mediated cell death, which process is more pronounced in the vascular niche. Furthermore, our results may provide a different explanation for the poor outcome of FXIII-A-negative cases. Patients assigned to the 'B-other' genetic subgroup displayed significantly more often FXIII-A-negative lymphoblasts according to both the uni- and the multivariate analysis, suggesting that FXIII-A-negative cases require a detailed molecular genetic evaluation. Recently several studies have focused on the examination of 'B-other' ALL. Approximately 50% of 'B-other' ALL cases were classified as *BCR-ABL1*-like ALL, which is associated with a 5-year EFS of 60%, similarly to that observed in *BCR-ABL1*–positive BCP-ALL [6, 34, 35]. *IKZF1* deletion, which is a secondary abnormality, often occurs in 'B-other' ALL alone or in association with other abnormalities, such as *BCR-ABL1*-like characteristics [36]. *BCR-ABL1*-like cases can be identified by gene expression profile examination and copy number alterations determined by multiplex ligation-dependent probe amplification (MLPA), which are too time-consuming and expensive methods [6] to be used in daily routine. Establishing the FXIII-A expression profile by FC may prove to be a cost-efficient and easy-to-perform assay for the pre-selection of cases requiring more sophisticated analysis.

The present study has several limitations. We wanted to examine a homogenous population of children with BCP-ALL, yet we found for our retrospective analysis only a relatively small sample of 55 patients enrolled in the BFM ALL-IC 2002 clinical trial. Furthermore, we had suitable DNA samples only from a limited number of patients, and we did not collect RNA samples to be examined by modern methods such as gene expression profiling or MLPA. Therefore, we were unable to investigate the hypothetical correlation between the lack of FXIII-A expression and *BCR-ABL1*-like ALL.

In conclusion, we found an excellent correlation between long-term survival and the FXIII-A-positive phenotype of lymphoblasts, which proves that FXIII-A expression character in lymphoblast is not only a useful LAIP but also a powerful prognostic factor in childhood BCP-ALL. In addition, FXIII-A expression is associated with the 'B-other' characteristics, therefore, FXIII-A can help to identify those cases that may require further detailed genetic examination using expensive methods. The retrospective results obtained from this cohort of patients treated in two BFM ALL-IC 2002 centers should be confirmed by a larger, prospective analysis involving several centers participating in the ongoing BFM ALL-IC 2009 clinical trial.

Acknowledgements The authors thank Dr. Erzsébet Balogh for performing the cytogenetic analyses and Csaba Antal for his administrative help. They are also grateful to all the physicians and assistants participating in the clinical care of patients and in the execution of flow cytometry investigations at the departments of the University of Debrecen. The authors express their gratitude to Dr. Kálmán Nagy and their coworkers at the Borsod-Abaúj-Zemplén County Hospital and University Hospital for providing bone marrow samples for flow cytometric analysis. Finally, they thank Attila Kárai for proofing and editing the manuscript. This study was supported by an OTKA K-108885 grant (CsK).

Compliance with Ethical Standards

Conflict of Interest Statement The authors declare no conflict of interest.

References

1. Conter V, Arico M, Basso G, Biondi A, Barisone E, Messina C, Parasole R, De Rossi G, Locatelli F, Pession A, Santoro N, Micalizzi C, Citterio M, Rizzari C, Silvestri D, Rondelli R, Lo Nigro L, Ziino O, Testi AM, Masera G, Valsecchi MG, Associazione Italiana di Ematologia ed Oncologia P (2010) Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 24(2):255–264
2. Moricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, Ludwig WD, Ritter J, Harbott J, Mann G, Klingebiel T, Zintl F, Niemeyer C, Kremens B, Niggli F, Niethammer D, Welte K, Stanulla M, Odenwald E, Riehm H, Schrappe M (2010) Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 24(2):265–284
3. Stary J, Zimmermann M, Campbell M, Castillo L, Dibar E, Donska S, Gonzalez A, Izraeli S, Janic D, Jazbec J, Konja J, Kaiserova E, Kowalczyk J, Kovacs G, Li CK, Magyarosy E, Popa A, Stark B, Jabali Y, Trka J, Hrusak O, Riehm H, Masera G, Schrappe M (2014) Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. J Clin Oncol 32(3):174–184
4. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114(5):937–951
5. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127(20):2391–2405
6. Moorman AV (2016) New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. Haematologica 101(4):407–416
7. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 446(7137):758–764
8. Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E (1998) Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer–childhood leukemia cooperative group. N Engl J Med 339(9):591–598
9. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, Sandlund JT, Rivera GK, Rubnitz JE, Ribeiro RC, Pui CH, Campana D (2000) Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 96(8):2691–2696
10. Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N, Fritsch G, Gadner H, Austrian Berlin-Frankfurt-Munster Study G (2002) Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 99(6):1952–1958
11. Basso G, Veltroni M, Valsecchi MG, Dworzak MN, Ratei R, Silvestri D, Benetello A, Buldini B, Maglia O, Masera G, Conter V, Arico M, Biondi A, Gaipa G (2009) Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. J Clin Oncol 27(31):5168–5174
12. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, Linda S, Martin PL, Pullen DJ, Viswanatha D, Willman CL, Winick N, Camitta BM, Children's Oncology G (2008) Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology group study. Blood 111(12):5477–5485
13. Mejstrikova E, Fronkova E, Kalina T, Omelka M, Batinic D, Dubravcic K, Pospisilova K, Vaskova M, Luria D, Cheng SH, Ng M, Leung Y, Kappelmayer J, Kiss F, Izraeli S, Stark B, Schrappe M, Trka J, Stary J, Hrusak O (2010) Detection of residual B precursor lymphoblastic leukemia by uniform gating flow cytometry. Pediatr Blood Cancer 54(1):62–70
14. Dworzak MN, Gaipa G, Ratei R, Veltroni M, Schumich A, Maglia O, Karawajew L, Benetello A, Potschger U, Husak Z, Gadner H, Biondi A, Ludwig WD, Basso G (2008) Standardization of flow cytometric minimal residual disease evaluation in acute lymphoblastic leukemia: Multicentric assessment is feasible. Cytometry B Clin Cytom 74(6):331–340
15. van Dongen JJ, van der Velden VH, Bruggemann M, Orfao A (2015) Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood 125(26):3996–4009
16. Boldt DH, Kopecky KJ, Head D, Gehly G, Radich JP, Appelbaum FR (1994) Expression of myeloid antigens by blast cells in acute lymphoblastic leukemia of adults. The southwest Oncology group experience. Leukemia 8(12):2118–2126
17. Invernizzi R, De Fazio P, Iannone AM, Zambelli LM, Rastaldi MP, Ippoliti G, Ascari E (1992) Immunocytochemical detection of factor XIII A–subunit in acute leukemia. Leuk Res 16(8):829–836
18. Kappelmayer J, Simon A, Katona E, Szanto A, Nagy L, Kiss A, Kiss C, Muszbek L (2005) Coagulation factor XIII-A. A flow cytometric intracellular marker in the classification of acute myeloid leukemias. Thromb Haemost 94(2):454–459
19. Kiss F, Simon A, Csathy L, Hevessy Z, Katona E, Kiss C, Kappelmayer J (2008) A coagulation factor becomes useful in the study of acute leukemias: studies with blood coagulation factor XIII. Cytometry A 73(3):194–201
20. Simon A, Bagoly Z, Hevessy Z, Csathy L, Katona E, Vereb G, Ujfalusi A, Szerafin L, Muszbek L, Kappelmayer J (2012) Expression of coagulation factor XIII subunit a in acute promyelocytic leukemia. Cytometry B Clin Cytom 82(4):209–216
21. Kiss F, Hevessy Z, Veszpremi A, Katona E, Kiss C, Vereb G, Muszbek L, Kappelmayer JN (2006) Leukemic lymphoblasts, a novel expression site of coagulation factor XIII subunit a. Thromb Haemost 96(2):176–182
22. Katona EE, Ajzner E, Toth K, Karpati L, Muszbek L (2001) Enzyme-linked immunosorbent assay for the determination of blood coagulation factor XIII A-subunit in plasma and in cell lysates. J Immunol Methods 258(1–2):127–135
23. Simons A, Shaffer LG, Hastings RJ (2013) Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res 141(1):1–6
24. Muszbek L, Yee VC, Hevessy Z (1999) Blood coagulation factor XIII: structure and function. Thromb Res 94(5):271–305
25. Muszbek L, Adany R, Mikkola H (1996) Novel aspects of blood coagulation factor XIII. I. Structure, distribution, activation, and function. Crit Rev Clin Lab Sci 33(5):357–421
26. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneitte G, Modis L, Muszbek L, Inbal A (2005) Impaired wound healing in factor XIII deficient mice. Thromb Haemost 94(2):432–437
27. Richardson VR, Cordell P, Standeven KF, Carter AM (2013) Substrates of factor XIII-A: roles in thrombosis and wound healing. Clin Sci (Lond) 124(3):123–137
28. Bagoly Z, Katona E, Muszbek L (2012) Factor XIII and inflammatory cells. Thromb Res 129:S77–S81
29. Serrano K, Devine DV (2002) Intracellular factor XIII crosslinks platelet cytoskeletal elements upon platelet activation. Thromb Haemost 88(2):315–320

30. Jayo A, Conde I, Lastres P, Jimenez-Yuste V, Gonzalez-Manchon C (2009) New insights into the expression and role of platelet factor XIII-A. J Thromb Haemost 7(7):1184–1191
31. Kulkarni S, Jackson SP (2004) Platelet factor XIII and calpain negatively regulate integrin alpha(IIb)beta(3) adhesive function and thrombus growth. J Biol Chem 279(29):30697–30706
32. Malara A, Gruppi C, Rebuzzini P, Visai L, Perotti C, Moratti R, Balduini C, Tira ME, Balduini A (2011) Megakaryocyte-matrix interaction within bone marrow: new roles for fibronectin and factor XIII-A. Blood 117(8):2476–2483
33. Guerrouahen BS, Al-Hijji I, Tabrizi AR (2011) Osteoblastic and vascular endothelial niches, their control on normal hematopoietic stem cells, and their consequences on the development of leukemia. Stem Cells Int. doi:10.4061/2011/375857
34. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, Van Zutven LJ, Beverloo HB, Van der Spek PJ, Escherich G, Horstmann MA, Janka-Schaub GE, Kamps WA, Evans WE, Pieters R (2009) A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 10(2):125–134
35. Harvey RC, Mullighan CG, Wang X, Dobbin KK, Davidson GS, Bedrick EJ, Chen IM, Atlas SR, Kang H, Ar K, Wilson CS, Wharton W, Murphy M, Devidas M, Carroll AJ, Borowitz MJ, Bowman WP, Downing JR, Relling M, Yang J, Bhojwani D, Carroll WL, Camitta B, Reaman GH, Smith M, Hunger SP, Willman CL (2010) Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. Blood 116(23):4874–4884
36. Clappier E, Grardel N, Bakkus M, Rapion J, De Moerloose B, Kastner P, Caye A, Vivent J, Costa V, Ferster A, Lutz P, Mazingue F, Millot F, Plantaz D, Plat G, Plouvier E, Poiree M, Sirvent N, Uyttebroeck A, Yakouben K, Girard S, Dastugue N, Suciu S, Benoit Y, Bertrand Y, Cave H, European Organisation for R, Treatment of Cancer CsLG (2015) IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's leukemia group study 58951. Leukemia 29(11):2154–2161