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Use of quantitative polymerase chain reaction (qPCR) for the diagnosis and monitoring of CNS leukaemia

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Main text

Despite the excellent overall survival in childhood acute lymphoblastic leukemia (ALL), central nervous system (CNS) disease continues to pose challenges. Currently, only 3–5% children with ALL present with cytological evidence of CNS involvement [1], however CNS-directed therapy is essential for cure, and the majority of CNS relapses occur in children who had negative CNS cytology at diagnosis [2]. This implies that the frequency of patients with CNS involvement may be higher than currently detected. There is a clear need for improving diagnostic accuracy of CNS involvement. Leukemic cells, being clonal in origin, carry VDJ gene rearrangements unique to individual patients. Using allele-specific oligonucleotides (ASO) primers TaqMan qPCR accurately estimates minimal residual disease (MRD) and predicts bone marrow relapse [3]. However, bone marrow MRD status does not predict the risk of CNS relapse.

We investigated if qPCR could identify patients with submicroscopic levels of CNS involvement at diagnosis and to track therapy response. Our research questions were: i) can amplifiable DNA be extracted from leftover cerebrospinal fluid (CSF) samples obtained for routine CSF cytology? ii) Can allele-specific PCR primers designed for bone marrow MRD detect leukemic in the CNS? iii) Is CSF qPCR able to track treatment response in the CNS?

The study was approved by the West of Scotland Research Ethics Committee (WoSREC reference: 09/SO703/77). Between July 2011 and July 2014, children (1–18 years old) diagnosed with ALL at the Royal Hospital for Sick Children, Glasgow were enrolled in the study. Diagnostic and follow-up CSF samples during induction therapy were collected. DNA was extracted using Qiagen QIAmp DNA Micro kit and quantified using TaqMan Real-Time qPCR assay for Albumin [4]. ASO primers were designed at the Glasgow MRD laboratory according to Biomed-II guidelines [5].

During the study period, 57 patients were enrolled. Out of these 19 were excluded from analysis (see supplementary Table 1 for details). This left samples from 38 patients for analysis (31 patients with CNS-1 disease (no blasts in CSF cytology), 5 with TLP + ve (traumatic lumbar puncture with identifiable blasts) and 2 with TLP − ve (traumatic lumbar puncture without blasts)) (supplementary Tables 2 and 3).

Real-time qPCR revealed detectable leukemic DNA in 15/38 patients (39.5%) (designated qPCR positive). Among these, 11 were CNS-1 patients and 4 were TLP + ve patients. 1/5 TLP + ve and 2/2 TLP-ve patients were CSF qPCR negative. Comparison of CSF qPCR positive *vs.* negative patients showed an association with a higher WCC (mean 26.6×10^9/l *vs* 19.4×10^9/l) and high-risk cytogenetics ($p = 0.04$) – factors recognized as high-risk for CNS relapse. Three out of 4 patients with T-ALL were CSF qPCR positive (Table 1).

Next, serial samples from 18 patients during the induction phase of treatment were analysed (11 patients were CSF qPCR negative and 7 were CSF qPCR positive at diagnosis). All patients were CSF qPCR negative on day 29 (end of induction). Two day 8 samples (patient #22 and #37), and one day 15 sample (patient #37) were CSF qPCR positive. (Fig. 1). Overall, these results indicate that qPCR positive disease is rapidly cleared from the CSF during induction.

Twenty patients had sufficient template DNA to test two primer/probe sets, 13 patients were negative and 4 patients were positive with both sets, whilst 3 patients were positive with one set but negative with the other (supplementary Fig. 1).

CSF qPCR positivity at diagnosis was not associated with BM MRD risk status at the end of induction. In this small study, no CNS relapses were seen during the period of follow up (71 ± 10 months, median ± SD). One CSF qPCR negative patient suffered isolated BM relapse at 4-years (Supplementary Table 3).

Our findings indicate that the use of qPCR detects occult CNS involvement in more than one-third of children with acute lymphoblastic leukemia. This is consistent with other newer methods such as flow-cytometry [6]. However, this technique also has important limitations. Firstly, 19/57 (33%) patient samples could not be used for ASO qPCR testing due to lack of amplifiable DNA or suitable primers. Some of these patients may represent true negative cases with no circulating blasts in the CSF, alternatively the small volume of CSF obtained may have yielded inadequate DNA. Secondly, 1/5 TLP + patients was negative by qPCR although this may reflect either false positive cytology or false negative qPCR. Thirdly, 3/20 patients had discordant results when testing two primer/probe sets. This is most likely to be due to differing sensitivity of the two assays. Alternatively, there could be subclonal selection of cells bearing only one of the two VDJ

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Table 1
Comparison between CSF qPCR + ve and qPCR –ve patients.

Variable	Category	QPCR + ve		QPCR – ve		
		n	%	n	%	
		15	39.5%	23	60.5%	p-value*
Age at diagnosis	< 10 year	12	39%	19	61%	0.839
	> 10 years	3	43%	4	57%	
Sex	Male	12	50%	12	50%	0.101
	Female	3	21%	11	79%	
Immunophenotype	BCP-ALL	12	35%	22	65%	0.280
	T-ALL	3	75%	1	25%	
WCC	< 10	4	21%	15	79%	0.043
	10-50	9	64.3%	5	35.7%	
	> 50	2	40%	3	60%	
CNS status	CNS-1	11	35%	20	65%	0.084
	TLP+	4	80%	1	20%	
	TLP-	0	0%	2	100%	
Cytogenetic risk**	Low risk	7	28%	18	72%	0.04
	High risk	3	100%	0	0%	
	Others	5	50%	5	50%	
Day 29 MRD	MRD risk	8	50%	8	50%	0.258
	Low risk	7	35%	15	65%	

* calculated using Chi squared test.

** Cytogenetic risk: Low risk: t(12;21), High hyperdiploid; High risk: t(9;22), *MLL*-rearranged; Others: t(7;9), del 12p, t(9;18), complex cytogenetics of uncertain origin.

rearrangements in the CNS. A body of experimental evidence from our previous work [7], and others [8] argue against this. Finally, the real power of developing biomarkers of CNS leukemia would be the ability to track response to treatment within the CNS compartment. Thus, an ideal test should be able to separate patients into rapid and slow responders, with subsequent follow-up needed to confirm that this has prognostic significance. In our study, all patient samples became negative by the end of induction chemotherapy, suggesting that it is too insensitive for disease response assessment.

Given these limitations, qPCR of CSF is unlikely to be clinically useful. Alternative strategies such as flow-cytometry or NGS still rely on detection of circulating cells within the CSF. Historical reports of autopsies [9] and our previous data [10] show that leukemic blasts are often embedded in the meninges and not present in a free-floating state. Future research into soluble biomarkers released by leukemic cells into circulating CSF might be the best way forward.

Contributions

1. Yasar Mehmood Yousafzai: Performed research, analysed data and wrote manuscript. 2. Linda Smith: Contributed to essential reagents and tools, analysed data, and approved manuscript 3. Amanda Smith: Contributed to essential reagents and tools, analysed data, and approved manuscript 4. Saeeda Bhatti: Contributed to essential reagents and tools, analysed data, and approved manuscript 5. Mary Gardiner: Contributed to essential reagents and tools, analysed data, and approved manuscript 6. Antony Cousins: Contributed to essential reagents and tools, analysed data, and approved manuscript 7. Frances Fee: Contributed to essential reagents and tools, analysed data, and approved manuscript 8. Sandra Chudleigh: Contributed to essential reagents and tools, analysed data, and approved manuscript 9. Alison Spence: Contributed to patient recruitment, analysed data, and approved manuscript 10. Wendy Taylor: Contributed to patient recruitment, analysed data, and approved manuscript 11. Nicholas Heaney: Contributed to patient recruitment, performed laboratory analysis of samples, and approved manuscript 12. Brenda Gibson: Contributed to patient recruitment, performed critical analysis of research and approved manuscript 13. Gerard Graham: Performed critical analysis of research and approved manuscript 14. Christina Halsey: Conceived research, performed research, wrote manuscript.

Declaration of Competing Interest

None.

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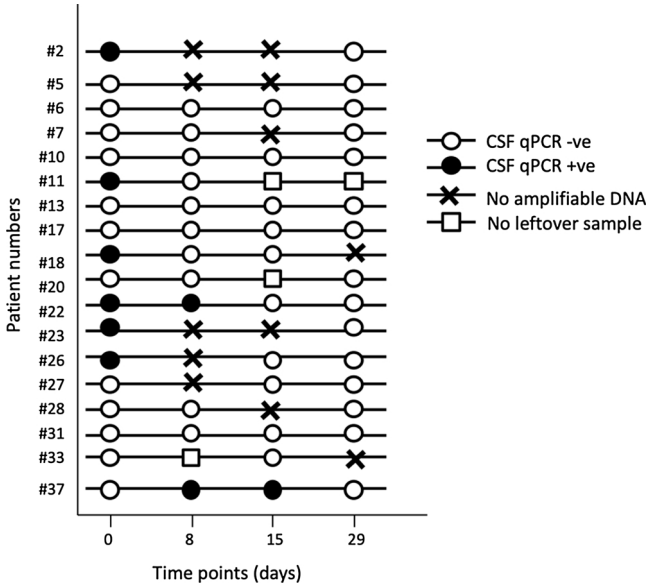

Fig. 1. qPCR positivity of representative set of patients at diagnosis and during induction period. Time points: days post diagnosis, open circles = qPCR negative, closed circles = qPCR positive, cross = No amplifiable DNA, open box = no leftover samples available.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.leukres.2019.106232.

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