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High-throughput sequencing in acute lymphoblastic leukemia: Follow-up of minimal residual disease and emergence of new clones

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ABSTRACT

Minimal residual disease (MRD) is known to be an independent prognostic factor in patients with acute lymphoblastic leukemia (ALL). High-throughput sequencing (HTS) is currently used in routine practice for the diagnosis and follow-up of patients with hematological neoplasms. In this retrospective study, we examined the role of immunoglobulin/T-cell receptor-based MRD in patients with ALL by HTS analysis of immunoglobulin H and/or T-cell receptor gamma chain loci in bone marrow samples from 11 patients with ALL, at diagnosis and during follow-up. We assessed the clinical feasibility of using combined HTS and bioinformatics analysis with interactive visualization using Vidjil software. We discuss the advantages and drawbacks of HTS for monitoring MRD. HTS gives a more complete insight of the leukemic population than conventional real-time quantitative PCR (qPCR), and allows identification of new emerging clones at each time point of the monitoring. Thus, HTS monitoring of Ig/TR based MRD is expected to improve the management of patients with ALL.

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1. Introduction

1.1. Assessment of minimal residual disease in ALL

Minimal residual disease (MRD) monitoring has proven to be one of the strongest independent prognostic factors in patients with acute lymphoblastic leukemia (ALL) [1,2]. Sequential monitoring of MRD using sensitive and specific methods, such as quantitative real-time polymerase chain reaction (qPCR) or flow cytometry, has improved the assessment of treatment response [3–5] and is currently used for therapeutic stratification and the early detection of relapses [6,7]. These methods allow the detection of a single leukemic cell among many normal cells.

MRD monitoring based on immunoglobulin (Ig) and T-cell receptor (TR) gene recombinations has been standardized, and has become the gold standard method in routine practice [3,4]. Ig and TR recombinations occur in the early stages of B-cell and T-cell development. As a consequence, each lymphoid cell contains unique V(D)J recombinations resulting from random coupling between one of many possible V, (D) and J genes (combinatorial diversity), as well as imprecise joining of gene segments and the addition of nucleotides to the DNA sequence at splice sites (junctional diversity) [8]. Identical recombinations thus reflect the clonal nature of a population, rather than being derived from independent cells.

Monitoring of Ig/TR-based MRD in ALL consists of step-by-step analysis of V(D)J DNA recombinations in lymphoblasts, and their subsequent detection, with very high sensitivity, during follow-up. Leukemic clonal recombinations can be amplified by PCR and examined by capillary electrophoresis, and can then be isolated

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by polyacrylamide gel electrophoresis and finally sequenced by direct Sanger sequencing. Overall, clonal V(D)J recombinations occur in >95% of ALL cases. Allele-specific oligonucleotides (ASOs) are designed to quantify MRD in follow-up samples by qPCR. However, this approach is time consuming in routine practice, and monitoring is therefore limited to the major leukemic clones (usually 1–3 markers per patient). This strategy can therefore yield false-negative results.

Failures of this MRD-monitoring strategy can occur as a result of absence of the initial marker, problems with the design of the customized primer sets (for ASO-PCR), clonal evolution during the course of the disease, or the emergence of a new clone at the time of relapse [9].

1.2. Next-generation MRD monitoring by high-throughput sequencing

Repertoire sequencing (RepSeq) involves detailed sequencing of a lymphoid population, focusing on the V(D)J recombinations [10]. Several recent studies focused on MRD quantification by high-throughput sequencing (HTS) in patients with acute and chronic lymphoid disorders [11,12]. Overall, HTS revealed higher sensitivity and precision in bone marrow and peripheral blood samples compared with multi-parameter flow cytometry or ASO-PCR. Notably, low MRD positivity by HTS was found in samples that were MRD-negative by standard methods [12]. In patients with ALL, MRD monitoring by HTS could predict the risk of relapse in both childhood [12,13] and adult ALL [14], and both pre- and post-allogeneic stem cell transplantation [15,16]. One of the benefits of the high sensitivity of HTS was the possibility of detecting MRD in peripheral blood, instead of bone marrow [15]. The use of HTS for MRD quantification may also allow the molecular heterogeneity of the lymphoid repertoire to be investigated. Interestingly, Kotrová et al. showed that patients with lower immunoglobulin heavy chain (IgH) diversity at day 78 had significantly lower relapse-free survival rates [13].

1.3. Challenges

HTS is a valuable technique at the time of ALL diagnosis, and allows the sequencing of clonal recombinations of multiple Ig/TR genes by pooling several PCR systems in one experiment. It is therefore possible to identify leukemia-specific sequences from one or more clones in one or more PCR systems in a single step, without the need for Sanger sequencing [17].

HTS is an accurate, reliable, fast and relatively affordable technology, applicable to many routine practices. It is expected to improve MRD monitoring and enable the detection of multiple clones and subclones simultaneously, faster and with higher sensitivity than standard methods based on V(D)J sequencing. The use of HTS is also expected to reduce the failure rate of MRD quantification associated with clonal evolution or the emergence of new clones [18–20].

ASO-PCR is a standardized technique that usually focuses on 1–3 clones, while HTS is an emerging field, able to evaluate millions of sequences in a single experiment. The study by van Dongen et al. [1] recently listed the key challenges for high-throughput MRD technologies as broad availability, easy implementation, applicability in most patients, adequate sensitivity, fast, affordable, and standardized, with quality-assurance programs. It is essential to establish standardized protocols to limit variability among laboratories. These protocols should include the use of primer kits designed for multi-system PCR.

The quantity of data generated also raises several challenges. From a practical point of view, laboratories must increase their capacities to store and process the terabytes of data generated each year. Finally, it is also necessary to develop software for processing the data and synthetizing the results in an easily understandable way for its transfer into clinical practice.

1.4. Repertoire sequencing (RepSeq) analysis software

The high-throughput analysis of B or T cell receptor DNA sequences cannot be achieved using regular mapping software. This is because of the specificity of the problem, with a read usually containing a recombination between three genes with dozens of potential insertions and deletions [21]. These recombinations, non-templated insertions, deletions, and somatic hypermutations need to be integrated into the design of the algorithm.

RepSeq software usually performs optimized comparisons of HTS reads against a germline database. The international ImMunoGeneTics information system (IMGT®, http://imgt.org/) has developed several tools for the in-depth analysis of V(D)J recombinations [22–25]. New software to deal with HTS data (i.e., millions of sequences) has recently been developed, including [26], IgBlast [27], Decombinator [28], miTCR [29], Vidjil [30], TCRKlass [31], miXCR [32], and IMSEQ [33]. Several of these software tools cluster HTS reads into clones allowing relative quantification. However few of them offer interactive analysis features. The Vidjil open-source platform enables an autonomous usage in an immunology or hematology lab, from raw sequence files to analysis, annotation and storage [34].

2. Material and methods

We conducted HTS-based MRD monitoring of IGH and/or T-cell receptor gamma chain (TRG) loci in patients with ALL. Data were analyzed using the Vidjil web application [30], which is directly usable by hematologists without the need for any dedicated bioinformatics support. This approach allowed us to identify new emerging clones at the time of relapse that were different from the main clones highlighted at diagnosis. We also assessed the reproducibility of our approach and compared our results with conventional ASO-PCR.

2.1. Patient selection

The clonality of 11 pediatric patients (8 B-ALL/3 T-ALL, 5 female/6 male, 3–19 years) was studied retrospectively in 43 bone-marrow samples (11 diagnosis and 32 follow-up time points, see Supplementary Material, Table 1). Approval for this study was obtained from the Institutional Review Board of CHRU of Lille (CSTMT093) and informed consent was obtained from the patients in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient or their parent or legal guardian.

2.2. DNA extraction, library preparation and HTS

We sequenced *TRG* (VGf1-10) and/or *IGH* by HTS in 11 patients with ALL at diagnosis and at several follow-up points. For every sample, 5×10^6 cells were extracted from bone marrow or blood using a QIAamp® DNA Mini Kit (Qiagen). DNA was measured using a Nanodrop system®, and 500 ng were amplified by PCR with non-fluorescent BIOMED-2 TRG and/or IGH primers (Supplementary Material, Table 2). A total of 58 (11 diagnosis and 44 follow-up) samples were analyzed.

The samples were initially purified with MinElute® PCR Purification Kit (Qiagen). Libraries were prepared using an Ion Xpress™ Plus gDNA and Amplicon Library Kit (Life Technologies). Each library was barcoded using the Ion Xpress™ Barcode Adapters 1-96 Kit. The concentration of each barcoded library was controlled

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