EDITORIAL

Running chromosome analysis in leukemia or not, upfront or later?

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In a letter by Li *et al.*,[1] published in this issue of *Haematologica*, an alternative clinical cytogenetic testing strategy is proposed to help overcome the shortage of skilled cytogenetic technologists. The proposed workflow integrates multiple fluorescence *in situ* hybridization (FISH) panels, genomic microarray, next-generation sequencing (NGS) panel-based gene mutation profiling and RNA fusion testing, followed by reflex karyotyping in some patients to confirm or define the chromosome and genetic abnormalities indicated by the initial tests. The results obtained with this workflow were compared with those of traditional karyotyping analysis of a pediatric leukemia cohort, consisting predominantly of patients with B-cell acute lymphoblastic leukemia. The intention was to help overcome the shortage of skilled cytogenetic technologists in clinical diagnostics by skipping chromosome analysis upfront in a vast number of cases, while improving the turnaround time. This pilot strategy may be applicable for some hematologic neoplasms, such as pediatric acute leukemia with simpler karyotypes or defined gains and losses, and those leukemias such as myeloma, and chronic lymphocytic leukemia, often with a low mitotic index in cell culture. It could provide a short-term benefit of requiring fewer trained technologists; however, this approach may in fact compound the challenges in the long-term in training new cytogenetic technologists and advancing the analytical skills of current cytogeneticists.

At many large cancer centers, numerous leukemia clinical trials are offered, and the eligibility for these trials and stratification to appropriate study arms often depend on cytogenetic findings, and molecular gene mutation profiling. Detecting recurring chromosome abnormalities and gene mutations listed in the current classification and guidelines, i.e., International Consensus Classification, World Health Organization 5th edition and National Comprehensive Cancer Network, is critical in clinical decision-making and management.[2-4] Whether a patient with newly diagnosed acute myeloid leukemia has a CBF leukemia, or a complex karyotype may guide entry into an appropriate clinical trial, as outlined in Beat AML and MyeloMATCH, which require these results by day 5 or sooner. Thus, a rapid workflow with appropriate integrated testing that includes karyotyping, selected FISH testing, and a rapid, small NGS panel for the most common gene mutations is in place in many cancer centers to meet clinical needs.

With prompt notification from clinicians and an efficient triaging and workflow in the laboratory, we provide a preliminary karyotyping result within 3 days for the majority of newly diagnosed patients with acute leukemia at the Memorial Sloan Kettering Cancer Center. Once an overnight culture of bone marrow or peripheral blood cells is set up, followed by harvesting, slide preparation, and chromosome banding the next morning, chromosome analysis is initiated in the afternoon to obtain a preliminary chromosome result. In our experience, performing karyotyping analysis together with a few selected FISH tests, i.e., to detect *KMT2A/MLL* translocations, t(9;22)/*BCR::ABL1* fusion, and *TP53* deletions, has been excellent in most cases of leukemia. Additional FISH tests may be performed to clarify uncertain karyotype findings or to rule out recurring chromosome translocations with breaks at the relevant loci. The concordance of karyotyping results and the findings of FISH and fusion studies was extremely high, with a few exceptions due to cryptic translocations, such as t(5;11)/*NUP98::NSD1*. On the other hand, certain recurring chromosome translocations or fusions, typically related to enhancer or promoter swapping or hijacking, such as inv(3)/t(3;3)/*MECOM* rearrangement, and immunoglobulin or T-cell receptor-related translocations are often under-detected by NGS-based assays, including RNA-sequencing. FISH testing may be run upfront for specific disease concerns, such as the *PML::RARA* fusion in acute promyelocytic leukemia, and is needed for some

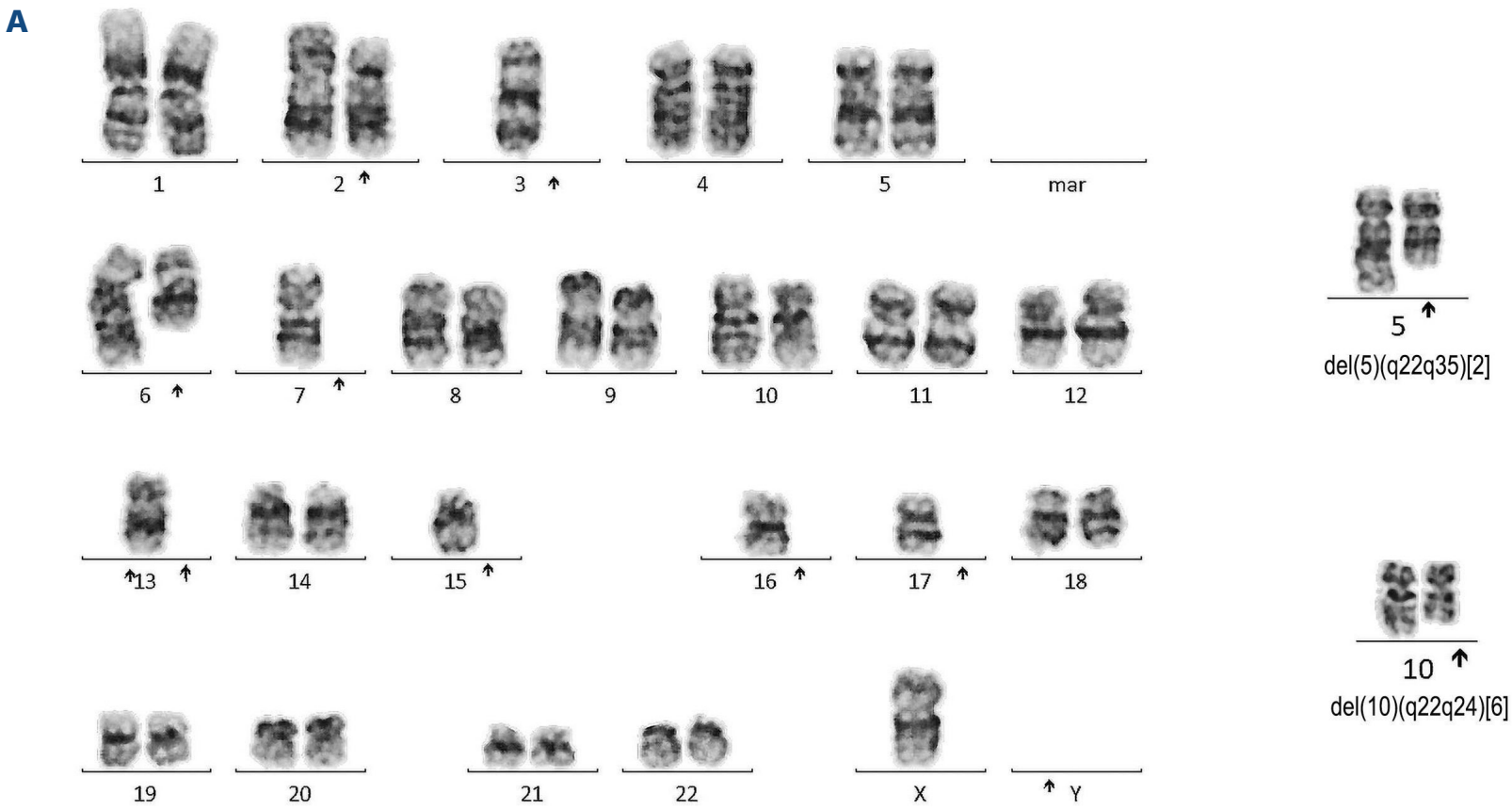

Figure 1. Coexistence of B-cell acute lymphoblastic leukemia and myelodysplastic syndrome in a 72-year-old male patient revealed by integrating chromosome and fluorescence *in situ* hybridization analysis and molecular testing. (A) Chromosome analysis of a bone marrow specimen detected a hypodiploid karyotype with clonal evolution, including doubling cells, and two cytogenetically distinct clones with deletion of the long arm of chromosomes 5 and 10. *TP53* and *TET2* mutations were detected earlier in a peripheral blood sample. Arrows point to loss of the relevant chromosomes and del(5q), and del(10q). (B) Fluorescence *in situ* hybridization testing confirmed del(5q) in granulocytes, supporting a diagnosis of myelodysplastic syndrome, despite minimal dysplastic morphological evidence. Two cells show del(5q) with one orange signal (EGR1, 5q31) and two internal control green signals (5p15.2). (C) Optical genome mapping study (circos plot) confirmed the hypodiploid clone with loss of chromosomes Y, 3, 7, 13, 15, 17 (red bar), der(2q;16q) and del(10q), but did not reveal del(5q). FISH: fluorescence *in situ* hybridization; OGM: optical genome mapping.

cryptic translocations, such as t(12;21*)/RUNX1::ETV6*, which is common in pediatric B cell lymphoblastic leukemia.

More importantly, with improved responses to chemotherapy and novel targeted treatments, many patients achieve a long survival; however, some of them are at risk of developing secondary hematologic neoplasms, such as a myelodysplastic syndrome or acute myeloid leukemia, following treatment for the primary cancer. These patients are often elderly and may present with a coexistent myelodysplastic syndrome. Detecting these diseases is critical, not only for a precise diagnosis, but also to guide management and treatment planning. In these situations, chromosome analysis is essential, and should be performed immediately.[5,6] As illustrated in Figure 1, chromosome analysis of a bone marrow aspirate specimen from a 72-year-old male detected a hypodiploid karyotype that is characteristic in B-cell acute lymphoblastic leukemia associated with a high risk and revealed two cytogenetically distinct clones with deletion of the long arm of chromosomes 5 and 10 (Figure 1A). FISH testing further confirmed the del(5q) in mature granulocytes (Figure 1B), supportive of coexistent myelodysplastic syndrome and B-cell acute lymphoblastic leukemia. Optical genome mapping confirmed the hypodiploid clone, the relevant translocations, and deletion of 10q but could not define the relationship of del(10q) to the hypodiploid clone, i.e., as a subclone or a separate clone as noted by chromosome analysis (Figure 1C). The del(5q) was not detected by optical genome mapping. This example nicely demonstrates some important technical features of karyotype analysis at the single-cell level, which may define clonal relationships, including clonal evolution, complexity, and heterogeneity, and pinpoint whether chromosome translocations or copy number alterations result from deletions, gains, or unbalanced translocations, which may involve gene fusions. It is particularly helpful in detecting early clones, or minor clones in myeloid neoplasms, such as myelodysplastic syndromes, characterized by only a few immature blasts in bone marrow or blood samples.

To provide a rapid cytogenetic result from samples from patients with newly diagnosed leukemia, it is necessary to have a combination of an efficient workflow within a triaging setting to select the test categories and targeted FISH testing menu, based on disease types, such as acute myeloid leukemia *versus* B-cell acute lymphoblastic leukemia, and disease status, along with hematopathology and flow cytometry findings, and cytogenetic history, if available. Receiving a fresh specimen quickly and including select cell growth factors in cell cultures will increase the success of chromosome analysis. Applying automation in various cytogenetic procedures, i.e., harvesting, slide preparation, and banding in karyotyping, and in post-hybridization washing in FISH testing will help to reduce staff time, and to ensure consistent quality.[7] Recently, some artificial intelligence-based karyotyping analysis modules, which rely on numerous algorithms and calibration, have been validated and applied in clinical cytogenetics diagnostics with an impressive improvement in chromosome analysis; only a few seconds are required to separate, identify, and compare the chromosomes. This technology will significantly help the cytogenetics laboratory to offer a rapid chromosome analysis result in many cases.

However, a precise chromosome analysis relies on skillful cytogenetic technologists who must examine, compare, and magically align chromosomes to match the banding patterns to define clones and their relationship. This can be achieved with extensive practice and work experience. Thus, there is an urgent need to advocate, introduce, and mentor new technologists to enter into this field, which for more than 50 years has been at the center in discovering recurring translocations in cancer, such as the t(9;22) in chronic myeloid leukemia, and t(8;14) in Burkitt lymphoma, defining their functional consequence, and developing targeted treatment.[8]

In summary, performing a high-quality karyotype analysis upfront, particularly in myeloid neoplasms, is the most efficient and economic strategy for a clinical cytogenetics laboratory. Integrating cytogenetic analysis with selected FISH tests, and NGS-based gene mutation profiling will provide an optimal service for clinical needs, particularly for various clinical leukemia trials. With additional molecular testing, such as optical genome mapping, and whole-genome sequencing, chromosome analysis will remain as one of the key genetic diagnostic technologies in the near future.

Disclosures

No conflicts of interest to disclose.

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