Editorial note:

Measurable residual disease (MRD) monitoring has emerged as an important indicator for risk stratification and treatment planning in patients with haematological malignancies. In the past decade, various techniques in measuring MRD have become available in Hong Kong. In this Topical Update, Dr. YIP Sze-fai provides an overview of the current techniques available for MRD monitoring. We welcome any feedback or suggestions. Please direct them to Dr. Alvin IP (e-mail: ihw426@ha.org.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Measurable residual disease (MRD) for Haematological Malignancy

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Introduction

Measurable residual disease (MRD) describes the application of assays for detection of submicroscopic level of residual disease burden which cannot be detected by morphology. Numerous studies have observed the association of MRD level and disease prognosis. It provides an objective parameter on the tumor burden, and guide stratified treatment including the application of haemopoietic stem cell transplantation (HSCT). Its ability to monitor disease and to detect molecular relapse enables preemptive therapy to prevent frank disease relapse [1]. For all these reasons, we see an increasing use of MRD in the field of haematological malignancy.

Different technologies are used for MRD measurement

1. Multiparametric flow cytometry (MFC)

MFC is commonly used for MRD detection in acute leukaemias. At diagnosis, the leukaemia-associated immunophenotype (LAIP) of the blasts can be determined by using a multitude of fluorochrome-labeled monoclonal antibodies against different cellular markers that aids identification of the leukaemic population as well as detecting the aberrant cellular marker expression. If the LAIP was not determined at diagnosis, a different-from-normal (DfN) approach can be used to detect the abnormal cells, as well as detecting any new or disappearance of

known phenotypic aberrancies [1,2]. With technological advancement, more fluorochromes are available and 8 to 12-colour panels are commonly used. Flow cytometry has the advantage of a short turnaround time which can provide timely results for clinical decision making. The sensitivity of MRD detection is at the level of 10^{-4} to 10^{-5}.

2. Next generation flow (NGF) for plasma cell myeloma

Novel Euroflow-based next generation flow (NGF) approach is being developed for highly sensitive and standardized MRD detection, primarily in plasma cell myeloma, using an optimized 2-tube 8-color antibody panel [3]. The NGF approach uses tools and procedures that are developed by the EuroFlow Consortium for a standardized sample preparation, antibody panel (including the type of antibody and fluorochrome), and automatic identification of plasma cells against reference databases of normal and patient BM using Infinicyt software. The sensitivity of MRD detection is close to 10^{-6}.

3. Quantitative polymerase chain reaction (qPCR) technique

a. Detection of leukaemia-specific fusion transcript

The MRD can be measured by detecting the amount of leukaemia-specific fusion transcripts present. The classical example is BCR-ABL1 fusion in chronic myeloid leukaemia (CML). The sensitivity is higher than that of flow cytometry, reaching the level of 10^{-4} to 10^{-6}. The test is relatively easy to be performed in hospital service laboratory. The MRD is represented in a ratio of normalized copy number of the fusion transcript and the control gene transcript (e.g. ABL1). For CML monitoring, an international scale (IS) ratio is developed for standardization of results among different laboratories [4]. Yet, this method is limited to cases with targetable fusion transcripts available for detection.

b. Allele-specific oligonucleotide (ASO) qPCR for immunoglobulin (IG) or T cell receptor (TCR) gene rearrangement

ASO qPCR can be employed to detect the disease-specific sequence of rearranged IG gene or TCR

gene in the sample. The sensitivity of this method is 10^{-4} to 10^{-5}. It is applicable to most of the cases of acute lymphoblastic leukemia (ALL) and plasma cell myeloma as long as a disease-specific rearrangement can be determined by sequencing. Patient-specific primers would need to be designed for each case. It has a disadvantage that if there is a clonal evolution, the disease-specific rearrangement can be lost and a false-negative result can be generated.

4. Digital droplet polymerase chain reaction (ddPCR)

In ddPCR, the sample is compartmentalized into very large number of separate small volume reactions. As a result, either zero or one target molecule could be detected inside any individual reaction. Thermal cycling would be performed to endpoint using same primer and probes as qPCR. Any target-containing compartments will become brightly fluorescent while compartments without targets will have only background fluorescence. Total number of 'positive' reactions is equal to the number of original target molecules in the entire volume, and the total number of reactions multiplied by the individual reaction volume equals the total volume assayed. Therefore, ddPCR provides an absolute quantification of the target molecules. The ddPCR has the advantage of very high sensitivity of $\sim 10^{-6}$, does not require a standard curve unlike qPCR, and is tolerant to PCR inhibitors due to small partition volume. The application of ddPCR includes monitoring of NPM1 and ASO IG or TCR gene rearrangement [5,6].

5. Next generation sequencing (NGS)

NGS is a robust method to perform multiple sequencing in parallel which can also be used for MRD detection apart from the detection of mutations that are of diagnostic, prognostic and therapeutic importance. For MRD detection, the LymphoTrack platform can be used to detect disease-specific IG or TCR gene rearrangements. The sensitivity of the method can be up to 10^{-5} or higher [7]. A diagnostic sample would be required for identification of the disease-specific rearrangement. However, this method is also capable of detecting clonal evolution.

Reference

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