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CURRENT DIAGNOSTIC METHODS FOR HEMATOLOGICAL MALIGNANCIES: A MINI-REVIEW

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ABSTRACT

Hematological malignancies are a group of diseases where several cancers are generated from blood-forming tissues/cells of the body. The cancer groups are highly heterogeneous, in that some diseases are biologically distinct in terms of protein expression, molecular progression, and biomarker characteristics, whereas other diseases are clinically variable in terms of treatments and prognostics. Recent improvements in neoplasm classifications have made significant in-roads towards a greater understanding of affected lineages, therefore hematological neoplasms can be divided into two primary categories; myeloid and lymphoid neoplasms, and each lineage can be further subdivided into several disease types. While the complexity of this disease group cannot be questioned, facilitating a rapid diagnosis and associated therapeutic intervention are key to reducing disease mortality and morbidity in patient groups. However, current diagnostic approaches for this disease group are equally complex and heterogeneous, therefore this comprehensive review aimed to catalog and appraise hematological characterization techniques to facilitate a greater understanding of disease classification, diagnosis, monitoring, and importantly, selection of appropriate therapies for hematological malignancies.

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Introduction

Hematological malignancies are clinically and biologically heterogeneous diseases [1]. The World Health Organization (WHO) has classified hematological neoplasms according to affected lineages, therefore hematological neoplasms are divided into two main categories: myeloid and lymphoid neoplasms. Each lineage is further subdivided into a wide range of disease [2] (Figure 1).

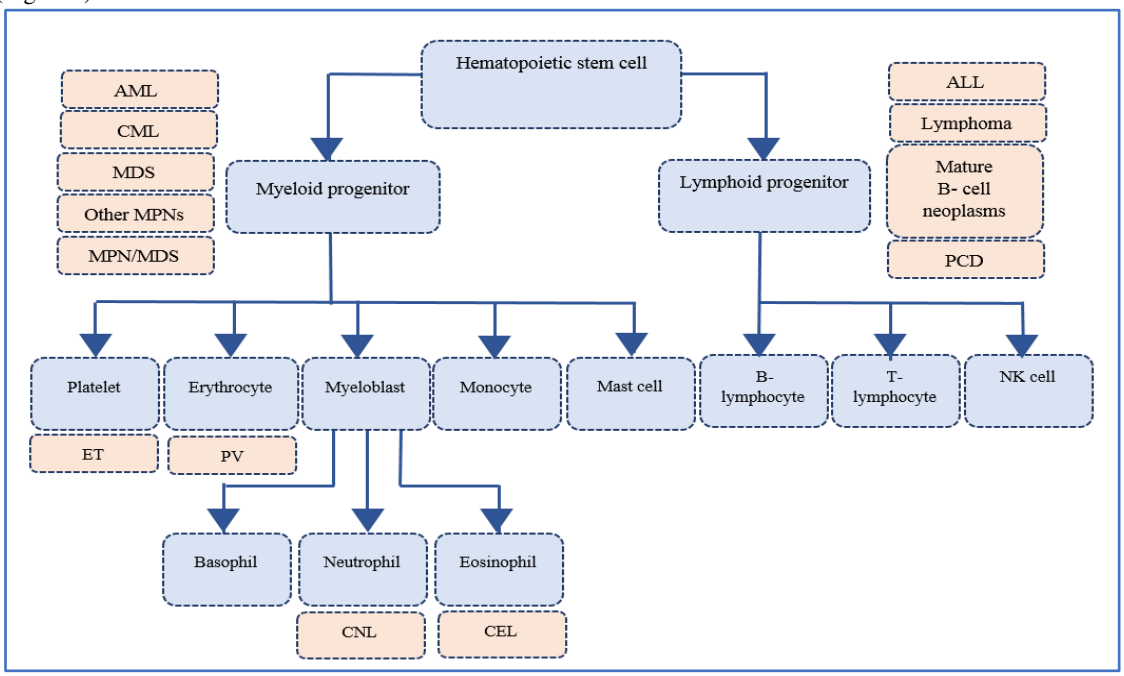

Figure 1: Hematological malignancies classification

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Abbreviations: Acute myeloid leukemia (AML), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs), acute lymphoblastic leukemia (ALL), plasma cell dyscrasia (PCD), natural killer (NK), essential thrombocytosis (ET), polycythemia Vera (PV), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL)

In terms of clinical heterogeneity, affected patients may require intensive treatments and therapeutic care, whereas other patients do not require such intensive therapies. Other patients will respond well to particular treatments, whereas other patients will develop resistance [3]. Similarly, biologically heterogeneous disease encompasses those patients where different molecular and biological etiologies account for variable disease characteristics, i.e. variability at the molecular level, molecular mediated resistance to therapies, and aberrant biomarker expression.

While it is accepted that disease classification, diagnosis, monitoring, and selection of appropriate therapies unequivocally contribute to the rapid and timely treatment of hematological malignancies, the associated diagnostics techniques used to characterize and diagnose these diseases are equally diverse and heterogeneous. To this end, we have reviewed and revised current methods available to bench scientists, clinicians, and diagnosticians to provide a comprehensive overview of techniques available in the fight against hematological malignancies (Table 1). This review is also timely given the huge and recent upsurge in 'omic' technologies in recent years [4]. We also emphasize recent advances in molecular techniques and how they are revolutionizing disease diagnostics.

Table 1: Laboratory techniques used in hematological malignancy diagnostics

Techniques
Complete blood counts (CBC)
Microscopic examination (blood film)
Bone marrow biopsy/aspirate
Cytochemistry
Histology
Immunohistochemistry
Flow cytometry (immuno-phenotyping)
Cytogenetic analysis
Fluorescence *in situ* hybridization (FISH)
Molecular genetic testing
Whole-genome scanning
Sequencing methods
Gene expression profiling

Complete Blood Count and Microscopic techniques

Upon suspicion of disease, a complete blood count (CBC) is typically the first step in the potential diagnosis of hematological malignancies. Typically performed using an automated cell counter, incorporating laser technologies and microfluidics, the process automatically quantifies red blood cells (RBCs), white blood cells (WBCs), and platelets. Under abnormal conditions, the instrument flags the presence/quantification of abnormal blood cells counts, i.e. blast cells or precursors. Abnormal CBC reports must be confirmed by blood film examinations [5]. Microscopic examination or blood film analyses allow for the determination and observation of cell abnormalities, in terms of cell morphology and aberrant staining characteristics under a microscope [6, 7]. This technique is somewhat limited in terms of definitive hematological malignancy diagnostics, due to differentiation difficulties between abnormal and normal cell morphology. This situation is particularly relevant for blast cells, as it is difficult to differentiate between myeloblasts, monoblasts, and lymphoblasts, based on morphology alone. However, this approach cannot be viewed as a standalone technique, as it often provides preliminary evidence for a differential diagnosis that requires confirmation by other, more complex techniques [8].

Bone Marrow examination and Histocytochemistry

Bone marrow (BM) biopsy/aspirate analyses are two complementary techniques essential for the diagnosis of some hematological malignancies [9, 10]. During aspirate sample collection, a highly specialized needle collects a sample from the bone, which contains a small amount of BM cells and fluid, whereas a bone marrow biopsy collects a tiny piece of the bone itself. Different downstream diagnostic tests can then be applied to these samples, such as morphological examination, cytochemistry, flow cytometry, cytogenetic analyses, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH). Cytochemistry assesses the micro-anatomical biochemical components of cells and organelles so constituents can be localized by different chemical components, and potential cell transformation disease dynamics assessed. While this technique is a preliminary step in deciding a definitive diagnosis, its role has become limited as other more advanced techniques have been developed [11, 12]. Cytochemical technology depends on the staining of specific cells under cell membrane dynamics and is used as a microscopic technique to examine sections of tissues/cells, other than BM. It is an important technique used

to diagnose several types of hematological malignancy [11]. Immunohistochemistry is a component of the histological process and identifies specific tissue molecules using commercially available antibodies targeting specific proteins implicated in hematological malignancies [6, 13]

Microfluidics and cell counting

Flow cytometry (immuno-phenotyping) is an advanced cell counting/quantifying approach with many technological advantages; the instrumentation is small and compact, it is a cost-effective approach to disease diagnostics, easy to use, specific and sensitive, with a rapid turnover time for definitive diagnoses [14-16]. The technique generates qualitative and quantitative data on different cell types, it measures cell physical and chemical properties, identifies the maturation stage of cells (i.e. mature cells and immature precursors), cell type (i.e. hematopoietic and non-hematopoietic) and cell lineages (i.e. erythroid, lymphoid or myeloid). The technique is also highly specific in that it detects, identifies, and quantitates specific hematological markers such as proteins, enzymes, or antigens expressed on cells. Once processed, cytometry data provides accurate quantitative information on specific cell types and groups that can help confirm disease status [17, 18]. A recent study highlighted the relevance of flow cytometry to hematological malignancies. Expression analysis of the surface immunoglobulin (Ig) light chain, which includes kappa (κ) and lambda (λ) chains, provided evidence for malignant B cell tumor proliferation in patients with persistent lymphocytosis [19].

Whole chromosome techniques

Cytogenetics (conventional cytogenetics, karyotyping, or chromosome analysis), analyzes chromosomes during metaphase growth when chromosomes are becoming more condense, visible, and distinguishable. Chromosome spreads are analyzed using differential staining techniques under a microscope, to help identify and quantify chromosomal numbers, aberrant morphologies, and chromosomal abnormalities implicated in the etiology of hematological malignancies [20]. Fluorescence *in situ* hybridization (FISH) is also used to detect structural chromosomal abnormalities and minimal residual disease (MRD); the detection of small numbers of neoplastic/leukemic cells that remain during or after treatment, when a patient is in a remission state. The technique detects MRD at very sensitive limits since MRD is the main cause of cancer relapse [20, 21]. Similarly, the development of FISH prognostic panels has greatly facilitated CLL diagnoses, i.e. the identification of five different mutational chromosomal states (del 17p, del 11q, trisomy 12q, normal karyotype and del 13q) allows CLL patients to be classified with good or poor prognoses. However, not all patients fit these qualifying criteria, therefore more FISH prognostic markers are required [22, 23].

Molecular techniques

Molecular genetic testing techniques include a large group of analytical assays, including Southern blotting, qualitative/quantitative PCR, and real-time polymerase chain reaction (RT-PCR) [24]. While the role of Southern blotting techniques in the preliminary diagnosis of hematological malignancies has become increasingly limited, the more molecular approaches, such as PCR and RT-PCR are widely used in diagnostic laboratories.

Traditional PCR and RT- PCR [25] are common molecular diagnostic techniques, with several key advantages over other diagnostics approaches; tiny amounts (ng) of DNA are required, dividing cells are not used for testing, and the techniques detect many chromosomal abnormalities, such as translocations, deletions, duplications and MRD detection [26, 27]. However, these techniques can only detect specifically targeted abnormalities, therefore in some instances, they provide limited information. MRD measurement is extremely important in monitoring hematological malignant patients and assessing their response to treatments [28]. Both RT-PCR and flow cytometry are routinely used for this approach [28, 29]. The limit for MRD detection by both flow cytometry and RT-PCR is 10^{-4}-10^{-5} copies, therefore both techniques lack sensitivity in MRD detection, especially at low levels [28]. Similarly, RT-PCR is not an ideal approach for marker identification, and sometimes it generates false negatives results, especially in cases of clonal evolution, in ALL cases [29].

Both RT-PCR and next-generation sequencing (NGS) (see below) for MRD detection in ALL patients show similar sensitivities [29]. While RT-PCR, flow cytometry, and NGS techniques are diagnostically useful, they require considerable improvements to sensitively detect MRD at minute levels. Once technologically achieved, these diagnostic tools could provide sensitivities that ultimately translate to decreased malignant relapses in patients.

A novel PCR application for the specific and sensitive quantification of MRD is a high annealing temperature - polymerase chain reaction (HAT -PCR). Recent studies have shown that increased annealing temperatures during HAT-PCR improves MRD sensitivity and quantification by approximately 2 log differences, when compared to conventional RT-PCR and flow cytometry techniques [28]. HAT-PCR appears to be a simple and cost-effective technique, with MRD quantifying capabilities down to 5 x 10^{-7} copies [28, 30].

Genome approaches

Whole-genome scanning techniques include microarray analyses, comparative genomic hybridization (CGH), and single nucleotide polymorphism (SNP) arrays [31, 32]. The main principle behind microarrays is the hybridization between two DNA strands (disease sample and the control). The fluorescently labeled sample sequences bind to the control and generate a signal that depends on the strength of hybridization between strands. The expression patterns of healthy and diseased genes are compared to genes responsible for a particular disease [33]. Microarray analyses can simultaneously process thousands of genes in one experiment; therefore, it is a convenient, high-throughput molecular technique, with a rapid turnaround. Thanks to its

ability to resolve changes in smaller gene regions, the technique is favored over the FISH approach. While these attributes are advantageous in terms of rapid disease diagnostics, microarray analyses generate huge data loads, making it difficult to ascertain structural arrangements and detect low mosaicism levels in some diseases [31, 32]. Microarray analysis is helpful in molecular diagnostics, the evaluation of patient prognosis, and influences the appropriate selection of relevant therapies (Severson 2016). In terms of CLL, SNP array profiling has contributed to the identification of deletion chromosomal mutations such as del 15q, del 8p, del 22q, and gain of function mutation, such as 20q and 2p [34].

CGH detects the gain or loss of important chromosomal fragments harboring genes involved in a disease, such as hematological malignancies. SNP analyses are used to detect single nucleotide changes (substitution) between two sequences. They are beneficial in genotyping analyses, distinguish between heterozygosity and homozygosity, the detection of gain or loss mutations, and the identification of acquired somatic uniparental disomy [24]. Gene expression profiling using microarray analyses have been used to measure changes in gene expression to better understand regulatory mechanisms. Expression patterns of healthy and diseased genes are compared to study genes responsible for the disease. This technique is helpful for diagnoses, prognostic evaluations, and the selection of appropriate therapies [20].

Sequencing approaches

Next-generation sequencing (NGS) is a high-throughput sequencing method that rapidly sequences DNA or RNA samples. The approach has improved our knowledge and understanding of hematological malignancies, particularly for CLL, where additional gene mutations have been detected in *SF3B1, BIRC3, NOTCH1, MYD88, SAMHD1, DDX3X, BIRC3, BRAF, MED12, FBXW7, XPO1, CHD2* and *KLHL6* [35]. NGS uses many different applications such as whole-genome sequencing, whole-exome, and targeted sequencing. Each approach has its advantages and disadvantages (Table 2).

Table 2 Comparisons between different NGS applications

	Whole-genome sequencing (WGS)	**Whole Exome Sequencing (WES)**	**Targeted sequencing**
Sequencing region	The full genome	- Exome regions (Protein-coding regions) - Coding regions contain 85% of mutations that relate to different diseases, but it represents 1% of the genome.	Specific targeted regions
Properties	- Expensive - Massive data generation which needs intensive analysis - Data storage is challenging	- Cost-effective - Less data so less analysis required	- Highly cost-effective - Least data so minimal analysis required
References	[36, 37]	[36, 38-40]	[36]

The relationship advances in laboratory method improvements in hematological malignancy diagnostics

Developments in laboratory techniques have to generate increased understandings of disease processes from diagnostic, prognostic, monitoring, and therapy selection perspectives. In considering the clinical and biological heterogeneity of CLL, the identification of diagnostic and prognostic markers, and potential treatment side effect, modern laboratory techniques must be robust and comprehensive for physicians to dispense their care. Such diagnostic contributions will undoubtedly provide for better decision making for patient treatments, what kind of treatments to administer, how to allocate treatments for different hematological diseases, and overall improved patient well-being [3, 41].

Conclusions

Multiple diagnostic methods are required to provide optimal diagnosis, monitoring, and treatments for hematological malignancy patients. Improvements in diagnostic methods have massively contributed to hematological malignancy characterization in recent years, especially with the advent of the 'omics' revolution. These approaches have increased our understanding of these diseases, improved diagnostic criteria, improved predictor and prognostic markers, and disease classification. Therefore, scientists at the bench must continue to explore new technological avenues to maintain their biological curiosity, while clinicians and physicians keep abreast of new molecular developments in hematological therapeutic areas.

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