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Research Article

Blood Diseases and *In vitro* Methods and Technologies

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Abstract

In vitro diagnostics, which appraise bodily functions or illnesses by extra-corporeal examination of materials like tissue and blood, have become increasingly important in our daily lives due to increased health consciousness and the advancement of new technology. Moreover, in addition to therapeutic applications, in-vitro diagnostics may also be used to screen for and prevent diseases. Laboratory methods and techniques have changed and have forwarded movements and developed. Laboratory hematology has a role in all aspects of patient care, from basic screening to more complex investigations in third-party care services. It also includes non-laboratory services like bone marrow transplantation and blood transfusion. Diagnosis of hematology patients correlated to the target and material that they want to find and rectify the reasons of illness, such as molecular assays that tested genetic materials and macromolecules, biochemistry assays that detected the metabolic disorders, which differentiated factors by biochemical properties and interactions, and biophysical assays that utilized the light and microscopically analyzed tools for tests. In this systematic analysis, the contemporary published articles that discuss the diagnosis of hematological diseases and demonstrate the factors of diseases that correlate with the Laboratory methods of assays. And want to indicate the correlation between the factor of illness in diagnosis analysis and laboratory methods and techniques that are utilized in diagnosis

Introduction

In vitro diagnostics, which evaluate illnesses or bodily functions by extracorporeal examination of materials like blood and tissue, have become increasingly significant in our daily lives due to the advancement of new technology and increased health consciousness [1,2]. In addition to therapy, *in vitro* diagnostics may be used to screen for and prevent diseases. This is particularly true in the prevention and control of the current COVID-19 pandemic. Patient acceptability and time consumption are dominated by *in vitro* diagnostics [3].

Focusing on blood cells, blood-forming organs, and the diseases of organs depends on hematology sciences. In the human body, each day, millions of erythrocytes, platelets, and leukocytes are produced and replaced by blood cells that are lost within the normal cell cycle [4]. Moreover, the laboratory diagnosis is one of the most critical steps for better diagnosis of different diseases than physical diagnosis, especially in hematology patients and their treatment. The hematologic diseases which have significant problems in health such as mild bleeding disorders [5], coagulation disorders [6], hereditary haemochromatosis [7], inherited platelet disorders [8], thromboembolic diseases [9], thalassemia [10], hematologic malignancies myelodysplastic/myeloproliferative neoplasms, myeloid/lymphoid [11], leukemias [4] and others that may be diagnosis in molecular, microscopic, biophysical, biochemistry assays and help the treatment departments to cure better by finding the reasons of diseases.

Laboratory hematology has a role in all aspects of patient care, from basic screening to more complex investigations in third-party care services. It also includes non-laboratory services like bone marrow transplantation and blood transfusion. Additionally, hematology labs support specialist facilities that offer integrated care for common hematological illnesses that call for multidisciplinary services and specialized experience, as well as national public health initiatives that target blood disorders. The function of the laboratory has evolved from that of a testing facility to that of a collaborator in healthcare, and technological advancements in hematology and personalized medicine have increased the dependence on laboratory data. But this is especially difficult in nations with low resources [12]. In this article, collected the hematological

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laboratory methods and techniques assays which indicated and measured the diseases factors with various types of assays which done *in vitro* such as molecular, biochemistry, biophysical, chromogenic, and microscopic, which done apporxcimnetally in last two decades, and one of facilitated way for *in vitro* researchers who want to find out the different laboratory results in among different homological diseases.

Method and materials

This systematic review article was formed by information which collected from acceptable international hematological and medical journals such as Google Scholar, PubMed, ScienceDirect, International Journal of Laboratory Hematology, etc. The flowchart (Figure 1) demonstrated more details about the steps of filtering and excluded articles that do not depend on electronic methods, indicating the number of articles used in this systematic review.

Types of laboratory methods

In this review, several articles are studied to indicate various methods and technologies (Table 1) that are utilized in hematology disease laboratory diagnosis, such as molecular biology testing, biochemistry testing, biophysical testing, chromogenic assays, and many others that are used in investigation and diagnosis in recent years.

Biophysical technology

The application of physical scientific concepts and methods to the study of biological systems has advanced significantly over the last 60 years, yielding amazing new understandings of the molecular foundation of life [13]. The process of transferring concepts and methods from physics to biology, which gave rise to the field of biophysics, has been repeated using a variety of other techniques, such as mass spectrometry (MS), electron microscopy, NMR spectroscopy, and a variety of optical spectroscopies, such as circular dichroism and various fluorescence measurements. Each of these techniques has seen dramatic methodological advancements, similar to X-ray crystallography, and its impact on structural biology has been revolutionary. A selection of recent articles in Annual Review journals provides insight into the variety of current applications of these techniques. As a result, for instance, cryo-electron microscopy can now produce the structures of even very large molecules and complexes at atomic resolution without the need for crystallization; this was thought to be impossible for many years, but as is frequently the case, a combination of new technology and scientific intuition has produced significant advancements [14]. Macromolecular structures in the solution environments where they function have been determined by NMR spectroscopy, and their conformations in other environments, such as membranes and pathological aggregates [15], have been probed through the use of specialized solid-state techniques. MS has now progressed to the point that it is possible to examine the structural characteristics of huge biological complexes in their functional settings [16]. The idea that optical methods could not reveal information about structural features smaller than the wavelength of light has been proven to be false by the development of super-resolution techniques [17]. Optical techniques, especially those involving fluorescence, have also advanced significantly, allowing them to be applied at the single-molecule level [18] and to produce images at a much higher resolution than was previously thought to be possible.

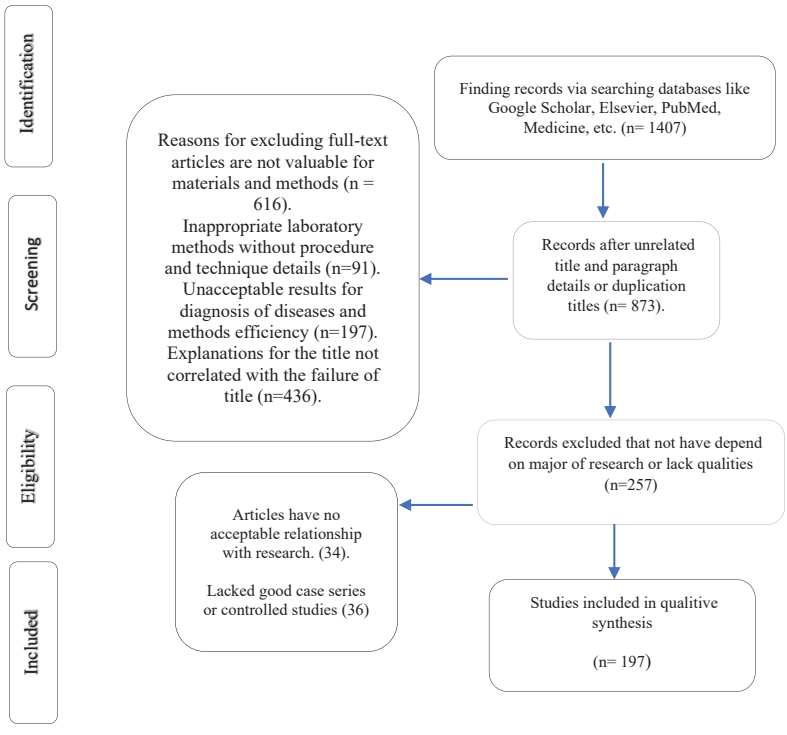

Figure 1: Flowchart of data extraction and search strategy finding.

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A short time after the initial structures were determined by X-ray diffraction, it was realized that proteins with great sequence similarity were probably similarly structured. In fact, the creation of a model of α-lactalbumin from that of lysozyme, whose function is entirely different but whose sequence is very similar, was a very early example of molecular homology modeling [19]; the viability of this approach was confirmed by the good agreement between this model and the experimental structure when it was determined. Since then, it has become possible to comprehend the relationships between sequence and structure in greater detail due to the massive increase in the number of structures in the Protein Data Bank and protein sequences in UniProt. This has allowed for the modeling of numerous unknown structures and, in fact, the creation of novel amino acid sequences that fold into particular structures [20]. Instrumentation advancements, from the building of ever-more powerful X-ray sources to the establishment of high-throughput sequencing facilities, have greatly benefited all of these chances. Furthermore, the use of simulation techniques that provide insight into the characteristics of macromolecules that are difficult to define by experiment, such as many aspects of dynamic behavior and reaction mechanisms, has been made possible by the rapid and ongoing advancements in computer power [21,22].

Microscopic technology

The foundation of hematological diagnostics is a growing level of precision in cellular and molecular analytical methods. A fundamental component remains the accurate interpretation of bone marrow and blood smears seen under an optical microscope [23]. The standard technique for analyzing hematological cells on peripheral blood and bone marrow aspirated smears, both qualitatively and quantitatively, is still an optical microscope. The variations in the nomenclature used to identify blood cells (BCs), the lack of staining techniques for the preparations to be examined under the microscope and a harmonized standardization of the preparation, and the subjectivity and methodological approach's variability [23] due to the degree of individual skill and experience under the microscope are all factors that limit the diagnostic effectiveness of the procedure [24].

The European Leukemia Net WP10.14 and the Working Group on Morphology of Myelodysplastic Syndrome (ISWMDS) [25-27] are two organizations that conduct international harmonization initiatives on blood cell morphology. Even though the World Health Organization [28], International Council for Standardization in Hematology (ICSH) [29,30], and Clinical and Laboratory Standard Institute (CLSI) [30] have established international guidelines and operational standards, inter-operator variability is still a critical factor [31] that necessitates additional training and group harmonization procedures. In the first edition of the WHO classification of hematopoietic neoplasms, the qualitative and quantitative microscopic diagnostic criteria of the FAB classification were revised, put into practice, and included. In a later edition, they were verified [32]. Specific qualitative and quantitative criteria have been explained: (i) For blasts, with precise indications on cytochemical aspects and morphological aspects and the minimum value required for the acute leukemia definition; and (ii) for dysplasia, with precise indications on the morphological criteria for each myeloid lineage and the minimum value required for the lineage dysplasia definition. Furthermore, depending on the situation, different quantitative criteria should be used to define lineage dysplasia. For example, the threshold for myelodysplastic syndromes and AML with dysplastic-related alterations is 10% and 50%, respectively [23,33].

Molecular technology

Isolating, identifying, and modifying genes is the fundamental idea of molecular techniques. Nucleic acids are extracted, isolated, and then DNA, RNA, and proteins are separated using ribonuclease, proteolytic enzymes, and various detergents [34]. As the gold standard for diagnosing some diseases, molecular diagnostics has gained a lot of recognition. The broad adoption of certain lab-based tests is, however, hampered by their reliance on large, costly equipment, particularly in settings with limited resources, such as centralized laboratory facilities, funding, and skilled people [3].

Result and discussion

Statistical analysis of methods and technologies

According to the qualitative statistics and information gathered from this assessment of techniques and methodologies, the materials of analysis that are examined at various levels are what determine the diagnosis of illness variables. Hematological diagnosis is based on more accurate cellular and molecular analytical techniques. A critical component remains the accurate interpretation of bone marrow and blood smears seen under an optical microscope [23]. The gold standard for both qualitative and quantitative studies of hematological cells on peripheral blood and bone marrow aspirate smears is still an optical microscope. Gene isolation, identification, and alteration are the goals of the other technique, which is based on molecular procedures. Following nucleic acid extraction and isolation, DNA, RNA, and proteins are separated using ribonuclease, proteolytic enzymes, and various detergents [34]. Molecular diagnostics is now widely regarded as the gold standard for diagnosing certain diseases.

Usage of methods in diseases

The types of laboratory diagnosis depend on the target of diagnosis that wants to find such molecular assays to diagnose the smallest parts of cells, like protein, DNA, and RNA [34], and microscopic analysis is utilized for morphological testing. Hematological diseases, including red blood cell membrane disorders, can be diagnosed *in vitro* by combining different types of techniques and methods, such as biophysical measurements [35-37], molecular tests [38-41], microscopic [42,43], physical, and biochemical tests [35,38,44-47]. This depends on the validity of the techniques and methods used and the length of time needed for diagnosis. This article

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gathered the most recent articles published in recent years to find the correlation between diagnostic factors and diseases. As an example, hepcidin, the iron regulatory factor, is another example that may be diagnosed using a variety of techniques, including chromogenic assays [48], antibody-based assays [49-55], biophysical measures assays [56,57], and biochemical tests [58]. However, other techniques are only employed to indicate the use of particular hematological materials, such as WCX-TOF MS, which indicates the use of hepcidin [55,58].

Conclusion

As a conclusion of this study, *in vitro* techniques and methods of diagnosis of hematological diseases are different and various, and they are correlated to targets and factors that researchers or doctors want to figure out, such as molecular detection, biophysical assays, and biochemistry, each of which has its own specific characteristics and validation.

Data availability statement

The data presented in this study are open sources available in Science Direct, Google Scholar, PubMed, Laboratory hematology, and other international journals.

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