EVALUATION OF THE HEMAVISION-28Q FUSION TRANSCRIPT FOR ACUTE LEUKEMIA SCREENING PANEL

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EVALUATION OF THE HEMAVISION-28Q FUSION TRANSCRIPT FOR ACUTE LEUKEMIA SCREENING PANEL

By

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DECLARATION

I hereby certify that this dissertation is based on my own research and investigations, except where otherwise noted and properly acknowledged. I also confirm that it has not been submitted, in whole or in part, for any degree at Universiti Sains Malaysia or any other institution. I grant Universiti Sains Malaysia the right to utilize this dissertation for teaching, research, and promotional purposes.

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(NOWSHIN TARANNUM)

Date: 27 January 2025

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ABBREVIATIONS

AML	Acute Myeloid Leukemia
ALL	Acute Lymphoblastic Leukemia
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
MRD	Minimal Residual Disease
NGS	Next-Generation Sequencing
FISH	Fluorescence In Situ Hybridization
ELN	European LeukemiaNet
FLT3	FMS-Like Tyrosine Kinase 3
NPM1	Nucleophosmin 1
TP53	Tumor Protein 53
BCR-ABL1	Breakpoint Cluster Region-Abelson Murine Leukemia
RUNX1	Runt-Related Transcription Factor 1
CBFB-MYH11	Core Binding Factor Beta-Myosin Heavy Chain 11
PML-RARA	Promyelocytic Leukemia-Retinoic Acid Receptor Alpha

TBE	Tris-Borate-EDTA
EDTA	Ethylenediaminetetraacetic Acid
FBP	Full Blood Picture
MFC	Multiparameter Flow Cytometry
ddPCR	Digital Droplet PCR
cDNA	Complementary DNA

PENILAIAN PANEL FUSI TRANSKRIP HEMAVISION-28Q UNTUK PENYARINGAN LEUKEMIA AKUT

ABSTRAK

Leukemia akut adalah suatu sel hematopoietik yang malignan dicirikan oleh pembiakan berlebihan sel darah yang belum matang, yang mengakibatkan gangguan yang teruk pada hematopoiesis normal. Penemuan yang tepat dan bersesuaian bagi kelainan genetik, khususnya translokasi kromosom, adalah penting untuk diagnosis, prognosis, dan perancangan terapi untuk leukemia akut yang berkesan. Kajian ini membandingkan prestasi kit HemaVision-28Q, iaitu ujian PCR kuantitatif berasaskan masa (RT-qPCR), dengan ujian HemaVision-28N, yang menggunakan PCR bersarang untuk mengesan transkrip gen fusi berkait dengan leukemia akut. Objektif kajian ini adalah untuk menilai sensitiviti, spesifisiti, dan nilai klinikal HemaVision-28Q sebagai alat diagnostik, serta keupayaannya untuk mengesan kes transkrip fusi positif dan negatif. Sampel RNA dari darah periferal dan sumsum tulang pesakit leukemia akut telah diuji menggunakan ujian HemaVision-28Q dan HemaVision-28N. HemaVision-28Q menunjukkan prestasi cemerlang dengan mengesan 28 transkrip gen fusi yang penting secara klinikal dengan cepat dan konsisten, termasuk t(9;22) [BCR-ABL1], t(15;17) [PML-RARA], dan inv(16) [CBFB-MYH11]. Ujian ini didepati sangat sensitif dan spesifik, dengan masa pusingan yang lebih cepat berbanding HemaVision-28N. Alur kerjanya mengurangkan keperluan untuk langkah-langkah yang memerlukan tenaga keija intensif seperti elektroforesis gel, mengurangkan risiko pencemaran, dan menjadikannya pilihan yang berpatutan dan praktikal untuk diagnostik klinikal rutin. Sebaliknya, ujian HemaVision-28N, yang menyediakan analisis tahap eksot gen

fusi yang menyeluruh, memakan masa dan memerlukan lebih banyak pengetahuan teknikal. Hasil kajian menekankan kit HemaVision-28Q sebagai pilihan diagnostik yang boleh dipercayai untuk leukemia akut, terutamanya dalam persekitaran klinikal di mana kepantasan dan kecekapan adalah kritikal.

EVALUATION OF THE HEMAVISION-28Q FUSION TRANSCRIPT FOR ACUTE LEUKEMIA SCREENING PANEL

ABSTRACT

Acute leukemia is a hematopoietic cell malignancy characterized by excessive proliferation of immature blood cells, resulting in severe disruption of normal hematopoiesis. The accurate and timely discovery of genetic abnormalities, particularly chromosomal translocations, is crucial for effective acute leukemia diagnosis, prognosis, and therapy planning. This study compares the performance of the HemaVision-28Q kit, a real-time quantitative PCR (RT-qPCR)-based assay, to the HemaVision-28N assay, which uses nested PCR to detect fusion gene transcripts associated with acute leukemia. The goal was to evaluate HemaVision-28Q's sensitivity, specificity, and clinical value as a diagnostic tool, as well as its capacity to detect both positive and negative fusion transcript cases. Archived RNA samples from the peripheral blood and bone marrow of acute leukemia patients were examined using the HemaVision-28Q and HemaVision-28N assays. The HemaVision-28Q performed admirably, detecting 28 clinically important fusion gene transcripts quickly and consistently, including t(9;22) [BCR-ABL1], t(15;17) [PML-RARA], and inv(16) [CBFB-MYH11]. The assay was found to be highly sensitive and specific, with a faster turnaround time than HemaVision-28N. Its workflow reduced the need for labor-intensive stages such as gel electrophoresis, lowering the danger of contamination and making it an affordable and viable option for regular clinical diagnostics. In contrast, the HemaVision-28N assay, which provided thorough exon-level analysis of fusion genes, was time-consuming and required more technical knowledge. The study's findings

emphasize the HemaVision-28Q kit as a dependable diagnostic option for acute leukemia, especially in clinical settings where speed and efficiency are critical.

CHAPTER ONE: INTRODUCTION

1.1 Background of study

Acute leukemias are malignant disorders affecting blood-forming organs, characterized by abnormal growth of immature cells/ blast cells within the hematopoietic system. These conditions involve the extensive replacement of bone marrow with undifferentiated hematopoietic cells, leading to a reduction in erythrocytes and platelets in the blood. Acute leukemias are classified based on the origin of these abnormal cells, which can be lymphoid, myeloid, mixed, or undifferentiated (Tebbi, 2021).

Recurrent genetic abnormalities are important indicators for diagnosis, prognosis, and treatment in acute leukemia. Although few recurrent genetic abnormalities are shared across patients, they are expressed in 18% of cases of acute myeloid leukemia (AML). Since chromosomal translocations that result in fusion transcript expression are also a characteristic of acute lymphoblastic leukemia (ALL). Diagnosing Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) requires identifying specific genetic abnormalities to confirm the leukemia type and classify it. In AML, recurrent genetic abnormalities such as t(8;21) [RUNX1-RUNX1T1], inv(16) [CBFB-MYH11], and t(15;17) [PML-RARA] are detected using molecular techniques like RT-PCR or cytogenetic methods such as karyotyping and FISH. In ALL, these methods, along with flow cytometry for identifying cell markers, are used to detect translocations like t(9;22) [BCR-ABL1], t(12;21) [ETV6-RUNX1], and KMT2A rearrangements.

In AML, molecular and cytogenetic testing is critical for prognosis and classification into risk categories: favorable, intermediate, or poor. For example, mutations in NPM1 without

FLT3-ITD suggest a favorable prognosis, while FLT3-ITD or TP53 mutations indicate a higher risk of relapse and poor outcomes. Treatment for AML traditionally involves cytarabine-based chemotherapy, but targeted therapies have significantly improved outcomes. Patients with FLT3 mutations are now commonly treated with FLT3 inhibitors like gilteritinib or midostaurin. The accurate determination of minimal or measurable residual disease (MRD) during the early months of therapy in ALL is well established as the most important independent prognostic biomarker, predicting response to combination chemotherapy stratification based on MRD maximizes treatment effectiveness while minimizing adverse effects. Allele-specific real-time quantitative PCR for the detection of clone-defining immunoglobulin/T-cell receptor gene rearrangements in leukemic clones of patients, coupled with multiparametric flow cytometric tracking of leukemia, provides a comprehensive approach to monitoring and analyzing the disease (Batram 2020).

The diagnosis, prognosis, treatment, and monitoring of ALL are greatly impacted by recurrent genetic abnormalities, including t(12;21) [ETV6-RUNX1], KMT2A rearrangements, and BCR-ABL1 (Philadelphia chromosome). Cytogenetic and molecular testing are used to identify these genetic anomalies, which aid in the classification of the ALL subtype. Genetic defect affects prognosis: BCR-ABL1-positive The prognosis for ALL is often bad, although t(12;21) [ETV6-RUNX1] suggests a better outcome, whereas KMT2A rearrangements are linked to high risk and poor treatment response. Tyrosine kinase inhibitors (TKIs), such as imatinib, are used to treat BCR-ABL1-positive ALL, while aggressive chemotherapy is used to treat KMT2A rearrangements. Treatment is customized based on the genetic abnormalities. Patients with t(12;21) [ETV6-RUNX1] typically respond well to standard treatment. The goal of monitoring is

to identify minimal residual disease (MRD) through routine evaluations because the presence of MRD suggests a higher risk of relapse and helps guide further treatment (Gökbuget et al., 2024).

A hemavision 28Q offers quick qualitative screening of 28 chromosomal translocations associated with both acute and chronic leukemia. It employs RT-qPCR to identify fusion gene RNA transcripts from RNA extracted from blood or bone marrow samples, including splice variants. By detecting specific fusion transcripts, HemaVision-28Q provides insights into the type, aggressiveness, and responsiveness of leukemia to targeted treatments, aiding in both diagnostic and prognostic decision-making. The test is time-efficient, yielding results within 4 hours, and cost-effective, making it a valuable diagnostic tool (HemaVision-28Q, n.d.). Previously, HemaVision-28N, was employed and no longer in the market used to detect over 145 breakpoints, 28 chromosomal translocations, and associated mRNA splice variants. With a sensitivity and specificity exceeding 99%, HemaVision-28N can detect even very low levels of fusion RNA. This test uses reverse transcription and multiplex nested PCR to analyze total RNA from bone marrow or blood samples. The detailed information on fusion gene exon structures provided by HemaVision-28N supports personalized treatment plans for leukemia patients, facilitating treatment selection and disease progression prediction (HemaVision-28N, n.d.).

This study focuses on the evaluation and the verification method of HemaVision-28Q kit for detecting fusion transcripts in acute leukemia (AL) patients, with the objective of confirming its accuracy by comparing results to those obtained from the previous gold standard method HemaVision-28N kit. Validation ensures that the HemaVision-28Q kit can accurately identify both positive and negative fusion transcripts, confirming its clinical suitability by aligning with previously established methods such as HemaVision-28N. In contrast, kit method verification refers to the evaluation of accuracy, sensitivity, repeatability, and robustness to guarantee a

commercial kit operates precisely, consistently, and dependably. Alternatives include manual protocols from published methods and in-house assays such immunoassays. Every approach needs to be properly validated to guarantee accuracy and reliability.

1.2 Problem Statement

Genetic changes might affect therapy choices and patient outcomes. Thus, accurate fusion gene transcript detection is essential for leukemia diagnosis and management. In this context, reverse transcriptase PCR (RT-PCR) and real-time PCR (qPCR) have different purposes. Real-time PCR (qPCR) provides quantitative information on the amount of amplified product by measuring DNA amplification in real time. This distinction emphasizes the importance of choosing the suitable approach for precise fusion transcript detection. Test kit verification of HemaVision-28Q is necessary to validate the assay's accuracy, sensitivity, and specificity in identifying fusion transcripts. This verification procedure fills a major vacuum in the trustworthy detection of fusion gene transcripts for leukemia diagnosis by confirming that the kit satisfies predetermined performance parameters and can reliably deliver correct results in clinical situations (Xue, 2021).

1.3 Objectives

The general objective of this study is to evaluate and correlate the HemaVision-28Q assay in comparison to the HemaVision-28N, DNA DIAGNOSTIC assay for detecting fusion gene transcripts associated with acute leukemia

The specific objectives are

1. To measure the agreement between HemaVision-28Q assay with the HemaVision-28N assay in accurately identifying positive fusion transcripts in acute leukemia patients.
2. To compare the HemaVision-28Q assay with the HemaVision-28N assay in accurately identifying negative fusion transcripts in acute leukemia patients.
3. To verify the specificity and sensitivity of the HemaVision-28Q assay in detecting fusion transcripts in acute leukemia cases**.**

1.4 Hypothesis

The null hypothesis (H0) are

1. The HemaVision-28Q assay does not show a significant agreement in the identification of positive fusion transcripts when compared to the HemaVision-28N assay
2. The sensitivity and specificity of HemaVision-28Q in diagnosing acute leukemia are not significantly better than those of the HemaVision-28N assay.

The alternative hypothesis (HA) are

1. The HemaVision-28Q assay demonstrates a significant agreement in the identification of positive fusion transcripts when compared to the HemaVision-28N assay,
2. HemaVision-28Q assay exhibits superior sensitivity and specificity in diagnosing acute leukemia.

1.5 Rationale of the study

This study aims to evaluate and verify the usage of HemaVision-28Q in comparison with HemaVision-28N assays in detecting fusion gene transcripts linked to acute leukemia. By examining these assays, the research seeks to meet the urgent need for more precise diagnostic tools. Evaluating the sensitivity of the HemaVision-28Q assay will help determine its capacity to accurately identify true positive and negative cases of fusion transcript among acute leukemia, thereby enhancing diagnostic precision. Additionally, assessing the assay's specificity will ensure it correctly identifies fusion transcripts while minimizing false positives. The findings from this study are expected to evaluate diagnostic practices and potentially lead to more effective and reliable monitoring and treatment strategies for acute leukemia patients.

1.6 Research Questions

1. What is the level of agreement between the HemaVision-28Q assay and the HemaVision-28N assay in accurately identifying positive fusion transcripts in acute leukemia patients?
2. What is the sensitivity of the HemaVision-28Q assay in detecting fusion gene transcripts in patients with acute leukemia?
3. What is the specificity of the HemaVision-28Q assay in ruling out fusion gene transcripts in patients who do not have acute leukemia?

CHAPTER TWO: LITERATURE REVIEW

2.1 Acute Leukemia

Acute leukemia is characterized by the presence of high numbers of immature white blood cells in both the bone marrow and blood which inhibits the production of normal blood cells, resulting in anemia, easy bruising or bleeding, and increased susceptibility to infections. Acute leukemia is divided into two main types: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), differentiated by the specific cell types involved. The aggressive nature of acute leukemia makes prompt diagnosis and treatment critical, as survival without intervention is typically measured in weeks to a few months. Diagnosis usually involves blood tests showing elevated blast levels and bone marrow biopsies to confirm the presence of leukemia. Treatment often consists of chemotherapy, targeted therapies, and sometimes stem cell transplants, tailored to the specific type of leukemia and individual patient factors. Understanding these characteristics is essential for early detection and effective management, ultimately improving patient outcomes (Eleni 2022).

2.1.1 Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is a condition characterized by the abnormal proliferation of lymphoid cells that are stuck in an early stage of differentiation. This impairment in lymphocyte maturation leads to the accumulation of immature blast cells, which originate from tumor-transformed precursor cells in the bone marrow. The presence of these leukemic cells in the bone marrow disrupts normal hematopoiesis, resulting in anemia, thrombocytopenia, and neutropenia. ALL is known for its aggressive nature, with survival times often ranging from

just a few weeks to several months without proper treatment. Historically, ALL has been treated with a combination of chemotherapeutic drugs, leading to a median overall survival rate of around 80% for newly diagnosed patients. Those at higher risk for relapse typically undergo more intensive treatment, while patients with a better prognosis can often avoid harsher side effects. Multicenter randomized controlled trials conducted by international collaborations are contributing to improvements in survival rates by investigating novel therapies. Future advancements in leukemia treatment may come from understanding the molecular pathways involved in the disease and identifying pharmacogenetic factors in patients. Successful research could lead to the discovery of new genes and candidate proteins for targeted therapies. Environmental risk factors for ALL may include significant radiation exposure or previous chemotherapy, while the evidence surrounding electromagnetic fields or pesticides remains inconclusive. Some theories suggest that an atypical immune response to common infections might play a role in their development. The underlying mechanism involves multiple genetic changes that result in rapid cell division, with an excess of immature lymphocytes in the bone marrow hindering the production of new red blood cells, white blood cells, and platelets. Diagnosis typically relies on blood tests and bone marrow examinations (Zuzzana 2023).

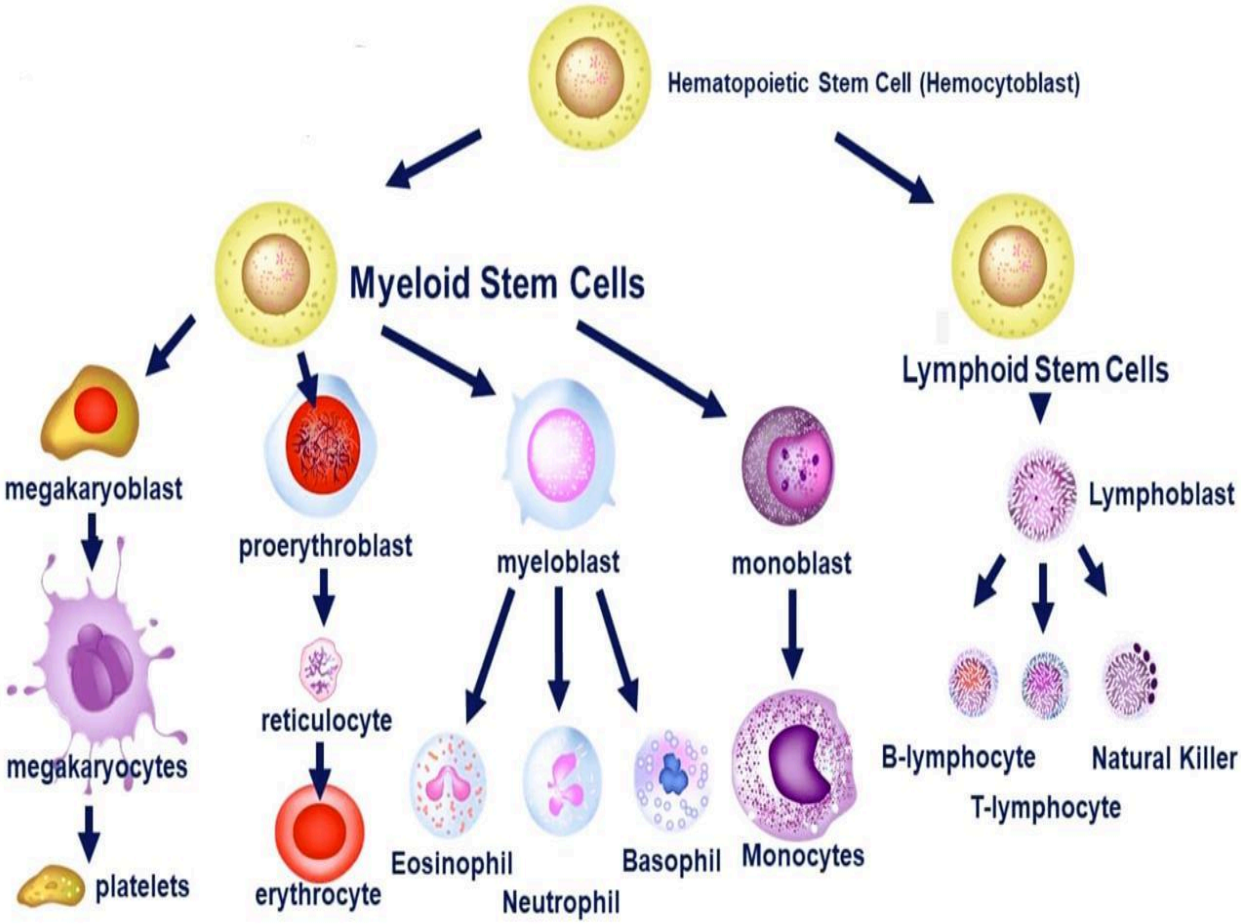

Figure 2.1: Several steps for a blood stem cell to become a red blood cell, platelet, or white blood cell. (Samuel, 2022)

2.1.2 Acute myeloid leukemia (AML)

Acute myeloid leukemia (AML) is a condition in which the malignant cell is the myeloblast, a precursor to myeloid leukocytes that normally matures into fully developed white blood cells. In AML, myeloblasts undergo genetic alterations that cause them to remain in their immature state, preventing normal maturation. This “differentiation arrest” alone does not lead to leukemia; however, when combined with other genetic changes that disrupt the regulation of cell proliferation, it results in the uncontrolled expansion of a young cell clone, manifesting as leukemia. The heterogeneity of AML arises because leukemic transformations can occur at various stages of the differentiation pathway. Current classification systems for AML recognize that the characteristics and behavior of leukemic cells—and the disease itself—can depend on the specific stage at which differentiation is halted. Most individuals with AML exhibit distinct cytogenetic abnormalities, which often have prognostic significance. These chromosomal abnormalities typically involve translocations that create abnormal fusion proteins, frequently transcription factors with altered functions that contribute to differentiation arrest. For instance, in acute promyelocytic leukemia, the translocation produces the PML-RARα fusion protein, which binds to retinoic acid receptors in the promoters of myeloid-specific genes, inhibiting their differentiation. The initial treatment for AML primarily consists of chemotherapy, divided into two phases: induction and post-remission therapy. Induction therapy aims to achieve complete remission by drastically reducing the leukemic cell count, while consolidation therapy focuses on eradicating any remaining undetectable disease to achieve a cure. Hematopoietic stem cell transplantation is often considered if induction chemotherapy fails or after relapse, and it is also utilized in certain situations (Obeagu 2020).

2.2 European LeukemiaNet (ELN) CLASSIFICATION

Identifying fusion transcripts is critical for understanding its molecular pathogenesis and guiding clinical management. Fusion transcripts, which result from chromosomal rearrangements such as translocations that include *RUNX1-RUNX1T1* (t(8;21)), *PML-RARA* (t(15;17)), and *CBFB-MYH11* (inv(16)), each associated with distinct clinical and prognostic implications. Advances in molecular diagnostics, such as reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS), have significantly enhanced the ability to detect these fusions with high sensitivity and specificity. These advancements have facilitated their integration into the ELN classification system, which stratifies AML patients into favorable, intermediate, and adverse risk groups based on genetic and molecular features (Döhner, 2022).

Fusion transcripts play a pivotal role in ELN risk stratification. For instance, *RUNX1-RUNX1T1* and *CBFB-MYH11* are associated with favorable risk, characterized by higher complete remission rates and improved overall survival. Conversely, fusions such as KMT2A rearrangements (e.g., *MLL* fusions) and *NUP98-NSD1* are linked to adverse outcomes, including higher relapse rates and treatment resistance (Döhner, 2022). Intermediate-risk fusions, which are less well-defined, underscore the complexity of AML heterogeneity and the necessity for further research. Beyond risk stratification, fusion transcripts have therapeutic implications. The *PML-RARA* fusion, for example, is the molecular basis for the use of all-trans retinoic acid (ATRA) in acute promyelocytic leukemia (APL), a paradigm of targeted therapy in AML. Additionally, emerging treatments are being developed to target specific fusions, such as FLT3 inhibitors for *FLT3*-rearranged AML.

Fusion transcripts are essential biomarkers for monitoring minimal residual disease (MRD), which allows for risk classification, early relapse, and the identification of remaining leukemic cells. Positive MRD is linked to a lower overall survival rate and an increased risk of relapse in AML and ALL. MRD detection is made easier by methods like qPCR, flow cytometry, and NGS; qPCR has a high sensitivity but has difficulties such as PCR inhibition. By directing relapse therapies and influencing choices like the necessity of allogeneic stem cell transplantation, the incorporation of MRD monitoring into clinical practice improves individualized treatment and improves patient outcomes through customized therapeutic approaches (Ip et al., 2024).

Highly sensitive molecular and cellular techniques are used in minimal residual disease (MRD) detection to find any remaining cancerous cells that are not detectable using traditional techniques. In MRD, quantitative PCR (qPCR) is frequently used to amplify and quantify particular genetic targets with high sensitivity and specificity, such as fusion genes (e.g., BCR-ABL1), immunoglobulin/T-cell receptor (Ig/TCR) rearrangements, or mutations like NPM1. By dividing the sample into thousands of nanodroplets, digital droplet PCR (ddPCR) increases sensitivity and enables absolute quantification of target DNA or RNA without the need for standard curves. This makes it very helpful for low-frequency mutation detection. Furthermore, multiparameter flow cytometry (MFC) detects abnormal antigen expression patterns on residual cells, and next-generation sequencing (NGS) provides a thorough investigation of MRD by detecting a variety of genetic changes and clonal evolution at an ultra-deep resolution. Together, these cutting-edge approaches offer vital information about treatment response, recurrence risk, and disease prognosis, serving as the foundation for MRD monitoring in hematological malignancies. Despite these advancements, challenges remain in

detecting cryptic or rare fusion events and understanding their clinical significance. Integrating multi-omics approaches, including transcriptomics and proteomics, is essential for developing more comprehensive risk models. The future of AML management lies in expanding personalized therapeutic strategies, including universal NGS panels and targeted therapies, to address the molecular diversity of this disease (Döhner, 2022).

2.3 Global Prevalence

Globally, leukemia accounted for approximately 2.5% of all new cancer cases and 3.1% of cancer-related deaths in 2020. Acute leukemia which includes ALL and AML contributes significantly to this burden. The age-standardized incidence rate (ASR) for leukemia worldwide was 5.4 per 100,000 people, with notable regional differences. Higher incidence rates were reported in North America (10.9 per 100,000), Australia and New Zealand (10.4 per 100,000), and Europe (8.5 per 100,000 in both Western and Northern regions). In contrast, the lowest rates were observed in parts of Africa, such as Middle Africa (2.2 per 100,000) and Western Africa (2.3 per 100,000). This disparity may reflect differences in healthcare infrastructure, diagnostic capabilities, and risk factor prevalence across regions (Huang et al., 2022).

The burden of leukemia is also influenced by socioeconomic factors, such as the Human Development Index (HDI) and Gross Domestic Product (GDP) per capita. Higher-income countries reported significantly higher leukemia incidence rates, likely due to improved diagnostic capabilities and longer life expectancy. Lifestyle and metabolic risk factors, including smoking, obesity, physical inactivity, and hypercholesterolemia, were associated with increased incidence and mortality rates. Men had a 40% higher global incidence (6.3 per 100,000)

compared to women (4.5 per 100,000), reflecting greater exposure to occupational and lifestyle-related risk factors (Huang et al., 2022).

Although global trends indicate a general decline in leukemia incidence and mortality over the past decade, certain regions exhibit increasing rates. For instance, incidence rates have risen in Germany, South Korea, Japan, Canada, and the United Kingdom. Conversely, mortality rates have increased in lower-income regions such as the Philippines, Ecuador, Belarus, and Thailand, highlighting gaps in access to effective treatment. These findings underscore the need for intensified public health interventions, such as smoking cessation, weight management, and early detection programs, particularly in high-risk and underserved populations (Huang et al., 2022).

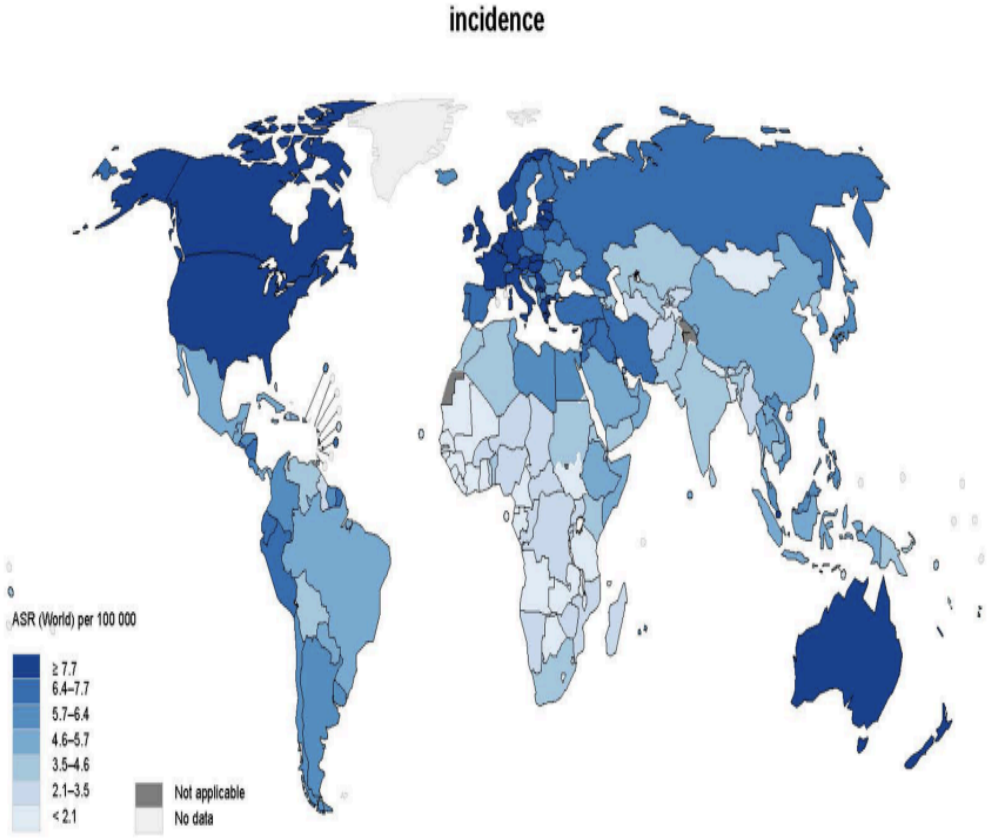

Figure 2.2: Global incidence of leukaemia, both sexes, all ages, in 2020 (Huang et al., 2022).

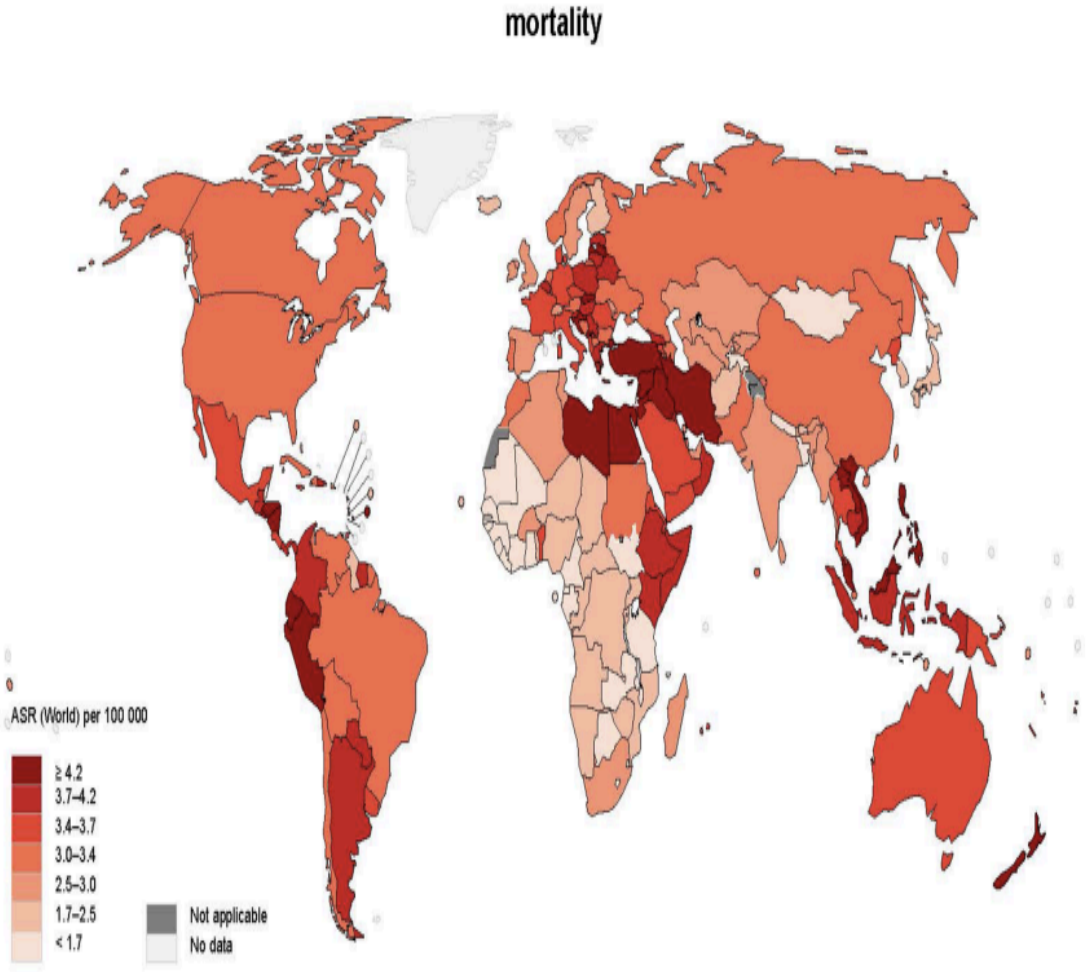

Figure 2.3: Global mortality of leukaemia, both sexes, all ages, in 2020 (Huang et al., 2022).

2.3.1 Prevalence in Malaysia

ALL is the most common type of leukemia among children in Malaysia. According to the Malaysia National Cancer Registry Report (2017-2021), nearly 400 children are diagnosed with blood cancer annually, with ALL being the predominant type. This high incidence rate underscores the critical need for effective pediatric oncology services and targeted public health interventions to manage and reduce the burden of ALL in the country. AML is the most prevalent form of leukemia among adults in Malaysia. The same national cancer registry report indicates that leukemia, including AML, accounts for approximately 3.7% of all cancer cases in Malaysia. The increasing incidence of AML, particularly among the aging population, highlights the necessity for enhanced diagnostic and treatment strategies tailored to this demographic (Fernandez 2022). Globally, the incidence of acute leukemia varies, with AML showing an increasing trend in many regions, including Malaysia. This trend is consistent with global data, which shows a rise in AML cases from 79,372 in 1990 to 144,645 in 2021. The age-standardized incidence rate (ASIR) of AML has also increased, reflecting similar patterns observed in Malaysia (Fernandez 2022).

2.4 Pathophysiology

The pathophysiology of acute leukemia involves genetic mutations that disrupt normal hematopoiesis. In ALL, mutations in lymphoid progenitor cells lead to the uncontrolled growth of lymphoblasts, which fail to mature into functional lymphocytes. Similarly, AML arises from genetic alterations in myeloid precursor cells, resulting in the accumulation of myeloblasts (Xue 2021). These immature cells crowd out normal hematopoietic cells in the bone marrow, leading to anemia, thrombocytopenia, and neutropenia. The genetic changes often involve chromosomal

translocations, gene fusions, and mutations in oncogenes and tumor suppressor genes. The clinical susceptibility for infections among patients with hematological malignancies is multifactorial (Xue 2021)l. The risk of development and the severity of infections are determined by a complex interplay between the pathogen and its virulence, and the degree of impaired defense mechanisms of the host. The risk of infection can broadly be divided into (i) disease-associated factors, (ii) patient-related factors, and (iii) treatment- related factors that is shown in Figure 2.4.

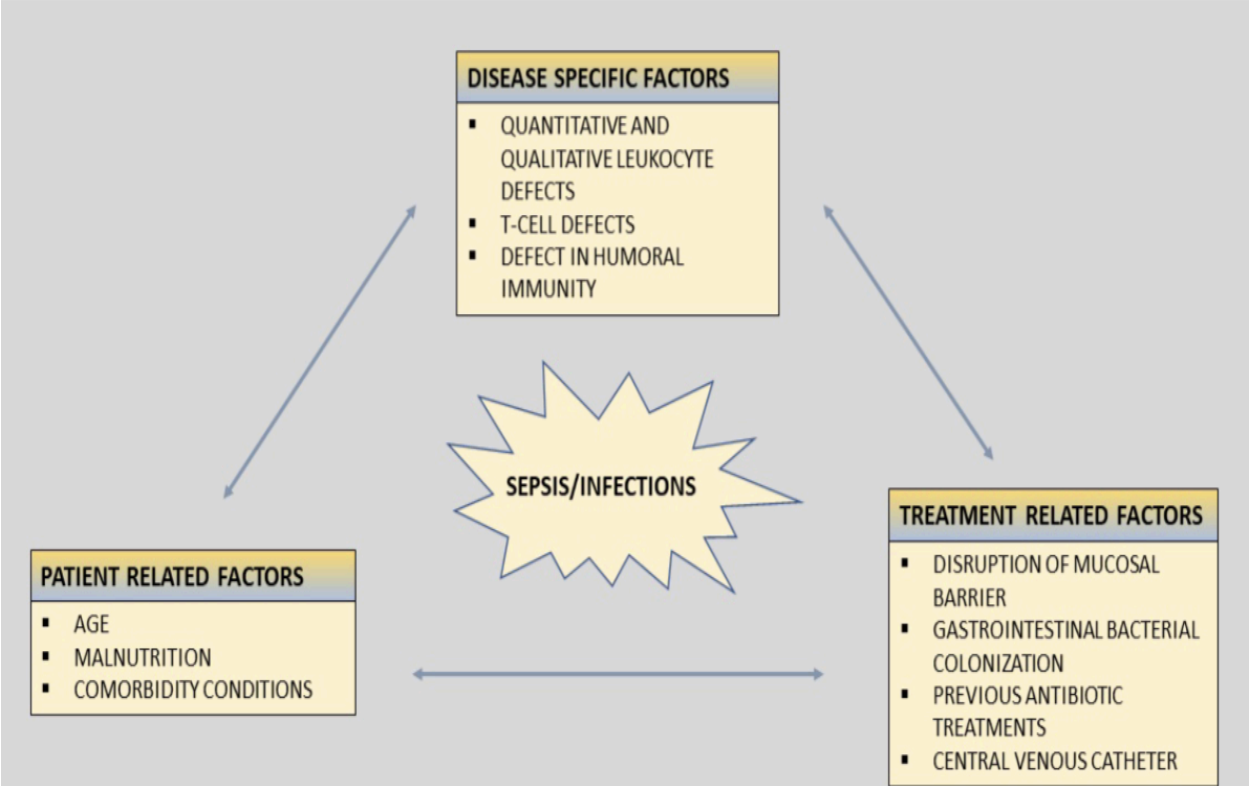

Figure 2.4: Risk factors for infections in patients with leukemia (Pitaloka et al., 2022)

2.5 Other Diagnostic Tools

A wide range of methods are used in the World Health Organization's (WHO) categorization system for acute leukemia disease diagnosis. Samples of bone marrow and blood

are examined to determine the quantity and shape of blasts, with a 20% lower cutoff point for AML diagnosis. Important characteristics that aid in diagnosis confirmation include Auer rods, which are a sign of myeloid differentiation. A crucial technique for immunophenotyping divides leukemia into discrete subtypes by identifying cytoplasmic and cell surface markers, such as CD19 for B-cell lineages and CD33 for myeloid lineages. Karyotyping and fluorescence in situ hybridization (FISH) are two examples of cytogenetic analysis that are essential for detecting chromosomal abnormalities. Some examples include t(15;17) in acute promyelocytic leukemia and BCR-ABL1 in Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL).

Next-generation sequencing (NGS) and polymerase chain reaction (PCR) have proved crucial in detecting genomic translocations and mutations in acute myeloid leukemia (AML), including t(8;21)(q22;q22.1);RUNX1-RUNX1T1 and inv(16). Core-binding factor leukemias are characterized by the RUNX1-RUNX1T1 fusion gene, which may be precisely detected using these methods. Significant prognostic and therapeutic implications arise from the discovery of such mutations. RUNX1-RUNX1T1, for example, is linked to a positive result and directs the creation of tailored treatments, including tyrosine kinase inhibitors and small compounds that target the fusion protein. Although molecular genetic testing has advanced, bone marrow biopsy is still essential for determining the architecture of the bone marrow and the degree of leukemic infiltration. Molecular methods offer in-depth information about genetic anomalies, but a biopsy gives a complete picture of the structural integrity of the bone marrow, the degree of leukemic infiltration, and other histopathological features (Al-Harbi et al., 2020). Leukemia subtypes can also be distinguished using cytochemical labeling, and recurrence prediction and treatment effectiveness depend on minimal residual disease (MRD) monitoring with PCR or flow cytometry. For acute leukemia to be accurately classified, prognosed, and treated individually,

these integrated diagnostic techniques are essential. The following table 2.5 states the advantages and the limitations of other diagnostic tools

Table 2.5: The advantages and the limitations of other diagnostic tools

Method	Advantages	Limitations
Flow Cytometry (FMP/BMA)	-Provides information on cell morphology and immunophenotype.	-Lower sensitivity
Immunophenotyping	-Identifies abnormal cells based on antigen expression. -Useful for monitoring response to therapy.	-Moderate sensitivity -Limited by the availability of markers specific to disease subtypes.
Cytogenetics	-Detects chromosomal abnormalities and karyotypic changes.	-Low sensitivity -Requires dividing cells, limiting applicability to certain cases.
FISH (Fluorescence In Situ Hybridization)	-Highly specific for known genetic rearrangements.	-Limited sensitivity- Can only detect pre-defined genetic abnormalities.
Molecular (RT-PCR/qPCR)	-High sensitivity -Effective for known genetic markers and rearrangements.	-May not detect all residual disease clones.
Next-Generation Sequencing (NGS)	-Ultra-high sensitivity -Detects diverse genetic abnormalities and clonal populations.	-Expensive and resource-intensive. -Turnaround time may be longer compared to other methods.

2.5.1 HemaVision Technology

HemaVision-28Q is a targeted RT-qPCR assay developed to detect fusion gene transcripts linked to leukemia, utilizing RNA obtained from whole blood or bone marrow samples.

The assay targets different clinically significant chromosomal breakpoints by utilizing preliminaries and tests that intensify districts containing translocation breakpoints. Taking after RNA filtration, switch translation changes over RNA into cDNA, which is at that point analyzed utilizing real-time qPCR with fluorescence discovery. The measure can distinguish RNA transcripts from 28 translocations covering over 145 clinically noteworthy breakpoints, offering fast screening inside four hours post-RNA extraction. The consideration of reference qualities such as ABL-1, B2M, and GUS guarantees the keenness of RNA tests and the usefulness of both cDNA union and qPCR responses, giving vigorous inside controls. Fluorescence location and cycle limit (Ct) examination are utilized to precisely recognize the nearness of combination transcripts, with safeguards taken to avoid defilement and guarantee dependable comes about, such as utilizing vaporized obstruction pipette tips and producing a Color Emolument record for the Roche LightCycler 480 framework. To improve the assay's accuracy and dependability, it uses fluorescent dyes such as ROX as a passive reference dye to normalize fluorescence variances, Cy5 for additional targets in multiplex processes, and FAM for target fusion transcript detection. (HemaVision-28Q, n.d.).

The HemaVision-28N test is RT Nested PCR, an advanced symptomatic apparatus planned for the fast discovery of 28 chromosomal translocations related to leukemia, utilizing add up to RNA extracted from blood or bone marrow tests. The test utilizes turn around translation to change over RNA into complementary DNA (cDNA), taken after by multiplex PCR to distinguish combination qualities and their joint variations. With an affectability and

specificity surpassing 99%, the HemaVision-28N test offers a solid and exact strategy for recognizing leukemia-related translocations, assembly CE-marked benchmarks for security and viability in in vitro diagnostics. The testing preparation includes several essential steps, beginning with RNA extraction, which is critical for ensuring the accuracy of the results. The Ace PCR intensifies target districts connected to known translocations, whereas the Split-out PCR encourages refining the distinguishing proof by recognizing particular breakpoints. Results are analyzed using gel electrophoresis, where the presence of translocation-specific bands indicates a positive result.

For instance, the detection of the t(9;22) translocation confirms the presence of the BCR-ABL1 fusion gene, which plays a critical role in therapeutic decision-making. One of the key features of the HemaVision-28N test is its ability to address the complexity of translocations, detecting over 145 breakpoints and associated mRNA splice variants. This capability ensures comprehensive coverage of the heterogeneity observed in leukemia translocations, enabling accurate clinical interpretation. Additionally, the test highlights the importance of adhering to safety protocols during sample handling to prevent contamination and ensure reliable results (HemaVision-28N, n.d.).

The sensitivity, applicability, and methodology of HemaVision 28N and 28Q are different. HemaVision 28N detects more than 145 breakpoints and mRNA splice variants using agarose gel electrophoresis and multiplex nested PCR. Because of its excellent sensitivity and specificity (>99%) and detection limit of 10^{-9} µg of fusion RNA in 1 µg of total RNA, it can be used to characterize fusion genes in detail at the exon level. Nevertheless, it is time-consuming and labor-intensive, involving electrophoresis and several PCR processes, which raises the possibility of contamination (HemaVision-28N, n.d.). On the other hand, HemaVision 28Q uses

RT-qPCR, which yields results quickly—within 4 hours of RNA extraction—and with little manual labor. It offers accuracy in treatment planning using amplification plots and is labor- and cost-effective. Though quick and effective, HemaVision 28Q could not be as sensitive or reach the same level of fusion gene characterization as 28N, which makes it less appropriate for in-depth investigations but perfect for making clinical decisions quickly (HemaVision-28Q, n.d.). Table 2.5.1 shows a detailed comparison table of HemaVision-28Q and HemaVision-28N.

Table 2.5.2: Detailed comparison table of HemaVision-28Q and HemaVision-28N

Feature	**HemaVision 28Q**	**HemaVision 28N**
PCR	RT- qPCR	RT Nested PCR
Technology	Newer	Older
Detection time	Fast	Time consuming
Sensitivity	High, suitable for clinical decision-making	Excellent (>99%)
Specificity	High, detects 28 translocations and over 145 breakpoints	High (>99%), detects 28 translocations and over 145 breakpoints
Labour and cost efficiency	More cost-effective and requires less labor	Less cost-effective due to time and labor intensity
Risk of contamination	Low	High
Quantification capability	Provides semi-quantitative Ct values	No quantification,
Detection method	Real-time fluorescence detection with Ct analysis	Gel electrophoresis to identify translocation-specific bands