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ARTICLE

MOLECULAR DIAGNOSIS: A BOON FOR LEUKAEMIA

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Abstract
In hematologic malignancies, leukaemia is categorized as the unusual proliferation of the hematopoietic cells. The confirmatory diagnosis is usually made on the aspirate of the bone marrow with or without trephine biopsy by visualizing the cell morphology and the architecture of the bone marrow. Furthermore, there are additional methods that are used to further categorize the variant of leukaemia that includes flow cytometry immune phenotyping, and genetic analysis. In India also use of molecular diagnosis is increasing rapidly for the detection of leukaemia. Techniques such as FISH and PCR are very sensitive and with accurate results have gained importance for cancer diagnosis. Further with the development of next-generation sequencing, it is likely to become an even more prominent practice in cancer diagnosis. This article focuses and elaborated on the molecular diagnosis of leukaemia
Keywords: Leukaemia, Molecular diagnosis, PCR, karyotyping, AML, CML, ALL, CLL

1 | INTRODUCTION

The hematologic cancers are related to a variety of aberrations in the gene that vary from a solo base-pair substitution to the entire abnormality of the chromosome. Leukaemia is categorized as the unusual multiplication of blood cells because of genetic errors. About 2.5% of the range of cancers, with a yearly incidence of 13 per 100000 individuals that are found in the UK suffer from Leukemia.[1] (1)Figure 1 illustrates the number of cases in 2020 and the number of cases predicted to occur in 2024 the above data has been derived from International Agency for Research on Cancer and WHO (World Health Organization). The above graph shows that the number of cases is predicted to rise in these four years from what it was in 2020.[2] (2)

There are four main types of Leukemia as described in Figure 2 . Out of these the most commonly oc-

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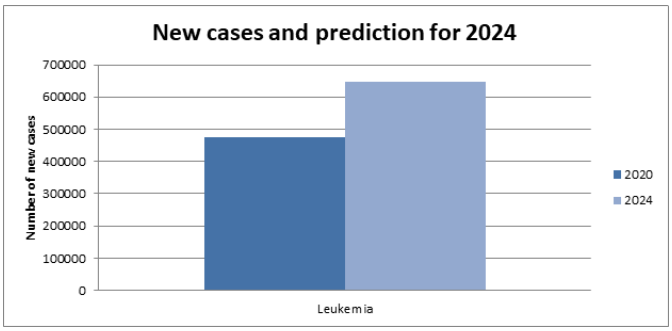

FIGURE 1: *Illustrationof new cases occurred in 2020 and the number of new cases predicted to occur in2024.*

curring is CLL, followed by AML, ALL, and the least common being CML. The World Health Organisation (WHO) proposed classification of the tumours of the hematologic origin has been widely accepted and it promotes a multi-parametric approach to the identification. [3] The molecular pathology in the myeloid lineage is suggested in many published works of literature. Ideally, when there is an alteration or a very high count of blood cell are seen in complete blood count (CBC) following the examination of the film of the blood, leukaemia is suspected. Final confirmation is done when the biopsy of bone marrow aspirate is done using trephine and there is an alteration in the structure of cells. The examination is usually affirmed on a bone marrow aspirate with or without trephine biopsy by visualizing the morphology of the cells and the architecture of the bone marrow. Furthermore, there are additional methods that are used to further categorize the variant of leukaemia that includes flow cytometry immune phenotyping, and genetic analysis. [4]

2 | GENETIC STUDIES IN LEUKAEMIA

Genetic analysis of leukaemia becomes extremely important while considering various therapeutic measures. Usually, the smear of peripheral blood or the bone marrow aspirate is the preferred sample of choice where the preliminary diagnosis is made looking at the cell/nucleus morphology and the findings of the immune phenotyping. Several other diagnostic protocols are followed to analyse the genetic defects in leukaemia, which majorly depends on the types of

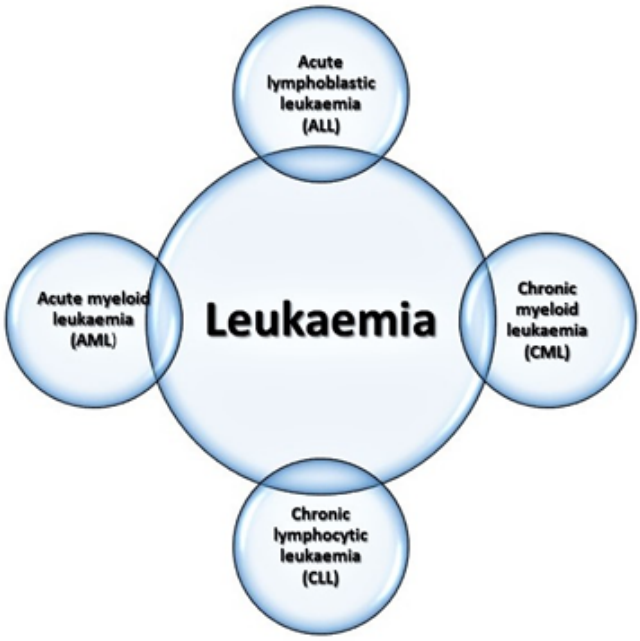

FIGURE 2: *Major types of Leukaemia*

leukaemia and other factors like the age, type, and sample size, the relevancy of the marker. [5]

2.1 | G-band metaphase chromosome analysis (`karyotyping')

For patients with a recent diagnosis of leukaemia, the presence of active mitotically dividing cells is the key for conventional cytogenic analysis, which infers that the bone marrow specimen should be sent for *in vitro* culturing, and if leukaemic cells are found in the circulation, then occasionally blood can be used for diagnosis. During the cell cycle, the cells halt at metaphase, in which the easily visible and more condensed chromosomes can be seen. Giemsa or Leishman stains are used to obtain a distinctive banding pattern that distinguishes the individual chromosomes in the metaphase stage for the numerical, structural abnormalities. [5] , [6] ,7 [7] , [8]

In CML, ALL, and AML, the detection of translocations and related changes like aneuploidies and inversion can be performed with a morrow specimen with the help of metaphase cytogenic analysis. This technique has the advantage of providing low-resolution whole-genome scans as well as balanced rearrangements detection, which is commonly seen in cases of leukaemia. The limited resolution of 3-

5 Mb, 5-10% sensitivity depends on the number of cells analysed, as well as the presumptuous presence of disease in the marrow and the presence of low mitotic index cells of leukaemia are the major drawbacks of the technique. It is remarking here, that gross abnormalities in chromosomes can only be estimated in proportion to leukaemia. [5], [6], [7], [8]

In studies of haematological malignancies, based on metaphase cytogenic analysis, many defects of chromosomes remain unidentified. [5] Metaphase cytogenetic analysis can provide a confirmative diagnosis upon detection of the pathognomonic rearrangements in CML, the 'Philadelphia [Ph] chromosome', acute promyelocytic leukaemia even in the absence of other diagnostic features, such as a blast count >20% in AML. In all other types of leukaemia, many other unique and recurrent abnormalities have been observed, and it is predicted that in the future some of these will be considered pathognomonic for all other subtypes of leukaemia. [6] Some of the other important information about the prognosis can be obtained with chromosomal aberrations. For establishing a prognosis in cases of AML, the most important parameter is cytogenic findings. Depending upon the diagnostic karyotype, a broad prognostic group is defined i.e. good, intermediate, and poor, which helps to define the patients that can be profited with transplantation of stem cells. [5], [8] In chronic myeloid leukaemia, the Ph chromosome or trisomy 8/19 or isochromosome, which is an abnormality of additional karyotype, presence my harm the survival and may imply that leukaemia has already increased to a phase that is accelerated or blasts crisis. [5], [9]

2.2 | Fluorescence in situ hybridization

FISH has proved to a useful adjunctive tool to cytogenic analysis, with a major advantage of unreliability on dividing cells and therefore, it can be performed with cells in their interphase stage of the cell cycle. In cases of unavailability of bone marrow, blood can be used and as there is no need for culture, the results are obtained much quickly. When compared with conventional cytogenic analysis, FISH has a sensitivity of 0.5 – 1% and a higher resolution of [10]0Kb which is dependent on the type of probe used. However, to evade false negative and false positive results because of the colocalization of signal or drop-out, caution should be taken depending upon the type of probe used. The cut-off value for each probe should be set by laboratories to definitive positive results. To detect a specific sequence of interest, a fluorescently labelled DNA probe is used in the FISH technique. And this probe can identify structural as well as numeric chromosomal changes counting balanced rearrangements as well as microdeletions. To identify the most common or aberrations that are clinically relevant in the subtypes of leukaemia, the use of a limited number of probes is a targeted approach, which is very unlikely with that of karyotype analysis, which normally does not provide a genome-wide assessment. [5], [10], [11]

In the diagnosis of leukaemia with cryptic cytogenetic abnormalities, FISH is predominantly useful. One such example of this is the translocation (t12;21) (p13;q22). As it is associated with a good risk prognostic group, the identification of aberrations for risk-adapted therapeutic protocols is vital. [9] Using a molecular technique or FISH, translocation can be detected. Genomic aberrations identification, using a probe with the disease-specific panel was successful for 80% of cases with CLL with interphase FISH technique. And it was predominantly useful for the identification of *TP53* (17p13) and *ATM* (11q23) deletions, as both are associated with poor prognosis. [5], [12]

2.3 | Microarray-based techniques Whole-genome scanning by molecular karyotyping: array comparative genomic hybridization

For comparing the genetic material from a test individual, like patients with leukaemia, to that of the normal subjects which are typically DNA pooled from several subjects, for identifying the presence of changes in the copy numbers of the test sample, comparative genomic hybridization(CGH) is used. For CGH both the test as well as reference DNA are assimilated into smaller fragments and each of the segments is labelled with a diverse fluorophore. The DNA probes on the array are most commonly oligonucleotides, or they can be bacterial artificial chromosomes (BACs). Factors like probe length,

the density of the probe, distribution of the probe, size of the clonal population, quality of the DNA software analysis algorithms determine the resolution as well as its sensitivity. A marked improvement in CGH array over karyotyping is its ability to detect 50kb small deletions or insertions. This whole genomic scanning array has helped to discover the chromosomal aberrations at an increased proportion in cases of leukaemia, there is a hope that this identification will lead to more detailed and accurate prognostic schemes. [5], [11] Although, CGH array has a discrete disadvantage of inability to detect balanced rearrangements, that are comparatively common in leukaemia. For cases of CLL, copy number array analysis is preferable because the genetic lesions with known clinical significance are chromosomal losses and gains. In CLL, to study the novel genomic imbalance the research tool used is the CGH array, however, moderately less affected tumour oncogenes and suppressors have been caught up in the disease. [5], [13], [14]

2.4 | Whole-genome scanning by molecular karyotyping: SNP arrays

Unlike CGH array, SNP arrays do not use reference DNA with competitive binding, but instead, it uses the test DNA directly for genotype polymorphisms, and the gene copy numbers are estimated from the hybridization signal strength of the individual probes. As the SNPs have a high density, they can assess a genome at a wide level, even a very region with copy number alteration can be determined, and in the study of CLL such arrays have gained huge popularity, for example, 250K SNP array. [5], [13] [14], [15], [16], [17] Here, 250K represents the number of SNPs distributed in the genome rather than their spacing, therefore, 250,000 SNPs are present in the 250K array, while the 500K array has twice the SNP density. Although, CGH arrays have their probes spaced evenly across the genome, whereas, in SNP array, it is dictated by the location of SNPs, because of that there is relatively poor resolution within the SNP deserts. [18], [19], [20] Both genomic probes, as well as SNPs arrays, have been created to intensify the resolution, [21] and Affymetrix CytoScan® HD is an excellent example. SNP array can identify a genomic breakpoint at the exonic level throughout the whole genome. Although, in the specimen of any leukaemia, both, the ability to resolve copy number changes as well as the loss of heterozygosity is dependent on the proportions of leukemic cells versus normal cells i.e., level of mosaicism. Within the genome, SNP arrays can detect the diploid stretches of homozygosity, measure the copy number as well as information about genotyping, and therefore, SNP arrays are capable of detecting CN- LOH (copy number- loss of heterozygosity). When SNP arrays are combined with routine metaphase analysis, the overall, karyotyping-based diagnostic yield is increased. [21] Unbalanced cytogenetic defects detection is dependent on enough cells sharing a clonal abnormality in both CGH array and SNP arrays. However, for the investigation of leukaemia, the most appropriate technique still is standard metaphase cytogenetics, despite its dependence on the dividing cells and limited resolution. [5], [21]

2.5 | Polymerase chain reaction

For confirmative and accurate molecular diagnosis of leukaemia, polymerase chain reaction (PCR) techniques become an important and widely followed method. The corrections and editing of the reaction in itself and the processing downstream have caused a wide variety of techniques. Some of the most effective and practised diagnosis of the disease using PCR has been mentioned below. [5], [21]

a. reverse-transcription PCR & reverse transcription-quantitative real-time PCR: While reviewing the genetic fusions molecular counterpart RNA has also been used as a choice of template apart from the other selected modalities within the gene lab. This occurs because the absence of introns in RNA makes the template more convenient. [5], [22]

By the use of the RNA template and finding primers which is present just outside the region of cluster i.e. specifically the breakpoint, a set of primers can be used to find the fused genes counterpart of the translocation of the chromosome in most of the patients, excluding those with the rare fusion category where the breakpoint is out of the regions that are usually common. The main drawback with the use of RNA is that it has a short shelflife of 23 days.

There might action of RNase enzyme that might degrade the RNA. The RT-PCR (endpoint reverse transcription) is where the results are found out after the ending of the reaction amplification, it delivers a non-quantitative analysis of the attendance or non-attendance of the particular product of interest. [5], [23]

b. Multiplex ligation probe amplification (MLPA)

The MLPA method was at first invented to find out the changes in the copy number from the entire chromosome aneuploidy to one exons deletion and duplication. This method was gradually improved for methylation profiling and detection of mutation. Subsequent ligation of two separate oligonucleotides leads to hybridization. When in the template annealing of both the oligonucleotides then only ligation will occur. [5], [24], [25]

c. Digital PCR

The digital PCR, by an array technology, changes its exponential, analogue nature into a signal digitally obtained that is favourable for finding earlier defined mutations that are found in a small fraction of the cell community. [5], [26]

In India also, the use of molecular diagnosis is rapidly increasing for the detection of leukaemia. Techniques such as FISH and PCR have gained importance for cancer diagnosis because they are high sensitivity and accuracy in results. Further with the development of next-generation sequencing, it is likely to become even more popular in cancer diagnosis. [27]

3 | CONCLUSION

A precise diagnosis is needed in the treatment of the neoplasms related to the hematopoietic system. With the new systems and the novel methods of diagnosing the disorder, an increase in the understanding of the carcinomas molecular biology, the explosion of the sensitive methods of testing, and the new target-oriented therapies that are developed to take benefit from these findings, it is even more crucial as this process prime aim is patient care. However, when conjugated with suitable clinical findings, molecular markers have shown to be effective and have been very important in the diagnosis, therapy, and prediction of hematologic carcinomas. The molecular findings also help in facilitating the probable outlook of the core pathophysiology of the carcinoma and help the healthcare provider in understanding why in certain cases the treatment fails. Finally, the aptitude to identify these signs of cancer allows for close monitoring of the relapse and the response

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