Interphase Chromosome Profiling (ICP) Assay in Peripheral Blood As a Viable Alternative to Bone Marrow Aspirate in the Initial Genetic Screening of Myelodysplastic Syndrome

Babu VR[1] and Rao N[2]

[1]InteGen LLC, Orlando, FL 32819; [2]David Geffen UCLA School of Medicine, Los Angeles, CA 90024

INTRODUCTION

Cytogenetics plays an important role in the diagnosis, classification and in the management of Myelodysplastic syndrome (MDS), and the testing is commonly performed on both peripheral blood and bone marrow samples. However, the failure rate to obtain informative chromosome results from peripheral blood is very high. A common practice in such situations is to reflex to FISH testing using a set of probes designed to detect the most common abnormalities involving chromosomes 5, 7, 8 and 20. While FISH is very valuable when less than 20 metaphase cells are available for analysis or when no metaphases are present, several studies in the literature unequivocally report that in MDS cases with a normal karyotype, FISH with currently used probe sets has no additional clinical value and does not detect any additional chromosome abnormalities. Therefore, classical karyotype studies remain the most important line of testing for MDS. Though, the bone marrow samples often have a higher success rate in producing the necessary diagnostic karyotype, suggestions have been made in the literature about the value of noninvasive approaches such as peripheral blood sample in the initial screening of patients with various anemias and suspected MDS, utilizing FISH probes if necessary when the peripheral blood cytogenetics is unsuccessful (Am J Clin Pathol 2011;135:915-920). According to this study, such noninvasive approaches will be very useful in the initial screening and would avoid or at least postpone the bone marrow aspirate in a significant number of patients resulting in substantial cost savings.

Interphase chromosome Profiling (ICP) is a new novel molecular cytogenetic technology capable of producing a complete molecular karyotype from interphase nuclei of any tissue, including peripheral blood, without the need for culture methods. ICP is failure proof and more sensitive than classical cytogenetics and FISH, and can also characterize the chromosomal material of unknown origin (markers) in cytogenetics preparations (Cytogenet Genome Res 2014;142:226, Abstract #22; manuscript in preparation). Besides the common deletions and monosomies involving the chromosomes 5, 7 and 20; and trisomy 8, several balanced translocations have been identified in MDS. The current FISH panel of probes are not designed to detect balanced changes. Additionally, other chromosomal aberrations present as part of both simple and complex karyotypes such as markers and “derivative chromosomes” cannot be identified by the MDS-FISH panel. We propose that ICP will detect all the relevant diagnostic abnormalities even in peripheral blood samples.

OBJECTIVES

To test the efficacy of using peripheral blood as an alternative to bone marrow from suspected MDS patients using ICP technology and compare the results with standard cytogenetics and FISH methodologies.

MATERIALS & METHODS

A total of ten patients with a suspected diagnosis of MDS were selected for this study. The specimen type consisted of peripheral blood from eight patients and bone marrow from two. Six had the standard cytogenetic studies done and four samples had standard MDS-FISH panel. ICP was done on all samples in a blinded fashion. The ICP protocol is as described below.

The Interphase Chromosome Profiling design is based on the equidistant concept of placing the FISH probes along the whole length of the chromosome as depicted in the ICP Illustrations. The total number of bands in any chromosome arm was largely dependent on the overall length of that arm. Each chromosome arm consisted of a minimum of one and a maximum of six bands. Telomeres and centromeres were given pure color band and the interstitial bands were either pure or hybrid color as depicted in the next section. This configuration provides approximately a 600 band resolution and each band on any given chromosome is molecularly distinct from its adjacent band or any other band on that chromosome. Therefore, any deviation of the expected number and/or position of the bands signifies an abnormality. Based on the specific characteristics of an abnormality, it is classified either numerical or structural and further classified into particular category of abnormality.

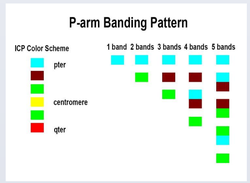
Individual chromosome hybridizations were done on four slides with six areas of hybridization on each slide, as per established standard FISH protocols. DNA probes labelled with fluorochromes DEAC, Fluorescein-12, Cyanine555, Cyanine647, and CF594 were detected using appropriate filters. A minimum of 20 interphase cells were analyzed for each chromosome. Since the entire chromosome was profiled as opposed to targeted site by the standard FISH techniques, the usual guidelines of metaphase analysis were followed with minor modifications in defining the abnormal clone i.e. four or more cells must show an identical structural or numerical abnormality.

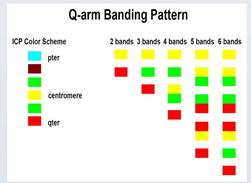
A typical hybridization slide is shown below.

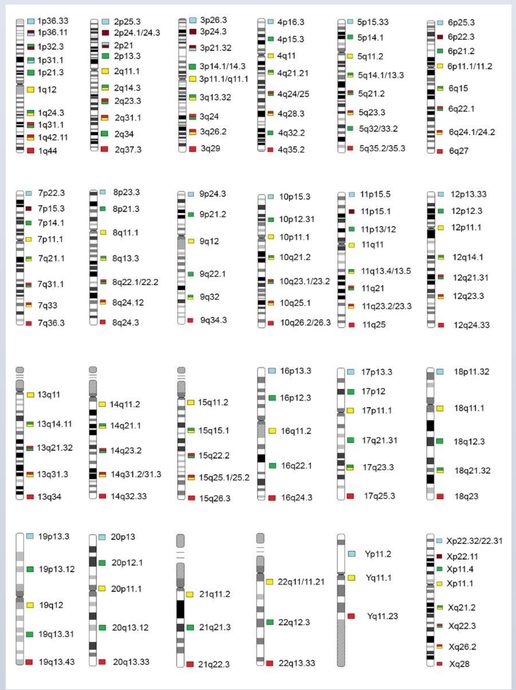
ICP ILLUSTRATIONS

Chr.		Chr.		Chr.	
1	p5/q5	9	p2/q4	17	p2/q4
2	p4/q6	10	p2/q5	18	p1/q4
3	p4/q5	11	p3/q5	19	p2/q3
4	p2/q6	12	p2/q5	20	p2/q3
5	p2/q6	13	p0/q5	21	p0/q3
6	p3/q5	14	p0/q5	22	p0/q3
7	p2/q5	15	p0/q5	X	p3/q5
8	p2/q5	16	p2/q3	Y	p1/q2

Number of bands in each arm of respective chromosomes

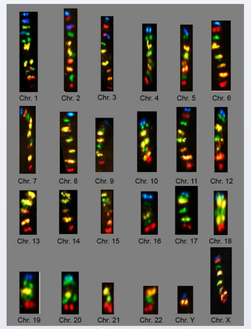




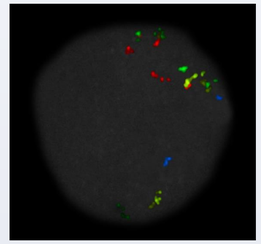


Chromosome Ideograms

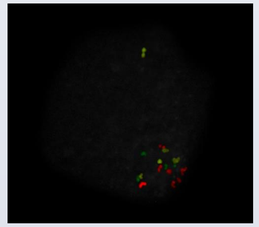
ICP ILLUSTRATIONS (CONT.)



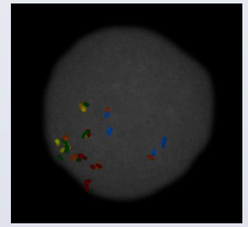
Composite Karyotype



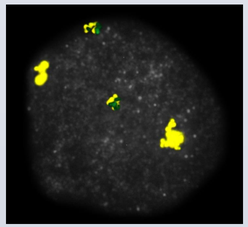
Case 4: Chr. 12 suspected rearrangement



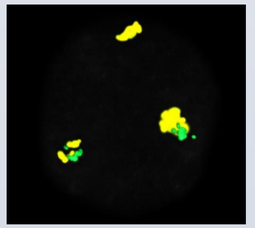
Case 4: Chr. 14 suspected rearrangement



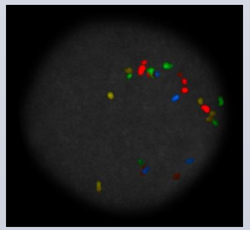
Case 7: Chr. 1 suspected rearrangement



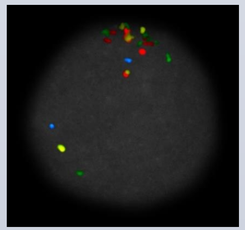
Case 4: Confirmation of normal pattern for Chr. 12 and 14



Case 4: Confirmation of t(12:14)(q13:q11)



Case 7: Chr. 2 suspected rearrangement



Case 7: Chr. 12 suspected rearrangement

RESULTS

Comparison of ICP Findings with Commonly Used Cytogenetic Methods

Case No.	Sample	Clinical Suspicion/Diagnosis	Cytogenetics	FISH *	ICP
1	Bone Marrow	MDS	46,XX[20] Normal		Normal
2	Blood	MDS	46,XY[20] Normal		Normal
3	Blood	MDS	Failed - No Karyotype		Normal
4	Bone Marrow	Relapsed AML with MDS changes	46,XY[20] Normal**		Abnormal icp.t(12;14)(q13;q11)[6]~
5	Blood	Anemia	46,XX[3] Normal Limited study		Normal
6	Blood	Unspecified Leukemia		MDS FISH panel - Normal	Normal
7	Blood	MDS/CLL		MDS/CLL panel - Normal	Normal***
8	Blood	Anemia	Failed - No Karyotpe		Normal
9	Blood	MDS		MDS FISH panel - Normal	Normal
10	Blood	Myeloma/MDS		MDS FISH panel - Normal	Normal

*MDS PANEL: Chromosomes 5, 7, 8, and 20. CLL PANEL: Chromosomes 6, 11, 12, 13, and 17.

**Previous trisomy 8 was not detected in this study.

***Initial suspected abnormalities (translocations) of chromosomes 1, 2, and 12 were not confirmed with concurrent region-specific probes.

~A control case of MDS with normal cytogenetics had the rearrangement in 3 cells with concurrent region-specific 12q13 and 14q11 probes.

DISCUSSION & CONCLUSIONS

Since the karyotype information is critical in MDS, it is imperative that all diagnostic laboratories employ suitable techniques to obtain results on every clinical sample. These techniques may include traditional karyotype, standard MDS-FISH panel or the recently introduced ICP methodology. Bone marrow is the tissue of choice for these studies; however, as demonstrated in a large study (Am J Clin Pathol 2011;135:915-920), a non-invasively obtained peripheral blood sample utilizing proper technology can provide equally important information for the patient management, resulting in significant cost-savings.

In the current study, all 8 peripheral blood yielded successful results with the ICP technique despite a failed karyotype by standard cytogenetics in two of these (cases 3 and 8). In case 5 only three cells were available for analysis with traditional karyotyping and is mostly considered as a failed or an uninformative karyotype result. Traditionally, standard FISH panel with targeted probes that can identify trisomies, monosomies or deletions of chromosomes 5, 7, 8 and 20, is the preferred reflex option for cytogenetically failed cases. However, numerous reports in the literature reveal that sometimes cryptic or balanced translocations involving many chromosomes exist in MDS.

Initial examination of 20 interphase cells in case 4 suggested a possible translocation between chromosomes 12 and 14 as evidenced by an aberrant signal pattern seen in three to four cells. The guidelines for defining a clone using ICP methodology are currently under development (manuscript in preparation). A more focused examination with probes selected close to the suspected breakpoints only on both chromosomes confirmed the presence of a balanced translocation – t(12;14)(q13;q11) in approximately 30% of the cells, whereas a control MDS case with normal karyotype had the abnormality in only 15% of the cells which is below the normal cut-off for this assay. Since this case had a history of AML with trisomy 8 which was not detected by any of the methods used, the presence of t(12;14) is most likely related to secondary MDS.

For case 7, initial analysis with the ICP technique identified four suspected rearrangement breakpoints as shown in the illustrations. However, a more focused attempt, did not confirm the suspected abnormalities. These two cases illustrate the utility of ICP in detecting potential “evolving clones” which will can be missed by current karyotype and FISH studies.

CONCLUSION

Interphase chromosome profiling is:

- Almost 100% failure-proof.
- More sensitive than standard cytogenetics and regular FISH.
- Well suited for the investigation of MDS cases using peripheral blood for initial screening as well as monitoring.

REFERENCES & CONTACT

1) Trask B, Pinkel D (1990): Fluorescence in situ hybridization with DNA probes. Methods Cell Biol 33:388-400.

2) Coleman JF, Theil KS, Tubbs RR, and Cook JR (2011): Diagnostic Yield of Bone Marrow and Peripheral Blood FISH Panel Testing in Clinically Suspected Myelodysplastic Syndromes and/or Acute Myeloid Leukemia A Prospective Analysis of 433 Cases Am J Clin Pathol 135:915-920.

Dr. Babu is the founder and CEO of InteGen LLC

contact@integenllc.com www.integenllc.com