

# Utilization Of Peripheral Blood For Diagnostic Testing For MDS/MPN Patients: Efficacy And Benefits Of A SNP Microarray Analysis

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## I. Introduction

The diagnosis of myeloid disorders is complex and has been traditionally been augmented in the laboratory by cytogenetics, FISH and molecular analysis. However, many specimens are normal based on these tests, or often have incomplete or no results. Technologies utilized for the detection of cytogenetic abnormalities in hematological malignancies has rapidly evolved over the past decade. Microarray analysis is now well established for the genetic evaluation of pediatric patients; however, its utilization for patients with neoplasia is still somewhat limited. Additionally, bone marrow, is the much preferred specimen for testing, but is not always available and only peripheral blood can be tested. The present report reviews a large laboratory's experience with the utilization and efficacy of microarray analysis for analysis of myelodysplasia (MDS) and myeloproliferative disorders (MPN) from peripheral blood.

**OBJECTIVES:** The overall objectives of this work were to determine if SNP microarray analysis from peripheral blood in in Oncology testing could:

Be utilized for the detection of abnormalities in chromosomally normal patients referred to determine if they had myelodysplasia (MDS) and myeloproliferative disorders (MPN).

Determine if microarray analysis had a diagnostic component in patients in which flow cytometry was suspicious, but could not conclusively make a diagnosis of MDS and MPN.

Delineate the presence of copy-neutral loss of heterozygosity (CN-LOH) and determine its implications in peripheral blood samples.

Delineate the more common abnormalities detected by the array analysis in these samples and determine if these results could provide prognostic information.

Emphasize the importance of carefully considered guidelines for reporting abnormalities in Oncology patients.

## II. Materials and Methods

**SPECIMENS AND ASCERTAINMENT:** This study addresses 945 MDS/MPN and 42 AML patients in which peripheral blood was studied by microarray analysis to detect aberrations in myeloid disorders. Peripheral blood samples were obtained for standard cytogenetic analysis, FISH or microarray studies at the discretion of the referring provider. Cytogenetic and FISH studies were done using standard analyses, but were not done for all specimens. For microarray studies, DNA could be extracted from as little as 1 cc. In some cases, specimens were also grouped by Flow Cytometry studies, when the information was available. These were broadly grouped into specimens with no studies, those with no findings and those with either abnormal myeloid maturation, the presence of blasts or an increased presence of monocytes.

**ARRAY METHODOLOGY:** All studies were done utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of Affymetrix, Inc.]. This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 structural non-polymorphic probes (NPCNs). On the average there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250ng of total genomic DNA extracted was digested with NspI and then ligated to NspI adaptors, and amplified using Titanium Taq with a GeneAmp PCR System 9700. PCR products were purified using AMPure beads and quantified using NanoDrop 8000. Purified DNA was fragmented, and biotin labeled and hybridized to the Affymetrix Cytoscan® HD GeneChip. Data was analyzed using Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly. The SNP array analysis is utilized to detect both copy number changes, as well as, copy neutral changes. Criteria for reporting copy number changes were deletions greater than 1.0Mb and duplications greater than 2.0Mb. Deletions or duplications as small as 50kb were also reported in custom genes of known cancer significance. Possible CN-LOH was reported when a homozygous block greater than 20Mb was present on a single chromosome interstitially and 8 Mb if it involved telomeric regions.

## III. Results

Our initial evaluation studies clearly showed that using microarray technology with peripheral blood is effective for studying patients with myelodysplasia (MDS) and myeloproliferative disorders (MPN). Therefore, this analysis has been added to our standard evaluation of patients when requested. Thus far we have studied almost 1000 clinical patients and have correlated the array findings to other analysis in these patients including chromosome and FISH studies and flow cytometry studies. These studies have shown the following:

Peripheral blood, rather than bone marrow is submitted for analysis in approximately 15% of the MDS/MPN patients and 19.9 % of the AML patients.

When flow cytometry showed any anomaly that might be associated (238 patients) with MDS/MPN (abnormal myeloid maturation, increased frequency of blasts, increased frequency of monocytes), array analysis revealed abnormalities in 46.7% of the patients. If at least two of the these findings were abnormal MPN (abnormal myeloid maturation, increased frequency (60.8%) of the patients.

When flow cytometry demonstrated no immunophenotypic abnormalities (327 patients) the array analysis revealed abnormalities in 15.3% of the patients.

Overall, 313 patients (33.1%) of the 945 studied referred for MDS/MPN had abnormalities detected by the array studies. In this manner it has been shown to be effective diagnostically.

Cytogenetic analysis was attempted in 441 patients and was only successful in 43.1% of the patients. When chromosomes failed, microarray analysis was successful in all cases and demonstrated abnormal findings in 21.3 % of the patients.

When chromosomes were abnormal, the array provided additional information in ~59.7% of the patients, which in many cases could be used providing better prognostic information.

In numerous patients, abnormalities might be detected by chromosomes/FISH – but the array may provide additional (prognostic) information. In MDS 5q-, 7q-, 20q- OR +8 is often detected, when one of the above is detected then in ~64% of the cases additional abnormalities by the array.

CN-LOH has been shown to be prevalent in leukemia and is seen in the peripheral blood MDS/MPN samples in ~ 11.5% of the patients with abnormalities in this study; the majority (58.2%) were as individual changes, while 41.8% were present with copy number.

The most common CN-LOH regions are 4q, 7q, 9p, 11q, 14q, and 17p, most likely involving the following genes: TET2, EZH2, JAK2, CBL, TP53.

In addition to the abnormalities routinely detected by cytogenetics and FISH, the array detected numerous abnormalities including: deletions and CN-LOH of 4q (TET2); 21q (RUX1); 11q (Cbl); 17p (TP53), 9p (JAK2), 17q (NF1), 1p (likely MPL or NRAS), 7q(CUX1) and CN-LOH of 7q (EZH2). The most common changes seen involved the TET2, CUX1, and TP53 genes.

Twenty-two concurrent peripheral blood and bone marrow samples were studied in these patients and in all cases yielded the same result.

## IV. Case Examples

### CASE EXAMPLE 1:

A 57-year-old male with pancytopenia was referred for evaluation for MDS. Initial cytogenetic and FISH studies done one year earlier on bone marrow were normal. At that time flow cytometry evaluation was suggestive of chronic myelomonocytic leukemia (CMML); however, an array analysis was not done. At the current visit only peripheral blood was obtained. Flow cytometry evaluation on peripheral blood did not detect any diagnostic immunophenotypic abnormalities, but monocytosis (24%) with increased CD56 was detected suggestive of CMML. Microarray analysis of peripheral blood revealed a normal array analysis showed normal dosage, but mosaicism (~70%) for long arm chromosome 4 allele homozygosity (copy-neutral loss of heterozygosity; CN-LOH) suggesting a TET2 homozygous mutation.

### CASE EXAMPLE 2:

A 91-year-old female with anemia, thrombocytopenia and macrocytosis was referred for evaluation for MDS. Initial cytogenetic studies could only yield a limited study of 5 normal metaphases [46,XX(5)]. Flow cytometry evaluation did not detect any significant immunophenotypic abnormalities. Microarray analysis of peripheral blood revealed a 58.4 Mb interstitial deletion of 5q14.3->q33.3 [arr [hg19] 5q14.3q33.3(84,946,953-143,322,611) x1~2] in 80% of the cells. These findings revealed an MDS related clone consistent with 5q- syndrome. Because of the array findings a bone marrow study was done revealing an interstitial 5q deletion. Hematopathology revealed MDS with multilineage dysplasia

## V. Discussion and Conclusions

These findings have provided compelling results for the utilization of a SNP microarray with peripheral blood specimens for the evaluation of MDS/MPN. Using this technology, abnormalities in chromosomally normal patients or patients where cytogenetic studies failed, especially when flow cytometry studies couldn't effectively diagnose MDS/MPN. We were also able to clarify additional chromosomal anomalies and provide better prognostic information. In addition, SNP microarray technology provided for the detection of CN-LOH, which has been shown to be of extreme importance in MDS/MPN. With this delineation, candidate genes that may have mutations and importance in cancer can be identified; these are localized in the homozygous blocks on the SNP microarray. Additionally, it is important to carefully consider guidelines for reporting abnormalities detected by the SNP microarray. In Oncology studies, by obtaining complete clinical information, including previous karyotype and FISH findings, the lab is better able to interpret copy number and copy neutral changes identified by microarray. SNP microarray can be a useful tool in cancer analysis, and MDS/MPN, for patients where only a peripheral blood sample is available.

The results from the analysis of these 945 MDS/MPN patients, have revealed several important findings:

This study clearly demonstrates the improved efficacy of using the array analysis of peripheral blood specimens assist in the diagnosis of MDS/MPN.

In all concurrently studied patients the peripheral blood studies were equivalent to bone marrow analysis.

Arrays on unstimulated blood was more effective than chromosome studies, especially in samples with little or no blast cells, providing results in all patients studied.

Arrays successfully revealed the same abnormalities seen by a MDS FISH panel and in some cases containing other specific genes that may alter the prognosis yielding additional prognostic findings in more than a third of the patients (e.g. deletions of RUNX1, BCOR, TET2, and CN-LOH 4q, 7q, 11q).

The findings suggest that the higher diagnostic yield of the microarray can easily supplant the use of routine FISH that is restricted to copy number changes in peripheral blood samples and limited by 4 probes (5/5q, 7/7q, 8 and 20q) in a routine MDS FISH panel.

These findings also show that when a bone marrow and peripheral blood array analysis was done at the same time, the results were concordant (including both CNVs and CN-LOH).

Our studies suggest that the utilization of microarray analysis in patients with myelodysplasia (MDS) and myeloproliferative disorders (MPN) is essential for both diagnosis and in some cases prognostic evaluation.

## VI. Disclosure

All of the authors are employees of Laboratory Corporation of America® Holdings, a diagnostic laboratory company.