Reliability of Cell-Free DNA (cfDNA) Next Generation Sequencing in Predicting Chromosomal Structural Abnormalities and Cytogenetic-Risk Stratification of Patients with Myeloid Neoplasms.

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INTRODUCTION

Cytogenetic studies require fresh viable cells for culturing and performing cytogenetics analysis remains mainly manual, expensive and time consuming. FISH studies help in reducing time and can be used in FFPE samples. However, FISH can test for predetermined chromosomal abnormalities one abnormality at a time. Recent advances in high-throughput genomic technologies allowed broader evaluation of chromosomal abnormalities using arrays. Array technology offers wider genome coverage with higher resolution. However, this technology requires significant quantity of samples. Next-generation sequencing (NGS) is increasingly being implemented to evaluate chromosomal structural abnormalities including chromosomal translocations. whole-genome sequencing has been shown to be reliable in detecting various chromosomal abnormalities including amplifications, copy number variations (CNVs), uniparental disomy, mosaicism, small indels and single nucleotide variations (SNVs).

AIM

We explored the potential of using liquid biopsy and next generation sequencing (NGS) in detecting chromosomal structural abnormalities or copy number variation (CNV) in patients with hematologic neoplasms. We tested the reliability of using targeted sequencing for the determining the chromosomal structural abnormalities in cell-free DNA (cfDNA) at the same time capturing single nucleotide variants (SNV) with enough depth in sequencing for detecting mutations and minimal residual disease.

METHOD

Peripheral blood plasma samples from 144 patients with myeloid neoplasms were used to extract cfDNA for NGS testing. This included 49 patients with MDS, 31 with AML, and 64 patients with myeloproliferative neoplasms (MPN). The median age was 68.5 (range: 24-96). Female were 56 (39%). cfDNA was sequenced using 275 gene panel. The panel uses single primer extension (SPE) approach with UMI. Sequencing depth was increased to more than 1000X (after removing duplicates). CNVkit software was used for analyzing and visualizing copy number variations.

RESULTS

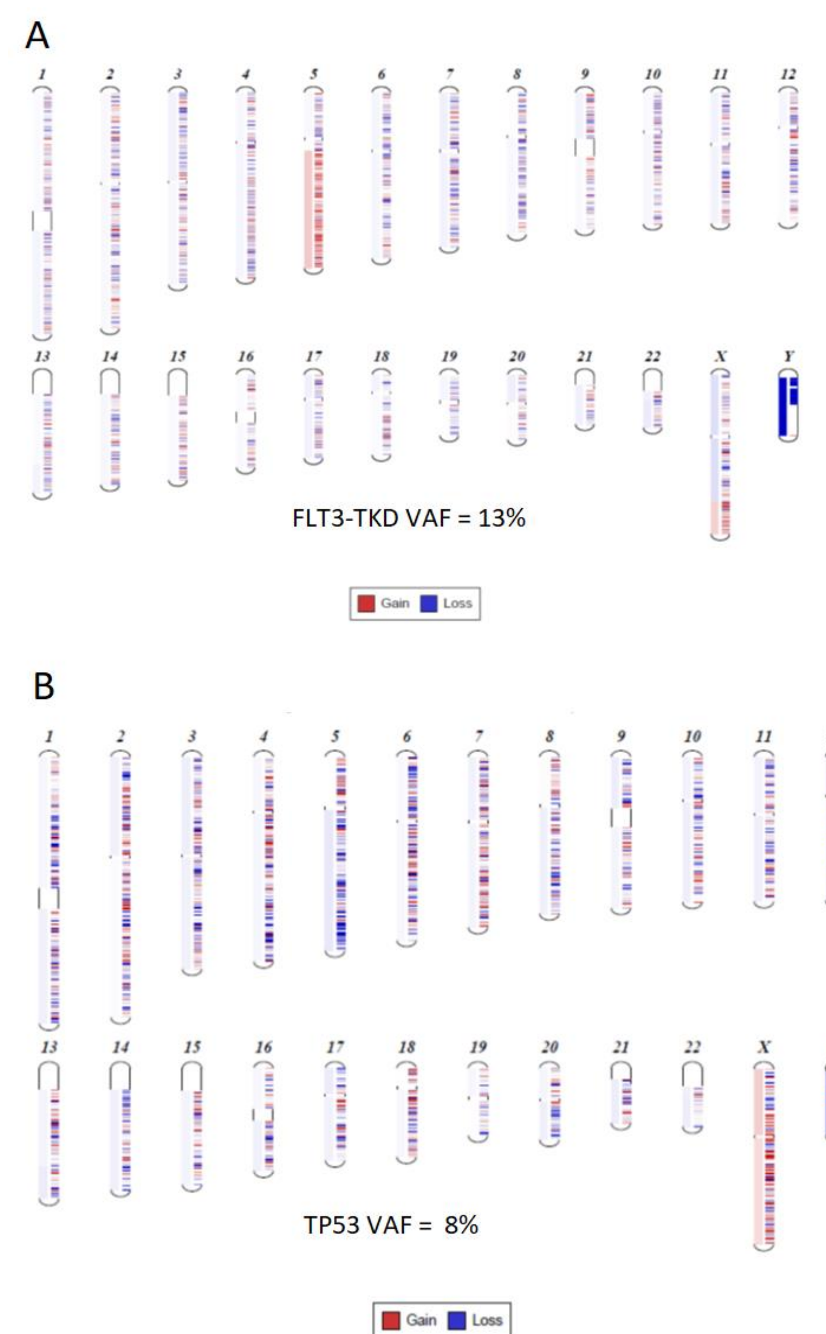
- Of the 1539 tested samples with evidence of neoplastic clone 633 (41%) had abnormalities associated with lymphoid neoplasms and 906 (59%) had myeloid neoplasms.
- Of the lymphoid neoplastic cases, 76 (12%) had chromosomal abnormalities detected in cfDNA. Of the 906 myeloid cases, 146 (16%) samples showed chromosomal structural abnormalities.
- Two samples showed no detectable mutations, but showed chromosomal structural abnormalities in cfDNA analysis, 5q deletion in one case of MDS and and trisomy 12 in one case of CLL.

Correlation between NGS chromosomal structural analysis and bone marrow cytogenetic studies

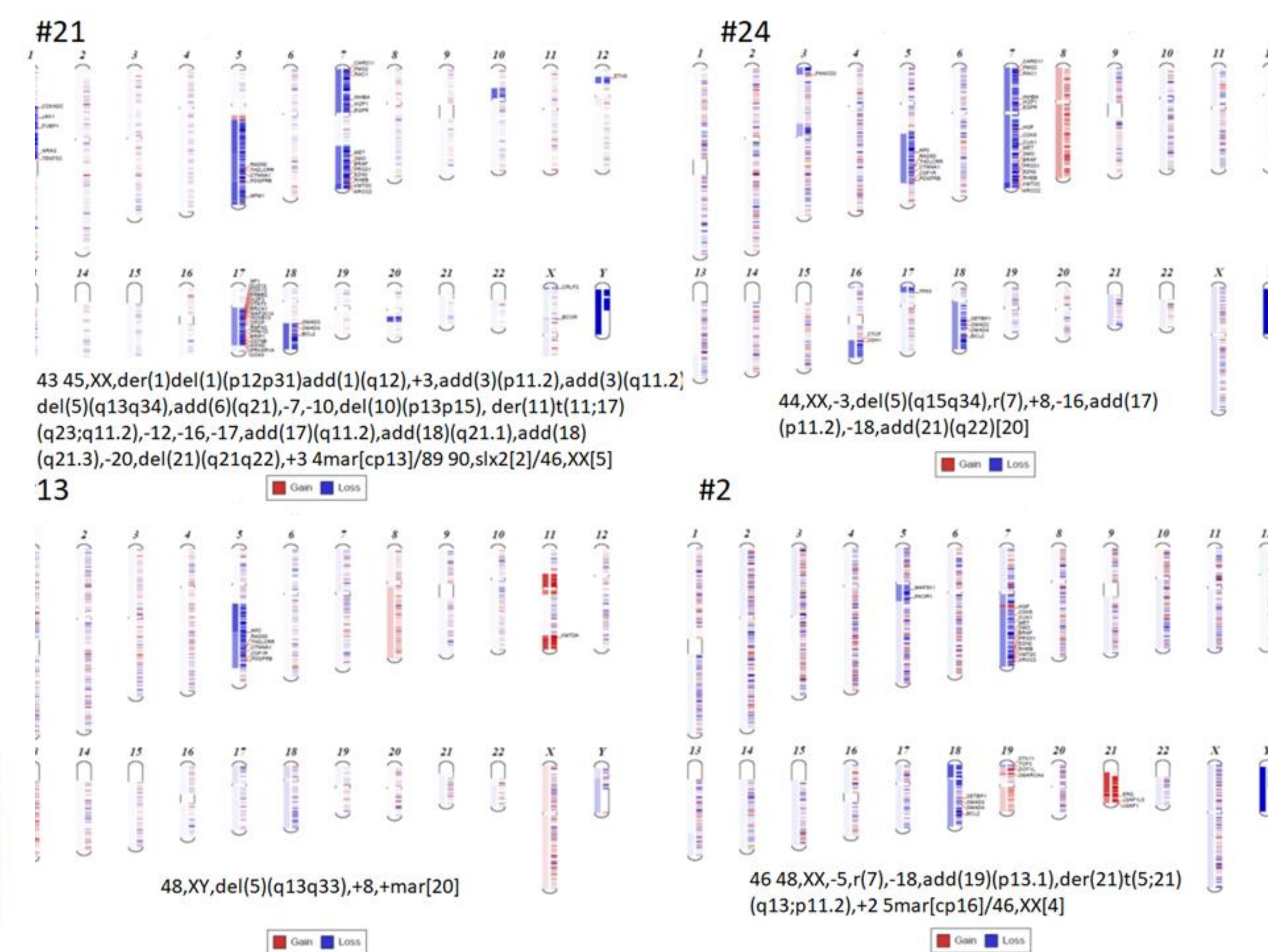
- 89 liquid biopsy samples had bone marrow cytogenetic data obtained within a week or two of the liquid Bx sample
- 33 sample (37%) showed chromosomal abnormalities
- 3 samples (3%) with fusion only abnormality
- 8 samples (9%) with "no metaphases detected'
- 45 (51%) with normal karyotype).

When the 89 cases were grouped into three myeloid risk groups: intermediate, poor and complex, there was 100% concordance between the NGS chromosomal structural analysis and cytogenetic data.

Sensitivity of NGS in detecting chromosomal structural abnormalities in cfDNA



Examples of cfDNA CNV findings as compared with karyotyping results of bone marrow samples.



Sensitivity:

- Chromosomal structural abnormalities easily detected in a sample with neoplastic clone at VAF 0f 13%
- Lower reliability of detection can be seen in a sample with neoplastic clone at VAF of 8%.

Sample #	CNV by Liquid Bx NGS	Cytogenetic report	Interpretation (agreement: Yes/No)
1*	5q-, 7q-, 18p-, 19p+, 19q+, 21q+(amplification)	46 48,XX,-5,r(7),-18,add(19)(p13.1),der(21)t(5;21)(q13;p11.2),+2 5mar[cp16]/46,XX[4]	complex (Yes)
2*	5q-, 7q-, -18 (bi-allelic 18p-), 19p+, 19q+, 21q+(amplification)	46 48,XX,-5,r(7),-18,add(19)(p13.1),der(21)t(5;21)(q13;p11.2),+2 5mar[cp16]/46,XX[4]	complex (Yes)
3	1q+, 7q-, 19q+, 21q+	46,XY,+1,der(1;7)(q10;p10)[2]/47,idem,+21[14]/48,idem,+8,+21[2]/47,idem,+8[1]/46,XY[1]	complex (Yes)
4	5q-, 8q+, and 17p-	46,XX,del(3)(q21q25),add(5)(p13),del(5)(q13q33),-17,der(17;21)(q10;q10),add(21)(p11.2),+mar[cp20]	poor (Yes)
5	5q-, 7q-, +8, 17p-, 17q1(proximal), 17q+(distal),-19, +21	46 47,XY,del(5)(q15q34),add(7)(q21),+8,del(11)(q22q23),-17,-19, -22,+2 3mar[15]/46,XY[5]	poor (Yes)
6	5q-; 7q-, 17p-, 18q-	45,XX,del(5)(q13q33),-7,der(17)add(17)(p11.1)add(17)(q23),del(18)(q21.3q23)[12]/45,XX,del(5)(q13q33),dic(7;17)(q11.2;p11.1),del(18)(q21.3q23)[3]/46,XX[5]	complex (Yes)
7	2p-, 3p-, 5q-, 17p-, 17q-(partial), 19p+, 21q+	42 43,XY,add(3)(p13),-2,-3,-5,add(16)(q12.1),-17,add(19)(q13.1),-20,+2mar,inc[cp3] LIMITED STUDY	complex (Yes)
8	3p-, 5q-, 7q-, 12p- and +22.	45,X,-Y,add(1)(q21),del(3)(p13p25),dic(5;12)(q11.2;p11.2),add(7)(q11.2),+mar[19]/46,XY[1]	complex (Yes)
9	monosomy 7	45, XX,-7[9]/46.xx[11]	poor (Yes)
10	5q-, +8, +11, +13, and 17q-	40 48,XY,+5,del(5)(q15q33),der(5;14)(p10;q10),der(5;17)(p10;q10),-7,+8,i(11)(q10),idic(13)(p11.2),add(14)(p11.2),add(16)(q24), i(17)(q10),-18,der(20)t(11;20)(q13;q13.3),-21,+22,+mar[cp17]/46,XY[3]	complex (Yes)
11	5q-, -7, 11q+, 12p-, 17p-, 18p-, 19q+	44,XY,-5,-7,inv(12)(p13q13),add(17)(p11.2),-18,der(19)ins(19;?)(q13.1;?),-20,+2mar[8]/43,idem,-11,add(13)(q32)[5]/44,idem,r(11)(p15q25)[2]/46,XY[7]	complex (Yes)
12	-5, 8p+, 9p-,11p-, 17p-, -18 and 20p-	45,XX,add(3)(q21) ,add(5) (q11.2)x2, der(6)t(6;17) (q27;q11.2) ,add(7) (q31) ,+8,der(13)t(5;13) (q15;q32) ,-17 ,1-18[10]/46,XX[4] INCOMPLETE STUDY	complex (Yes)
13	5q-, 8q+, 11p+(proximal amplification), 11q (KMT2A gene amplification), +13.	48,XY,del(5)(q13q33),+8,+mar[20]	poor (Yes)
14	3p-, 5q-, -7, and 12p-	43 44,XX,-3,dic(5;15)(q11.2;p11.2),-6,del(6)(p23p25),der(7;12)t(7;12)(p10;q10)ins(7;?)(p11.2;?),+der(?)t(?;3)(?;q12),+r[cp17]/46,XX[3]	complex (Yes)
15	4q-, 5q-, 7q-, +11, 12p-, 13p+, 13q+, -16, 17p-, -18, +21 and +Y.	44 50,XYY?c,-4,dic(5;17)(q13;p11.2),add(7)(q11.2),der(12)ins(12;?)(q13;?),-14,-21,-22,+2 8mar[cp14]/47 48,idem,+11,+13[cp3]/81 89,idemx2[2]/47,XYY?c[8]	complex (Yes)
16	7q-	46, XX, del(7)(q22q32)[19]/46,XX[1]	poor (Yes)
17	12p-	46,XY[20]	intermediate (Yes)
18	1q+, 2p+, 3q-, -4, -7, 9q+, 13q+, 17p-, 17q-, 20q+, 21q-	44 46,XY,add(2)(p11.2),-3,add(3)(q11.2),add(4)(q12),-5,der(5)t(3;5)(p13;p13)ins(5;?)(p13;?),del(6)(p23p25),-9,add(13)(q12),-17,-17,add(20)(q11.2),+3 5mar,inc[cp5] Limited Study	complex (Yes)
19	1q+ and trisomy 14.	46,XY,i(14)(q10)[20]	intermediate (Yes)
20	8p-, 9p- (PAX5, CD174, CDKN2A/B), 17p- and gain:1p+, 17p+.	45,XY,der(8)t(1;8)(q12;p21),inv(8)(p11.2q24.3),add(9)(p13),i(17)(q10),-20[4]/46,XY[20]	poor (Yes)
21	1p-, 5q-, -7, 10p-, 12p-, 17q-, 18q- and 20q-	43 45,XX,der(1)del(1)(p12p31)add(1)(q12),+3,add(3)(p11.2),add(3)(q11.2), del(5)(q13q34),add(6)(q21),-7,-10,del(10)(p13p15), der(11)t(11;17)(q23;q11.2),-12,-16,-17,add(17)(q11.2),add(18)(q21.1),add(18)(q21.3),-20,del(21)(q21q22),+3 4mar[cp13]/89 90,slx2[2]/46,XX[5]	complex (Yes)
22	3q-, 5q-, 7p-, 8q+, 12p-, 12q-, -16, 17p-, -18, -20	43,XY,del(5)(q13q33),del(7)(p13p22),add(9)(q13),der(12)add(12)(p11.2)del(12)(q14q21),-16,-18,-20[20]	complex (Yes)
23	+8 and 10q-	46,XY,der(4)t(4;8)(q33;q13),t(8;21)(q22;q22)[7]/47,XY,+8,t(8;21)(q22;q22)[5], LIMITED ANALYIS	intermediate (Yes)
24	3p-, 5q-, -7, +8, 16q-, 17p-, 18q-, +21	44,XX,-3,del(5)(q15q34),r(7),+8,-16,add(17)(p11.2),-18,add(21)(q22)[20]	complex (Yes)
25	Trisomy 21	47,XX,+21[13]/46,XX[3]	Intermediate (Yes)
26	8p+, 18p-	47,XY,+8[4]/46,XY[16]	Intermediate (Yes)
27	1q+, 8q+	47,XY,dup(1)(q11q44),+8[17]/46,XY[3]	Intermediate (Yes)
28	1p+, 5q-, +6, 7q-, -11, 17p- and others	44,XY,add(2)(p11.2),der(5)t(5;17)(q15;q21),add(6)(p21.3),del(7)(q22q36),-11,del(13)(q12q14),-14,der(16)t(14;16)(q24;q11.2),-17,add(21)(q22),+r[19]/46,XY[1]	Complex (Yes)
29	monosomy 7 and 12p-	46,XY,r(7)[3]/46,XY[1], LIMITED ANALYSIS	Poor (yes
30	Normal	92,XXXX,add(12)(q24.1)x2[20]	Intermediate (Yes)
31	9p-	46,XY,del(9)(q13q34)[3]/46,XY[17]	Intermediate (Yes)
32	2q+, 2q-(distal, IDH1 and ERBB4 deletion), 3p-, 5q-, 7q-, and 12p-.	39 45,XX,add(2)(q22),del(3)(p11),dic(5;7)(q11.2;q11.1),-12,-17,+mar[cp19]/46,XX[1]	Complex (Yes)
33	5q+ (gain)	46,XX[20]	intermediate (Yes)
34	9p-(deletion of CDKN2A and CDKN2B)	NO METAPHASES DETECTED	Poor (N/A)
35	7q- and 8q+	NO METAPHASES DETECTED	Poor (N/A)
36	12p-	NO METAPHASES DETECTED	Intermediate (N/A)
37	normal	No Metaphases Detected	Intermediate (N/A)
38	Normal	NO METAPHASES DETECTED	Intermediate (N/A)
39	Normal	NO METAPHASES DETECTED	Intermediate (N/A)
40	Normal	No Metaphases Detected	Intermediate (N/A)
41	Normal	NO METAPHASES DETECTED	Intermediate (N/A)
42	Normal	46,XX,t(9;22)(q34;q11.2)[4]/46,XX[16]	No Loss/Gain (Yes)
43	Normal	46,XX,inv(16)(p13.1q22)[22]	No Loss/Gain (Yes)
44	Normal	46,XY,t(9;22)(q34;q11.2)[20]	No Loss/Gain (Yes)
	45 sample with no chromosomal abnormalities	45 samples with Normal Karyotype	45 sample (Yes)

CONCLUSIONS

1) Targeted NGS is reliable in detecting chromosomal structural abnormalities in myeloid neoplasms without the need for whole genome sequencing.

2) Liquid biopsy is adequate for the detection of cytogenetic abnormalities if adequate tumor DNA is in circulation (VAF >10%)

3) This study suggests that using liquid biopsy and evaluating mutations chromosomal structural abnormalities is appropriate approach for screening patients suspected of having myeloid neoplasm.

4) liquid Bx and targeted NGS testing can be used when cytogenetic studies shows insufficient metaphases.

5) This study does not address chromosomal translocations and different approach needs to be developed to detect chromosomal translocation in liquid biopsy.

CONTACT INFORMATION

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