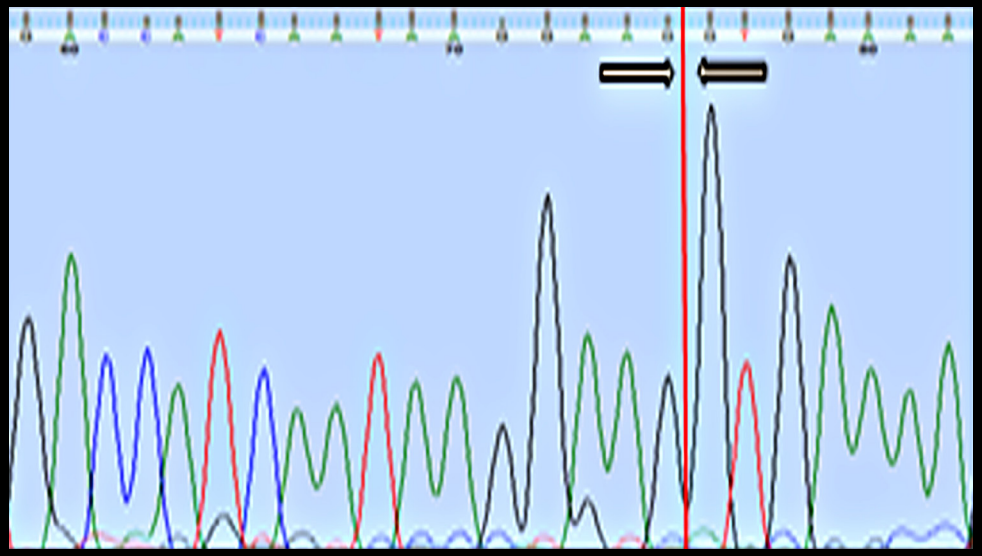
REAL TIME POLYMERASE CHAIN REACTION

All markers for AML were negative when tested by qRT-PCR Repeated. Surprisingly, BCR-ABL transcripts were also not detected in this assay. Our current taqman probe based assay detects the Major- e13a2/e14a2, minor –e1a2 and micro – e19a2 fusion transcripts in comparison to ABL1 as reference gene. Quantitative RT-PCR testing for all three BCR-ABL transcripts yielded negative results for this case.

RESULT INTERPRETATION AND SUBSEQUENT ACTION

Based on flow cytometry assessment and presence of Philadelphia chromosome in 100 % metaphases, this patient clearly had evidence for chronic myeloid leukemia in blast crisis. However, the failure of RT-PCR to capture the three common BCR-ABL transcripts led us to speculate the presence of a rare fusion variant.



We went on to amplify the BCR-ABL cDNA of the patient using primers spanning BCR exon 13 and ABL exon 7 to identify any rare breakpoint involving the M region of BCR. The PCR product involving the e13a2 fusion is expected to be 1.5 Kb in size, but we obtained a product of approximately 1.1 kb in size which was unusual. Hence, we subjected this PCR product to direct Sanger sequencing to identify the missing region. Sequencing data on analysis showed the fusion between BCR exon 13 and ABL exon 3, with the exon 2 missing. We came to the conclusion that the patient held a rare BCR-ABL fusion transcript **e13a3** found in less than 1% of CML cases.