



‘PHi-RACE: PGIMER In-House Rapid & Cost Effective Classifier for the detection of *BCR-ABL1*-like Acute Lymphoblastic Leukaemia in Indian Patients’

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Abstract

For the detection of *BCR-ABL1*-like ALL cases, two methodologies, specifically Gene expression profiling (GEP) or Next-generation targeted sequencing (NGS) and TaqMan based low-density (TLDA) card, are being used. NGS is very costly and TLDA is not widely commercially available. In this study, we quantified the expression of 8 selected overexpressed genes in 536 B-ALL cases. We identified 26.67% (143/536) *BCR-ABL1*-like ALLs using hierarchical clustering and principal component analysis. *BCR-ABL1*-like ALL cases were significantly older at presentation (p=0.036) and had male preponderance (p=0.047) compared to *BCR-ABL1*-negative ALL cases. Lastly, we built a PHi-RACE classifier (sensitivity = 95.2%, specificity= 83.7%, AUC= 0.927) using logistic regression to detect *BCR-ABL1*-like ALL cases promptly at diagnosis. This classifier is beneficial for haematologists in quick decision making at baseline to start tailored treatment regimes.

Background

A new provisional entity of “B-lymphoblastic leukaemia/lymphoma, *BCR-ABL1*-like” has been introduced in the 2016 revised edition of WHO classification of myeloid neoplasm and acute leukaemia. *BCR-ABL1*-like acute lymphoblastic leukaemia (*BCR-ABL1*-like ALL) exhibits similar gene expression profile to that of *BCR-ABL1*-positive ALL and does not express the RNA fusion transcripts and proteins resulting from Ph chromosome. To the best of our knowledge, there have been no previous studies in India regarding the actual heterogeneity and cost effective diagnostic approaches of *BCR-ABL1*-like ALL cases.

Objective

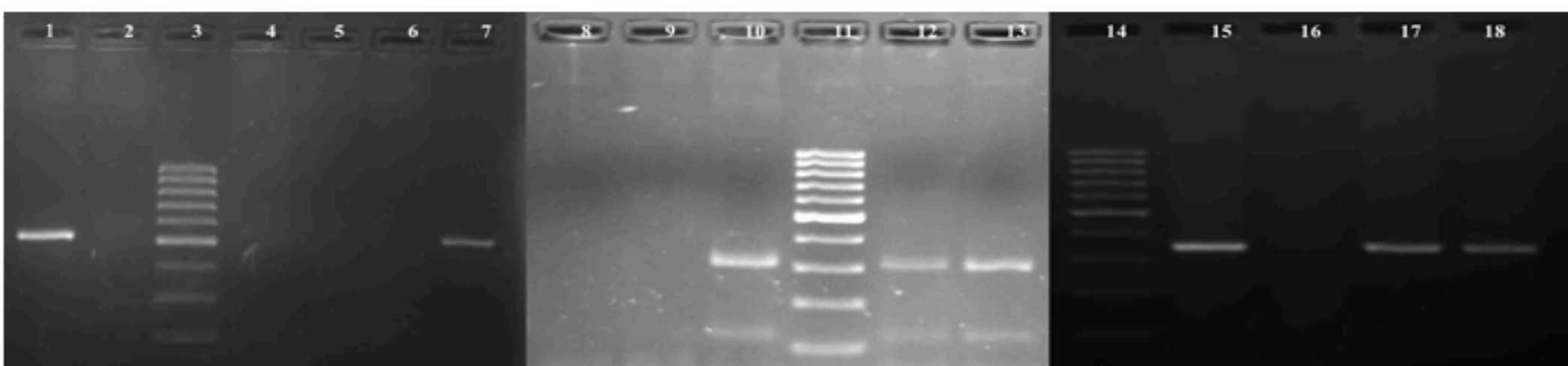
***BCR-ABL1*-like ALL signature identification, followed by the creation of a PHi-RACE classifier using machine learning approach.**

Materials & Methods

Flowcytometric immunophenotyping and multiplex RT-PCR were performed on 629 B-ALL cases, to detect the positivity of *BCR-ABL1* chimeric fusion transcripts. Further, 12 *BCR-ABL1*-positive cases were subjected to transcriptome profiling using Affymetrix microarray (Gene Chip™ Human Genome U133 Plus 2.0 Array). Selected 8 best classifier genes were quantified in 551 B-ALL cases to make a logistic regression model for the rapid detection of *BCR-ABL1*-like ALL cases, followed by PHi-RACE classifier generation and validation (n=93).

Results

In the cohort of 629 adult B-ALL cases, the incidence of *BCR-ABL1* fusion transcripts was 17.17% (108/629) {(b2a2 and b3a2 = 8.90% (56/629) & e1a2 = 8.26% (52/629)} as revealed by multiplex RT-PCR assay.



Lane 1	Positive Control	521 bp (e1a2)	Lane 10	Positive Control	310 bp (b2a2)	Lane 15	Positive Control	385 bp (b3a2)
Lane 3	Ladder	100 bp	Lane 11	Ladder	100 bp	Lane 14	Ladder	100 bp
Lane 7	Minor breakpoint (e1a2; p190kDa)	521 bp (e1a2)	Lanes 12 & 13	major breakpoint (b2a2; p210kDa)	310 bp (b2a2)	Lanes 17 & 18	Major breakpoint (b3a2; p210kDa)	385 bp (b3a2)

A total of 1574 differentially expressed (DE) genes (460 genes in patients with minor fusion transcript e1a2, 515 genes in patients with b2a2 major fusion transcript and 599 genes in patients with b3a2 major fusion transcript) have been identified.

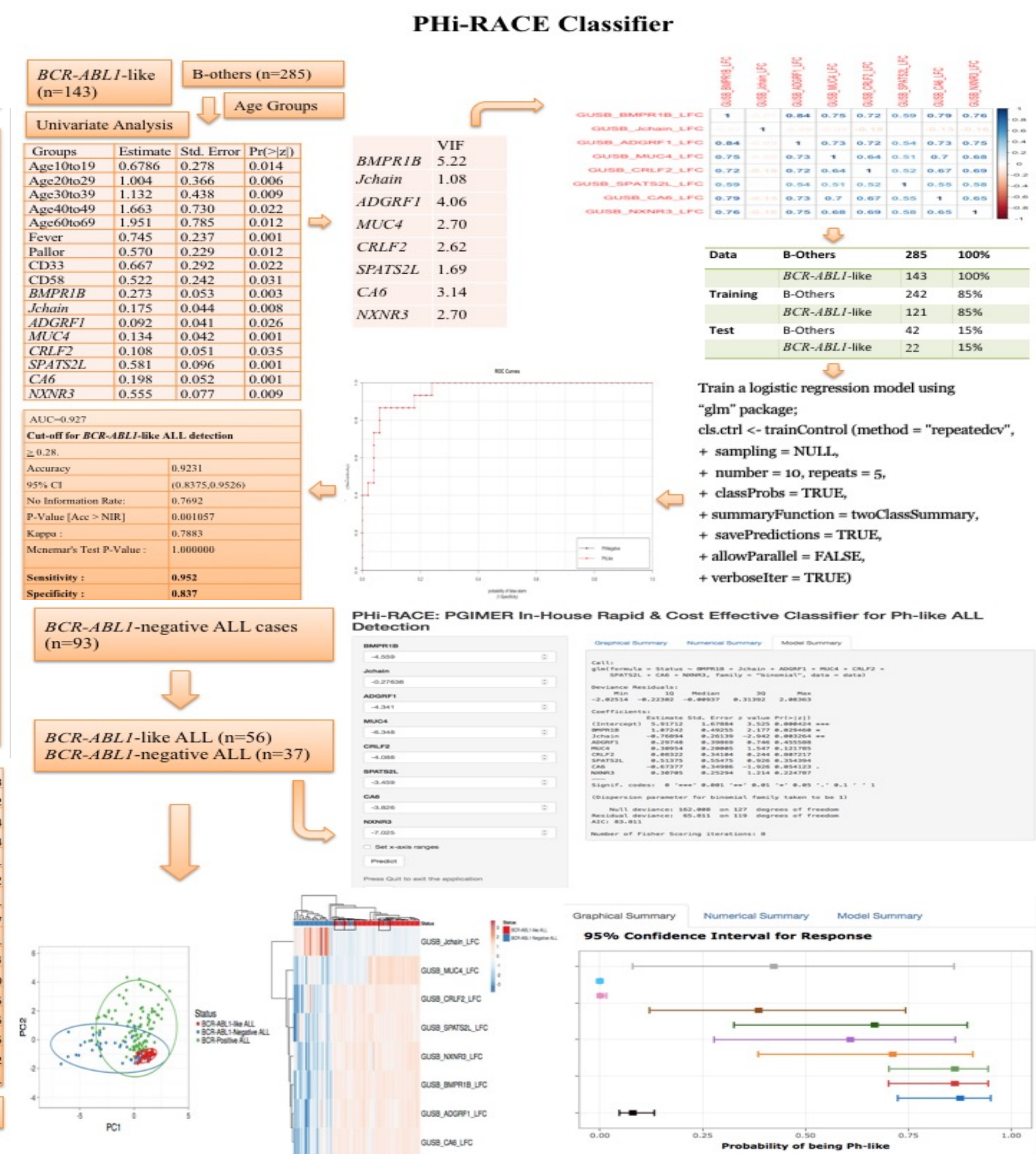
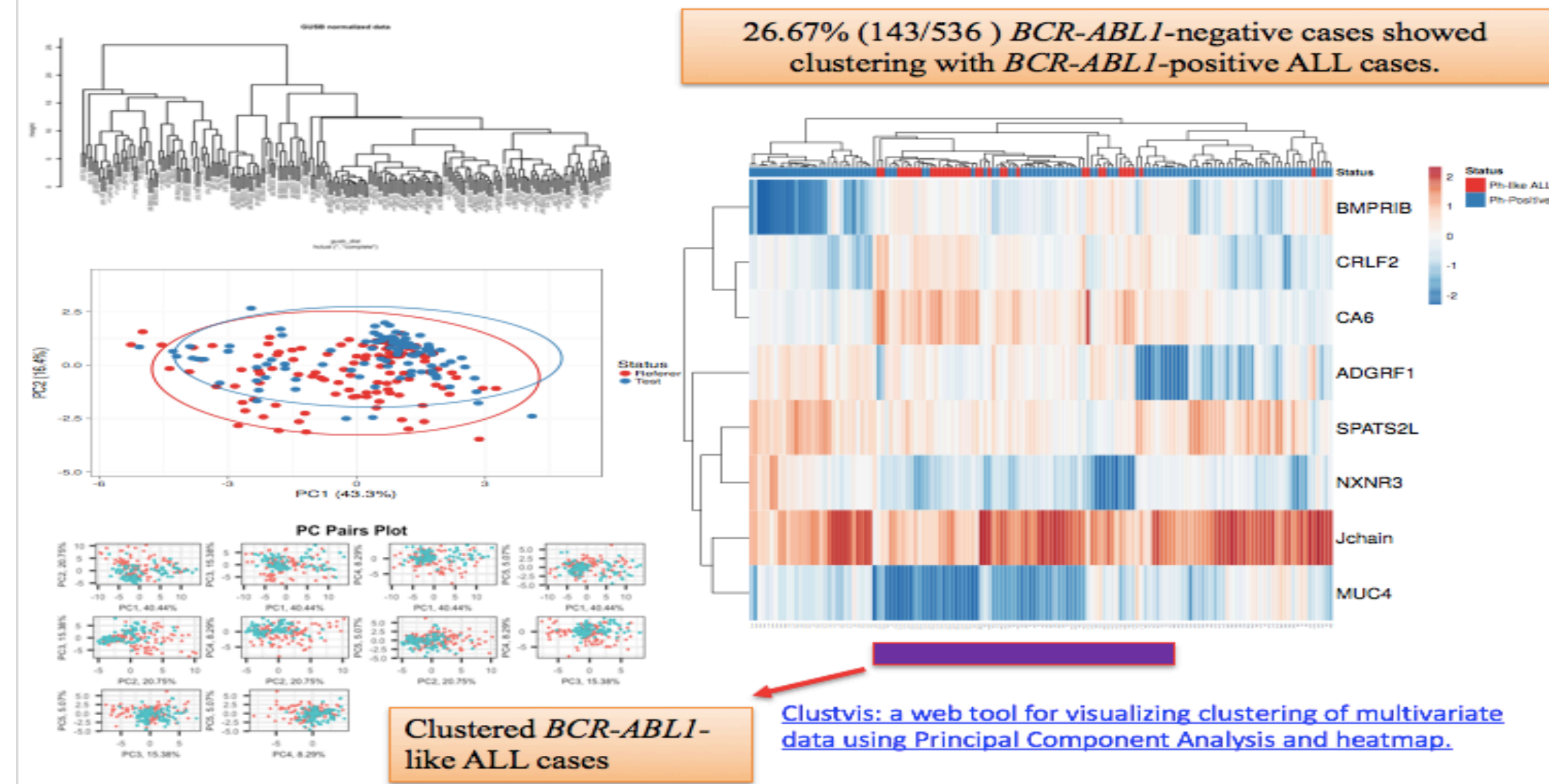
Differentially expressed (DE) genes were identified according to the criteria: Anova p (Corr) cut-off = 0.05 and log fold change cut-off of 2.0.

DE genes were further filtered through hierarchal clustering analysis and a total of 45 DE genes with 10- to -86-fold change were identified.

We refined 45 identified gene and selected 15 genes by performing penalized logistic regression analysis using glmnet R package based upon previous literature and their regression coefficient values.

Finally 15 genes were selected whose performance was individually analysed using receiver operating characteristic curve (ROC) and area under the curve (AUC).

For the detection of *BCR-ABL1*-like ALL cases from B-others we used hierarchal clustering and PCA analysis.



Summary

We developed and validated a PHi-RACE classifier for the first time in a developing country. This predictive classifier is rapid, cost-effective [USD 42.00 (INR 3,000.00)/sample], with short turnaround time (4 hours), including testing and interpretation. This classifier is advantageous over other approaches for the prompt detection at baseline to start tailored treatment regimes in *BCR-ABL1*-like ALL cases.

Discussion

Results of our predictive classifier are very different from the study conducted by Chiaretti S et al, who used 10 differently overexpressed genes, namely *SOCS2*, *IFITM1*, *CD99*, *TP53*, *INP1*, *IFITM2*, *JCHAIN*, *NUDT4*, *CD97*, *SEMA6A* to construct a *BCR-ABL1*-like ALL statistical predictor. Only *Jchain* was common to the genes selected in our study. We selected 8 overexpressed genes namely *JCHAIN*, *SPATS2L*, *CA6*, *NRXN3*, *MUC4*, *CRLF2*, *ADGRF1*, *BMPR1B*, and *EEF2* and these genes are also represented in other TLDA models devised by various authors. As TLDA cards are not widely commercially available, we used RQ-PCR data to make a logistic regression classifier to identify *BCR-ABL1*-like ALL among our B-ALL patients efficiently. Both Reshmi SC et al and Roberts KG et al used *EEF2* as control gene and in our study *EEF2* showed significant variation and was excluded for analysis. So we built a GUSB normalized PHi-RACE classifier, based upon quantification of overexpressed 8 selected genes.

References

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