Myelodysplastic syndrome (MDS) is a neoplastic disease of hematopoietic stem cells with abnormalities involving the immune system. Therefore abnormalities can be detected in myeloid lineage as well as reactive (lymphoid) cells. Diagnosis of MDS has improved significantly with the recent characterization of the molecular abnormalities of this disease. Detection of significant clonal molecular abnormalities typically detected in MDS is currently considered sufficient for the diagnosis of MDS. However, flow cytometry analysis remains the first line in the diagnosis of hematologic neoplasms. Absence of the expression of antigen on some cells, or increases or decreases in specific populations of cells can be diagnostic for MDS. Therefore, in principle, diagnosis of MDS relies on detecting these abnormalities in hematopoietic cells. However, other reactive processes can manifest with features overlapping with those of MDS, especially in early stages of the disease. Using pattern recognition-based approaches incorporating multiple variables from flow cytometry has been demonstrated to be the best approach for reliable diagnosis of MDS by flow cytometry. Multiple flow cytometry-based scoring systems have been developed for the diagnosis of MDS. However, most of these studies of various scores used conventional diagnostic confirmation of MDS diagnosis, which remains less objective.

The most commonly studied scoring system is the “Ogata score”, which uses the percentage of CD34+, percent of B- cell within the CD34+, intensity of CD45 on AML blast as compared with lymphoid cells, and the granularity in the mature granulocytes. The sensitivity of this score was reportedly between 65% and 89% and the specificity between 90% to 98%. However, this approach was reported to be very limited in hypoplastic BM and pediatric patients. Other studies reported more significant limitations in low-risk MDS.

However, most of these scores involve subjective parameters that are difficult to standardize. We developed a flow cytometry software with a capability to automatically capture relevant parameters of each gated cell population and use the generated metadata in an algorithm for the diagnosis and prediction of molecular abnormalities in MDS.

**RESULTS**

- 294 bone marrow samples were used for training
- 115 bone marrow samples were used for validation
- 108 samples referred with diagnosis of AML were also tested using the algorithm

All samples were referred for suspected diagnosis of MDS due to cytopenia
- All samples had molecular evaluation by NGS using 54 gene panel
- Majority had cytogenetic data
- Patients classified as having MDS if molecular studies or cytogenetic data showed one or more abnormalities associated with MDS

- Mutations at allele frequency >20% are considered adequate for the diagnosis of MDS

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- Flow panel and gating
  - Standard multiparameter panel for leukemia and lymphoma evaluation
  - Conventional gating to capture on the average 2623 different data points
  - Software which automatically captures and saves the following parameters from each sample: mean, % of cells, mean intensity, diagnosis, dispersions for each quadrant, correlation coefficient between X and Y dispersions

- RUNX1 mutations

- The most widely used scoring system for MDS diagnosis is the "Ogata score", which uses the percentage of CD34+, percent of B-cell in the CD34+, intensity of CD45 on AML blast as compared with lymphoid cells, and the granularity in the mature granulocytes. The sensitivity of this score was reported to be between 65% and 89% and the specificity between 90% to 98%. However, this approach was reported to be very limited in hypoplastic BM and pediatric patients.

- For further validation of this algorithm after integration into the software, we tested blindly an additional cohort of 115 patients that had bone marrow submitting for ruling out MDS. The algorithm correctly distinguished between MDS and non-MDS in 104 (90.4%) of these patients using a cut-off point at 0.5 and predicted the presence of cytogenetic abnormality or the presence of one or more genes mutated. However when corrected for cases misclassified, the sensitivity was at 97% and specificity at 95%.

- In addition, we tested cases with questionable diagnosis of AML. The same algorithm detected AML cases as abnormal with a sensitivity at 98% and specificity at 100% after correcting for misclassified cases.

- The algorithm classify AML cases with imt(16) or t(15;17) as normal.