# An assessment of the utility of CD15, CD16 and CD56 in evaluating dysplasia severity in myelodysplastic syndromes.

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## 1. Introduction

Myelodysplastic syndromes (MDS) are a group of heterogenous haemopoietic stem cell diseases. The patient presents with a hyper cellular bone marrow with the peripheral blood counterpart in a clinically cytopenic state. This is due to the ineffective and irregular haemopoiesis in the bone marrow resulting in an increased level of apoptosis causing cytopenias in the peripheral blood. The myeloid cells that are successful in reaching the peripheral blood can be defective and dysfunctional appearing morphologically 'dysplastic'. They are chronic diseases that progress to bone marrow failure. They have an increased chance of transforming to acute myeloid leukaemia in a third of patients.

- 4-5 per 100,000 65-70 year olds; 20 cases per 100,000 >70 year olds annually
- Minor male prevalence except in MDS (del 5q) subtype.
- 15% cases attributed to treatment for previous cancer secondary MDS
- 60-70% de-novo/primary MDS
- Risk factors: Family history of haematological malignancies, smoking, agricultural chemicals and benzene exposure

The diagnosis of MDS is multi-faceted and requires a combination of analyses immunophenotype, cytogenetics, immunohistochemistry, bone marrow morphology and molecular testing. It is hard to classify due to heterogenous nature with over 50 somatic gene mutations associated with MDS. It is currently classified by WHO 2017 under 8 subtypes using the blast cell percentage and the level of cellular dysplasia. The only subtype classified by genetic mutation is MDS(del 5q).

Dysplasia assessment requires specialist staff to assess which of the myeloid cell lines are dysplastic and microscopically quantify the level of cellular dysplasia. Not all dysplastic cells can be visually identified. Immunophenotypic aberrancies and altered myeloid maturation patterns can assist in the diagnosis of these diseases. CD15 and CD16 are 'normal' neutrophil maturation markers and can be lost or under expressed in MDS. CD56 aberrant expression on granulocytes and monocytes has been well documented in myelofibrosis, acute leukaemia and chronic myelomonocytic leukaemia. Differences in CD15/CD16 and aberrant expression in CD56 could represent dysplasia that cannot be viewed microscopically.

## 2. Neutrophil Maturation

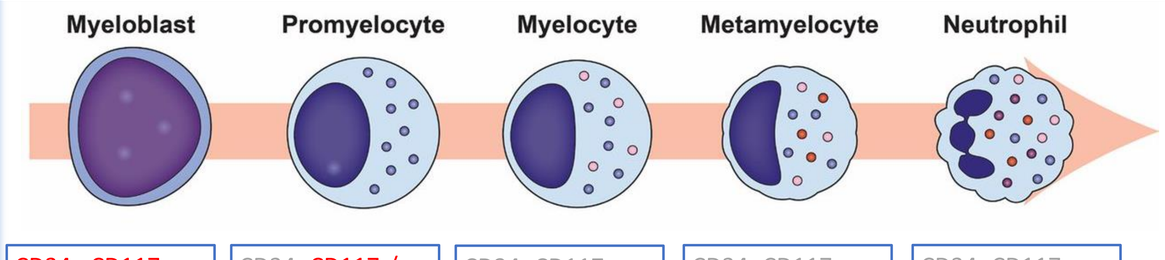


*Figure 1:Normal Neutrophil maturation showing the differing antigen expressions as the neutrophil matures. Adapted from Lawrence et al, (2019).*

## 3. Aims

The aim of this study is to use historical flow cytometry results from previously diagnosed MDS patients to assist the dysplasia assessment by isolating populations of granulocytes that do not exhibit the expected immunophenotype of CD15 and/or CD16 and assessing whether they have gained an aberrant CD56 expression. With the view that this could be developed and used to quantify dysplasia that may not be seen microscopically.

## 4. Methods

- This retrospective multicentre study analysed immunophenotyping dot plots from 192 adult patients >18 years old with a confirmed diagnosis of myelodysplastic syndrome using the current 2017 WHO classification.
- A list was gathered using the Haemato-Oncology Diagnostic Service database (version 2.24) date range from January 2018 – December 2020
- The granulocyte data of interest was gated and further plots were produced.

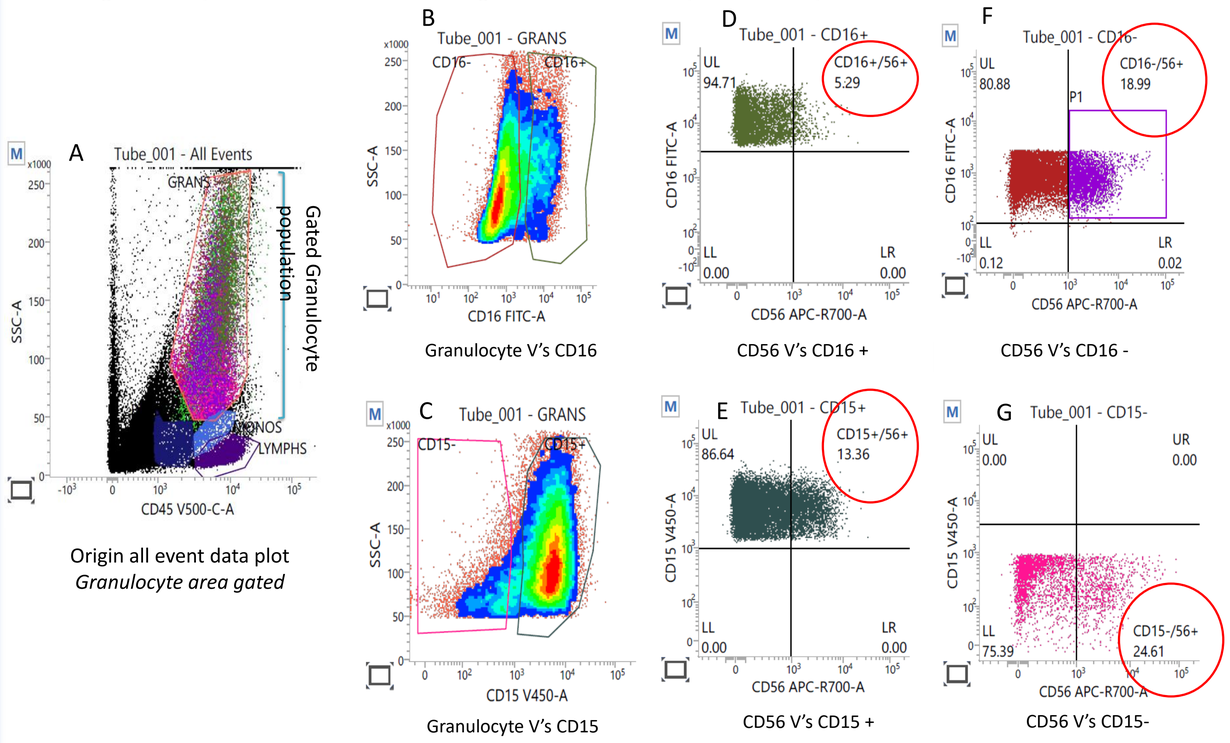


*Figure 2: Method for data collection. Dot plot A - original dot plot containing all of the events versus side scatter granulocyte area is gated. Dot plot B uses the granulocyte population versus CD16, the two populations produced are gated and named CD16- and CD16+. Dot plot D is the gated population of CD16 + versus CD56. Dot plot F is the gated population of CD16- cells versus CD56. Dot plot C uses the gated granulocyte population versus CD15 to produce two populations. These are gated and called CD15- and CD15+. Dot plot E uses the CD15+ gated population versus CD56. Dot plot G uses the CD15- gated population and versus against CD56. The data for CD56 positivity is collected.*

## 5. Results

Table 1: Samples included in study. Subtype percentage according to WHO incident figures. Highlighted are the cases not included due to low numbers. *WHO subtypes: MDS-Excessive blasts (MDS-EB), MDS-Multilineage Dysplasia(MDS-MLD), MDS-Multilineage dysplasia with ringed sideroblasts (MDS-MLD-RS), MDS-Single lineage dysplasia with ringed sideroblasts (MDS-SLD-RS), MDS-Single lineage dysplasia (MDS-SLD), MDS-Unclassified (MDS-U) MDS/Myeloproliferative neoplasm not otherwise stated (MDS/MPN NOS)*

| Diagnosis of MDS subtype | Number of cases | Percentage of sample cohort (%) | Percentage of Reported MDS cases according to WHO (Swerdlow et al., 2017) |
|---|---|---|---|
| MDS-Del(5q) | 1 | 0.5% | <5% |
| MDS-EB | 52 | 26% | 40% |
| MDS-MLD | 78 | 39% | 30% |
| MDS-MLD-RS | 42 | 21% | 35% |
| MDS-SLD-RS | 2 | 1% | 3-11% |
| MDS-SLD | 2 | 1% | 7-20% |
| MDS-U | 13 | 6.5% | 6.3% |
| MDS/MPN NOS | 7 | 3.5% | Not Stated |

- MDS-Del(5q), MDS-SLD-RS, MDS-SLD not included in study due to low numbers. As highlighted in table 1
- These eliminated subtypes are considered low grade due to their milder symptoms.
- Sample cohort subtype volume comparable with WHO incidence figures
- MDS-EB, MDS-MLD, MDS-MLD-RS most prevalent in our sample cohort
- MDS/MPN NOS subtype included in study as it meets both the criteria for MDS and MPN

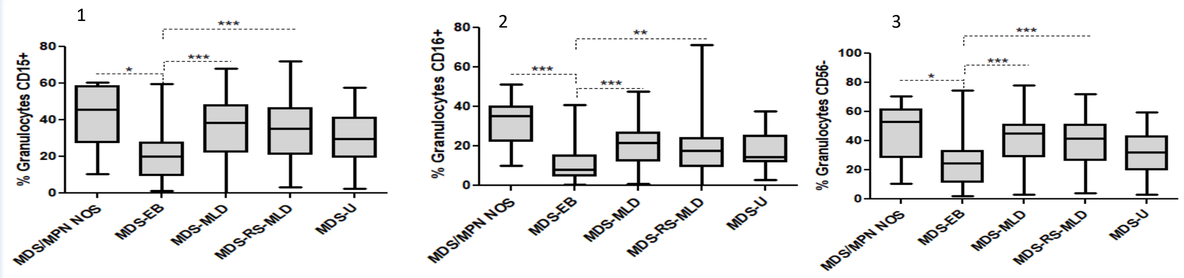


*Figure 3:Percentage of the cells within the granulocyte population (1) positive for CD15; (2) positive for CD16; (3) negative for CD56. Statistical analysis was performed using the Kruskall-Wallis with Dunn's post-hoc test. Significant differences are indicated. * represents p<0.05, ** represents p<0.01, *** represents p<0.001. MDS-EB subtype expressed lower levels of CD16+ and CD15+ granulocytes compared to the other groups. MDS-EB subtype has lower CD56- granulocytes than the other groups.*

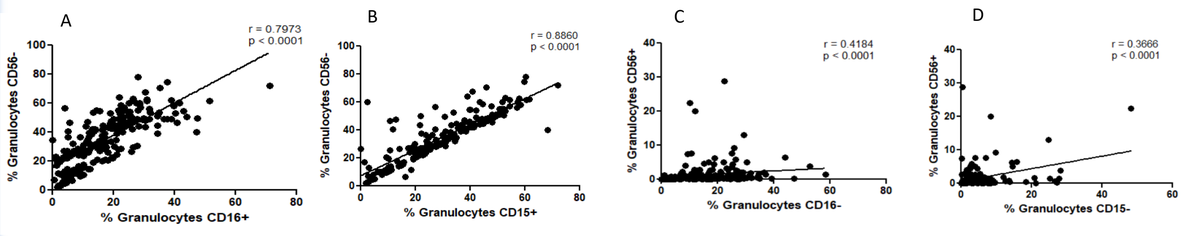


*Figure 4: Spearman's correlation analysis of (A) CD16+ versus CD56- granulocytes, (B) CD15+ versus CD56- granulocytes, (C) CD16- versus CD56+ granulocytes and (D) CD15- versus CD56+ granulocytes in all MDS patient samples (n = 192). Spearman's r and p values are indicated. There was significant correlation between CD16+ and CD56- granulocyte expression (A) and CD15+ and CD56- granulocyte expression (B). The very significant p values in (C) and (D) in combination with the significant correlations in (A) and (B )would suggest that if a granulocyte has lost CD15/CD16 expression it gains CD56 expression.*

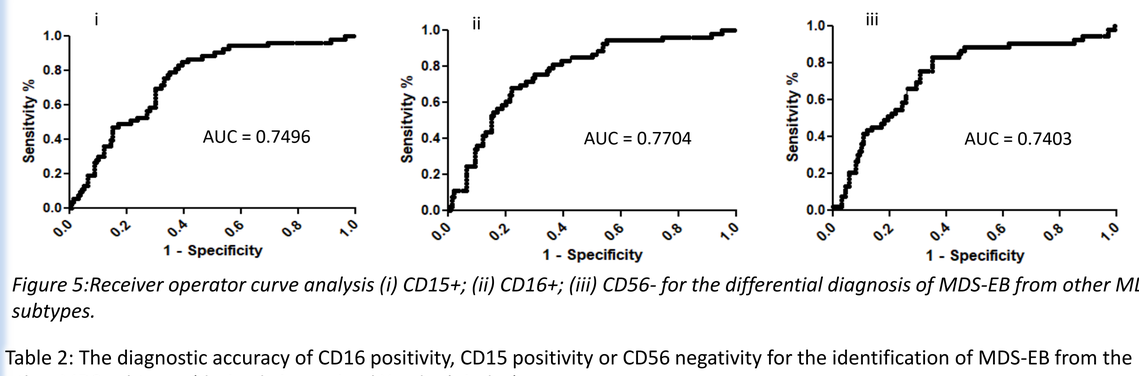


*Figure 5:Receiver operator curve analysis (i) CD15+; (ii) CD16+; (iii) CD56- for the differential diagnosis of MDS-EB from other MDS subtypes.*

Table 2: The diagnostic accuracy of CD16 positivity, CD15 positivity or CD56 negativity for the identification of MDS-EB from the other MDS subtypes (derived via maximal Youden's index).

| | Area Under Curve | Optimal Cut-off (%)* | Sensitivity at optimal cut-off | Specificity at optimal cut-off | Positive Predictive Value | 95% CI | Negative Predictive Value | 95% CI |
|---|---|---|---|---|---|---|---|---|
| CD16+ | 0.7704 | <11.82 | 67.92% | 77.86% | 53.73% | 44.72 – 62.51% | 86.51% | 81.10 – 90.55% |
| CD15+ | 0.7496 | < 30.94 | 84.91% | 60.71% | 45.00% | 39.27-50.86% | 91.40% | 84.70 – 95.33% |
| CD56+ | 0.7403 | < 34.76 | 83.02% | 65.0% | 47.31% | 41.00 – 53.71% | 91.00% | 84.63 - 94.89% |

## 6. Conclusions

- Correlation analysis suggests that if a granulocyte loses CD15/CD16 it gains a CD56 antigen expression
- MDS-EB subtype expressed lower levels of CD16+ and CD15+ granulocytes compared to the other groups
- MDS-EB subtype has lower CD56- granulocytes than the other groups

## 7. Limitations

- Lack of 'low grade' MDS sub types
- Full patient history of previous cancers and current treatment regimes not available
- Sample quality – Samples taken according to local procedures at the origin hospitals
- Sample timing – Assumption is made that the origin hospital has entered the accurate time taken
- Lack of 'normal' quality control – Only patients with obvious suspected haematological neoplasms are referred to the service

## 8. Future studies

- Extend the study to include other aberrant antigens that have been noted on granulocytes in MDS
- Assess the utility of routine full blood count samples as a screening tool in the detection of 'low grade' MDS patients
- Peripheral blood sample assessment of 'low grade' MDS using flow cytometry
- Next Generation Sequencing panels on full blood counts from cytopenic patients

**References:** 1. Ades, L., Itzykson, R. and Fenaux, P. (2014) 'Myelodysplastic Syndromes.' *The Lancet*, 383 pp. 2239-2252. 2.Bain, B. and Leach, M. (2021) *Immunophenotyping for Haematologists. 3.Gong, G., Metrebian, F., Dulau-Florea, A., Wang, Z., Bajaj, R., Gulati, G., Peiper, S. and Gong, J.(2013)'Aberrant expression of CD56 on granulocyte and monocytes in myeloproliferative neoplasm.' Journal of Hematopathology, 6pp. 127-134. 4. Swerdlow,S., Campo, E., Harris, N., Jaffe, E., Pileri, S., Stein, H., Thiele, J., Arber,D., et al.(2017) WHO classification of Tumours of Haemopoietic and Lymphoid Tissues 5.Lawrence,S., Corriden,R. and Nizet, V.(2019) 'The ontogeny of a neutrophil:Mechanisms of granulopoiesis and Homeostasis.'Microbiology and molecular biology reviews, 82(1) pp.1-22. 6. Boutault R, Peterlin P, Boubaya M, Sockel K, Chevallier P, Garnier A, Guillaume T, Le Bourgeois A, et al.(2018) 'A novel complete blood count based score to screen for myelodysplastic syndrome in cytopenic patients.' British Journal of Haematology, 183 pp. 736-746 7. Shanmugam V, Parnes A, Kalyanaraman R, Morgan EA, Kim AS. (2019)'clinical utility of targeted next-generation sequencing-based screening of peripheral blood in the evaluation of cytopenia.' Blood, 134(24) pp.2222-2225 8. Aires A, Teixeira MDA, Lau C, Moreira C, Spinola A, Mota A, Freitas I, Coutinho J, et al. (2018)'A pilot study on the usefulness of peripheral blood flow cytometry for the diagnosis of lower risk myelodysplastic syndromes: the "MDS thermometer". BMC Hematology, 18(6) pp. 1-20*