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# Presence of CD34+ in Megakaryocytes in Association With p53 Expression Predicts Unfavorable Prognosis in Low-risk Myelodysplastic Syndrome Patients

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**Abstract.** *Background/Aim: Although risk stratification using the Prognostic Scores Systems (IPSS, WPSS and IPSS-R) incorporate key information about prognosis of patients with Myelodysplastic syndromes (MDS), patients classified as low-risk may evolve rapidly and aggressively, despite a "favorable" prognostic stratification. The aim of this study was to identify biomarkers for predicting prognosis, and for better stratification and management of these patients. Materials and Methods: Expression of CD34 and p53 in megakaryocytes was examined by immunohistochemistry in 71 MDS patients classified as low-risk. Results: CD34 staining in megakaryocytes was associated with p53 expression (p=0.0166). CD34 and p53 expression were associated to worse overall survival in patients (p=0.0281). Conclusion: The presence of CD34 in megakaryocytes is associated with p53 expression and an adverse prognosis for MDS patients.*

Myelodysplastic Syndrome (MDS) comprises a group of clonal diseases of hematological progenitor cells, being characterized by ineffective hematopoiesis and risk of progression to acute leukemia (1). Risk Stratification Prognostic Scores Systems (IPSS, WPSS and IPSS-R) normally determine the prognosis of patients and are essential for adequate clinical management. Although these systems have advanced in the characterization of patients by refining the used criteria, providing better stratification capacity (2), a subgroup of patients classified as low-risk shows a rapid and aggressive disease evolution, demonstrating the need to identify auxiliary markers for risk and prognostic stratification systems and recognize these individuals at an early stage and to provide them with adequate treatment (3).

The presence of megakaryocytes positive for CD34 has been reported as an unfavorable prognostic factor in hematological malignancies. In MDS patients, the clinical impact of the expression of CD34 in megakaryocytes is still discussed (4).

Mutations in TP53 gene are found in approximately 10-12% of MDS patients and are associated with worse prognosis and lower survival in patients with high-risk MDS. The expression of p53 protein by immunohistochemistry is an available method to predict the presence of these mutations (5, 6).

This study evaluated the role of CD34 expression in mature megakaryocytes and p53 protein in bone marrow on the clinical features and the overall survival of patients classified as low-risk MDS.

## Materials and Methods

*Patients.* Seventy-one adult patients of both genders with low-risk MDS treated at a hematology center in Ceará, Brazil, were included in the study. The research was approved by the Ethics



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*Key Words:* Myelodysplastic syndromes, CD34 on megakaryocytes, p53 protein expression, prognosis, low risk.

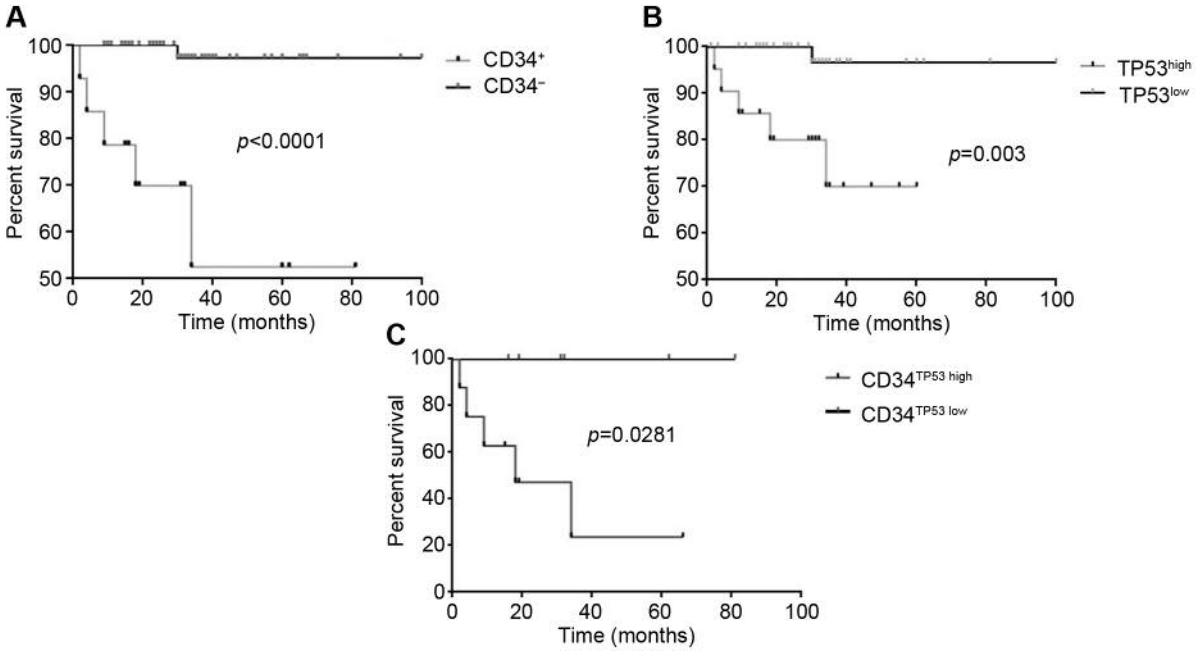


Figure 1. *Correlation of overall survival of low-risk MDS patients with the expression of CD34 and p53. A: patients with and without CD34 labeling. B: patients with and without p53 staining. C: patients with CD34+ and high or low p53 staining.*

Committee of the Federal University of Ceará with protocol number 129/12. Informed consent was obtained from all patients. Patients were stratified and classified as low-risk according to IPSS and IPSS-R criteria. Clinical data related to age, blood count, and bone marrow biopsy at diagnosis were collected from patient's medical records.

*Immunocytochemistry.* Immunohistochemical analysis of p53 was performed according to Shah *et al.* (2012) (7). Bone marrow biopsy sections were incubated with a monoclonal antibody specific for p53 (Clone DO-7; Dako, Santa Clara, CA, USA). To visualize the reaction, the slides were treated with 1 mg/ml diaminobenzidine (DAKO) solution, followed by counter staining with hematoxylin. Cover slips were mounted on slides using Canada balsam. The p53 protein expression was defined as positive or negative based on the level of nuclear staining. TP53 expression was defined as positive, according to the Modified Quick Score (5), when strong nuclear staining was observed in at least 1% of the cells analyzed (8). All samples with at least 1% strong p53 staining in myeloid progenitors were subsequently double-stained for CD34, glycophorin A and myeloperoxidase. Staining with glycophorin was employed to distinguish erythroblasts, avoiding miss diagnosis.

Immunohistochemical staining for CD34 and CD61 in megakaryocytes was performed on formalin-fixed paraffin embedded bone marrow core biopsy sections after heat-induced antigen retrieval using the avidin–biotin peroxidase technique on Dako Autostainer platform (Dako). The percentage of CD34+ megakaryocytes was calculated as CD34+ megakaryocytes/total megakaryocytes. CD34+ megakaryocytes ≥20% were considered as high-level or positive, and cases with CD34+ megakaryocytes <20% were considered as negative (9).

Table I. *Clinical and hematological characteristics of patients with MDS, with and without CD34 expression in megakaryocytes.*

| Parameters | Patients groups | | *p*-Value |
|---|---|---|---|
| | CD34 positive (n=14) | CD34 negative (n=57) | |
| Median of age at diagnosis, years (range±error) | 63.00±4.694 | 64.67±2,065 | 0.7386 |
| Gender | | | |
| Male | 6 (42.85%) | 21 (36.84%) | 0.7622 |
| Female | 9 (57.15%) | 36 (63.16%) | |
| Cytogenetics, n (%)† | | | |
| Normal karyotype | 13(92.86%) | 51 (89.47%) | 1.000 |
| Chromosomal aberrations | 1 (7.14%) | 6 (10.53%) | |
| Complete Blood Count | | | |
| Hemoglobin (g/l) | 100.4±75.26 | 111.4±37.30 | 0.1976 |
| Hematocrit (%) | 30.25±2.263 | 33.40±1.090 | 0.2078 |
| MCV (fl) | 91.81±2.339 | 89.98±1.563 | 0.5754 |
| Leukocytes ($\times 10^9$/l) | 3.64±3.85 | 4.53±4.05 | 0.2946 |
| Platelets ($\times 10^9$/l) | 171.45±32.25 | 170.02±21.02 | 0.9751 |
| Bone Marrow Blasts, (%) | 1.488±0.8808 | 3.416±0.9192 | 0.3855 |
| Tp53 expression | | | |
| Positive | 8(57.14%) | 12 (21.0%) | 0.0166 |
| Negative | 6 (42.86%) | 45 (79.0%) | |

*Statistical analysis.* Data analysis was performed using the Graph Pad Prism 5.0 statistics program (GraphPad Software Inc., La Jolla, CA, USA). Results were expressed as means±standard error. The Kolmogorov–Smirnov test was used to check for normal

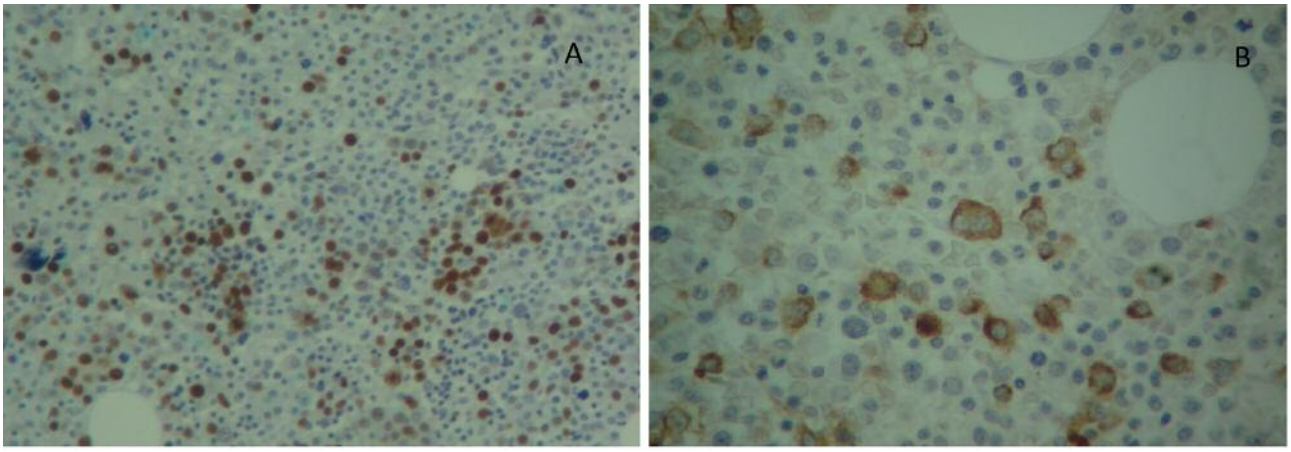


Figure 2. *Expression of p53 in bone marrow precursor cells and CD34 in megakaryocytes. (A) Bone marrow biopsy sections were immunocytochemically stained with a monoclonal antibody specific for p53 as described in the Materials and Methods section. (B) Formalin-fixed paraffin embedded bonne marrow core biopsy sections were immunocytochemically stained with an anti-CD34 antibody as described in the Materials and Methods section. Sections were counterstained with hematoxilin and eosin. ×400 magnification.*

distribution of data and statistical differences between groups were observed using a *t*-test or the Mann–Whitney test. The Kaplan–Meier curve was performed and the log-rank test was applied to estimate and compare overall survival between groups. The level of significance was $p<0.05$ for all analyses.

## Results

Forty-five patients were female and 27 were male. CD34 was found to be expressed in mature megakaryocytes (≥20 %) of the bone marrow of 14 (19.7%) patients. CD34 positive and CD34 negative patients. The median age and the hematological parameters of the patients were similar. However, a significant difference was observed in p53 protein expression, being more frequent in the CD34-positive group ($p=0.0166$) (Table I).

Positive labeling for CD34 in megakaryocytes and p53 expression were associated with lower overall survival (Figure 1). One interesting case of a patient was identified who presented positive CD34 labeling in megakaryocytes and 40% of p53 expression at diagnosis (Figure 2) and died six months after diagnosis from bacterial infection.

## Discussion

In the present study, the relevance of prognostic factors such as CD34 labeling in megakaryocytes and p53 expression in bone marrow precursor cells for patients classified as low-risk MDS was investigated.

Megakaryocytopoiesis is a complex process that involves up/down expression of signaling molecules in the bone marrow microenvironment, which promote lineage differentiation (10). CD34 is a glycophosphoprotein selectively expressed in hematopoietic precursors. In megakaryocytic lineages, normal expression of CD34 is found only in immature progenitors, such as promegakaryoblasts and megakaryoblasts, and its expression decreases progressively throughout cell maturation (4, 11).

Expression of CD34 in mature megakaryocytes in clonal and nonclonal hematological diseases, including MDS has been demonstrated in some studies. However, there is no consensus about its prognostic value. Pellegrini *et al*., (2000) (11) reported increased number of megakaryocytes expressing CD34 in 10 of 22 MDS patients. They observed an association between CD34 expression, platelet count and cytogenetic abnormalities. Tang *et al*., (2011) (9) also investigated CD34 expression on bone marrow biopsies from 202 MDS patients. Positive expression was found in 29 cases (14%) and high levels of CD34 on megakaryocyte were associated with severe cytopenia, number of blasts, cytogenetic abnormalities and shorter survival, indicating its potential as a strong and an independent unfavorable prognostic factor in MDS.

Torlakovic *et al*. (2002) (12) investigated the role of CD34 positivity on megakaryocytes in various hematologic diseases, including MDS. The authors found no association between CD34 labeling and clinical features and laboratory parameters in patients with MDS. Moreover, the number of CD34+ on megakaryocytes did not seem to have diagnostic value. However, they suggested that this finding should be further investigated in relation to clinical parameters.

In the present study, CD34 labeling in megakaryocytes was found in 14 cases (19.7%). The positivity for CD34 was associated with p53 protein expression and a lower overall survival ($p=0.0281$).

Mutations in *TP53* gene have been considered an independent factor of poor prognosis, being related to more severe clinical manifestations including thrombocytopenia, increase in the number of blasts, resistance to lenalidomide, relapse and worse survival (6). They occur in approximately 10% of *de novo* MDS cases. Of this percentage, about 30 to 50% correspond to those with a complex karyotype. One of techniques used in several hematological diseases as a predictive marker for mutations in the TP53 gene is the immunohistochemical expression of p53 protein. It is a low-cost assay and easy to perform (5).

Analysis of p53 expression is not yet considered a risk factor in prognostic scores for MDS (R-IPSS, WPSS, IPSS). However, the present study indicates that, although all patients were classified as low-risk, the expression of p53 at diagnosis is associated with lower overall survival. Both markers investigated in the present study were associated to unfavorable outcomes in low-risk MDS. There are at least two studies examining expression of p53 and CD34 in low-risk patients. Saft *et al.*, (2014) (8) investigated the expression of p53 protein in patients with 5q deletion and identified an abnormal CD34 expression in megakaryocytes in one patient with strong p53 labeling in the bone marrow in a cohort of 85 patients. Recently, Cheng and Ghang (13) reported the presence of CD34-positive megakaryocytes in bone marrow and in peripheral blood of an MDS patient with excess number of blasts. In the present study, p53 expression presented a cumulative effect on overall survival in the group of CD34 positive patients ($p$=0.0281).

## Conclusion

CD34 expression in megakaryocytes and p53 expression in bone marrow associate with adverse prognosis and lower survival in low-risk MDS patients. More studies with a large number of patients must be conducted for elucidating the value of this marker on the prognosis of MDS patients, as it seems to be a potential prognostic factor for this heterogeneous group of MDS patients and may, in the future, be incorporated in stratification score systems.

## Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

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