Regulatory T cells and progenitor B cells are independent prognostic predictors in lower risk myelodysplastic syndromes

Research has provided evidence for immune surveillance in patients with myelodysplastic syndromes (MDS). However, while the presence of immune surveillance in MDS has been observed, little is known about its prognostic implications. This study aimed to identify the prognostic impact of an immune active and/or immunosuppressed environment in patients with very low, low and intermediate risk MDS. We show that an immunosuppressive environment, characterized by a high percentage of regulatory T cells (Tregs) and low percentage of progenitor B cells, is associated with poorer survival in patients with lower risk MDS. Based on multivariate analysis, the percentage of Tregs and progenitor B cells impacted survival independently of known prognostic factors. Therefore, this study provides evidence for the independent prognostic value of progenitor B cells and Tregs in patients with lower risk MDS, underlining the importance of immunosuppression in the prognosis of MDS.

Myelodysplastisc syndromes are a group of heterogeneous clonal hematopoietic stem cell disorders characterized by peripheral cytopenias, myeloid dysplasia and an increased risk of progression to acute myeloid leukemia (AML). Prognostic scoring systems, including the recently revised International Prognostic Scoring System (IPSS-R), are instrumental in predicting the risk of progression to AML, segregating patients into very low, low, intermediate, high and very high risk groups.[1] While the pathogenesis of MDS remains poorly understood, it is believed to be a multistep process of which one of the factors is immune dysregulation. The hypothesis of a dysregulated immune environment is supported by the often associated autoimmune disease in patients with MDS, the improvement of hematopoiesis in a significant number of patients after treatment with immunosuppressive therapies and the dysfunction of several immune compartments.[2] The nature of immune dysregulation differs considerably between various MDS risk groups. In lower risk MDS, the immune system is characterized by an activated, pro-inflammatory state leading to a high apoptosis rate of hematopoietic progenitors.[2,3,4] In contrast, in higher risk MDS an immunosuppressive environment is observed, enabling dysplastic clones to expand and evade immune surveillance.[2,4-7] However, while the presence of immune dysregulation in MDS has been shown, little is known about its role in the prognosis of MDS. The aim of this prospective study is to identify the prognostic implications of immune (dys)regulation in patients with lower risk MDS.

Thirty-eight treatment naive patients with very low, low and intermediate risk MDS according to the IPSS-R were included in this prospective study.[1] Diagnosis of MDS was made in compliance with the World Health Organization (WHO) criteria. After informed consent, peripheral blood (PB) and bone marrow (BM) samples were drawn from all patients and analyzed by flow cytometry. After obtaining samples, patients were started on Epo/G-CSF treatment according to Dutch guidelines. Antibodies used for flow cytometry were purchased from BD Biosciences (San Jose, CA, USA) unless otherwise specified and included CD3 (clone SK7, PerCPCy5.5 and APC-labeled), CD4 (SK3, FITC and PerCP), CD8 (SK1, PerCP), CD10 (SS2/36, PE, Dako Cytomation), CD16 (DJ130C, FITC, Dako Cytomation), CD25 (2A3, APC), CD27 (L128, PE), CD34 (8G12, APC), CD45 (2D1, PerCP), CD45RA (4KB5, FITC, Dako Cytomation, Glostrup, Denmark), CD56 (My31, PE), FOXP3 (PCH101, eBioscience, San Diego, USA). We focused on immune cells that have been shown to be involved in immune (dys)regulation in MDS,[2,-5,8] including $CD4^+$ naïve T cells (defined as: $CD4^+CD45RA^{++}CD27^+$) , $CD4^+$ effector T cells (defined as: $CD4^+CD45RA^{++}CD27^-$), $CD8^+$ naïve T cells ($CD8+CD45RA^{++}CD27^+$), $CD8^+$ effector T cells ($CD8^+CD45RA^{++}CD27^-$), Tregs (defined as: $CD4^+CD25^{hi}FoxP3^+$), natural killer (NK) cells (defined as: $CD3^-CD16^+CD56^{dim}$) and progenitor B cells (defined as: $CD10^+CD34^+$) (*Online Supplementary Table S1*). Overall survival (OS) and progression-free survival (PFS) were calculated for all patients. OS was defined as the time between inclusion in our study and death or date of last follow up. PFS was defined as time between inclusion in our study until progression to at least RAEB-2, death or date of last follow up. OS and PFS survival curves were calculated using the Kaplan-Meier method. Statistical analyses were performed using the Cox proportional hazards regression

Table 1. Univariate analysis of overall survival and progression-free survival for all investigated cell subsets.

Overall survival	HR	95% CI	P
Naive $CD4^+$ T cells	1.00	0.98-1.03	0.849
Effector $CD4^+$ T cells	1.09	0.99-1.19	0.079
Naive $CD8^+$ T cells	1.00	0.97-1.02	0.781
Effector $CD8^+$ T cells	1.00	0.98-1.03	0.801
Regulatory T cells	1.33	1.04-1.67	0.021
Progenitor B cells	0.86	0.77-0.96	0.009
NK cells	0.91	0.73-1.13	0.369

Progression-free survival	HR	95% CI	P
Naive $CD4^+$ T cells	1.00	0.98-1.03	0.910
Effector $CD4^+$ T cells	1.07	0.99-1-17	0.092
Naive $CD8^+$ T cells	0.99	0.97-1.02	0.620
Effector $CD8^+$ T cells	1.00	0.99-1.03	0.498
Regulatory T cells	1.34	1.08-1.66	0.007
Progenitor B cells	0.87	0.79-0.97	0.010
NK cells	0.96	0.82-1.13	0.628

Table 2. Multivariate analysis of progression-free survival with IPSS-R and the percentage of Tregs and progenitor B cells, both separate (A) and combined (B).

A Progression-free survival	HR	95% CI	P
IPSS-R	1.92	1.22-3.02	0.005
Tregs	1.27	1.03-1.56	0.027
IPSS-R	2.02	1.24-3.31	0.005
Prog. B cells	0.88	0.80-0.98	0.014

B Progression-free survival	HR	95% CI	P
IPSS-R	2.37	1.36-4.12	0.002
Tregs $^+$	1.44	1.09-1.91	0.010
prog. B cells	0.88	0.80-0.97	0.012

PFS: progression free survival; IPSS-R: revised International Prognostic Scoring System; Tregs: regulatory T cells. Prog. B cells: progenitor B cells.

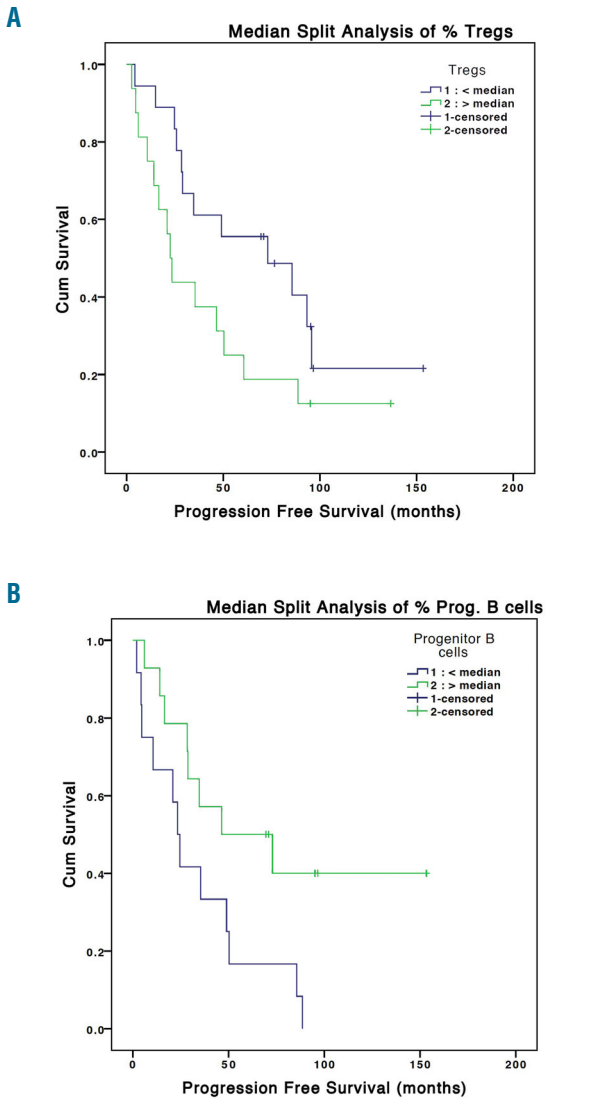

Figure 1. Median split analysis. Median split analysis of the percentage of Tregs (A), Progenitor B cell (B) and PFS. Tregs: regulatory T cells; Prog. B cells: progenitor B cells; PFS: progression-free survival.

model, with hazard ratios referring to an absolute increase of the independent variables of 1% for the different cell subsets. Multivariate Cox regression analyses were conducted to assess the added value of the results to the IPSS-R. The predictive power of regression models for survival was calculated with Harrell's c-concordance. In all analyses, $P<5\%$ was considered statistically significant.

The patient group had a median age of 70 years (range 40-88 years) and a WHO 2008 distribution as follows: RA n=5, RARS n=3, RCMD n=9, RCMD-RS n=8, RAEB-1 n=1, MDS-U n=1. Median IPSS-R score was 2.0 (range 1-4.5), the median OS 4.1 years (range 0.3-12.8 years) and the median PFS 2.9 years (range 0.2-12.8 years). Univariate Cox proportional hazard regression analysis was used to assess the predictive value of our markers in relationship to OS and PFS (Table 1). The results show the percentage of Tregs, assessed in the PB, to be predictive for PFS and OS, with a hazard ratio of respectively 1.34 (95%CI: 1.08-1.66; $P=0.007$) and 1.33 (95%CI: 1.04-1.67; $P=0.021$). In addition, the percentage of progenitor B cells, assessed in the BM of our patients, is a predictor of PFS and OS with a hazard ratio of 0.87 (95%CI: 0.79-0.97; $P=0.010$) and 0.86 (95%CI: 0.77-0.96; $P=0.009$). Neither the percentage of effector T cells, naïve T cells or NK cells was predictive for OS or PFS. Secondly, we conducted a median split analysis of the percentage of Tregs and progenitor B cells, with median values of respectively 5.8% and 9.7% (Figure 1 and *Online Supplementary Table S1*). The median PFS for patients with a percentage of Tregs above the median is 1.9 years *versus* a median PFS of 6.1 years for patients with a percentage of Tregs below the median ($P=0.06$). Patients with a percentage of progenitor B cells above the median have an increased median survival of 3.9 years *versus* 1.9 years for patients below the median ($P=0.027$). Finally, to assess the prognostic implication of the percentage of Tregs and progenitor B cells in relationship to other known predictors, we conducted a multivariate analysis. As expected, our univariate analysis identified (next to the above-mentioned immune markers) the IPSS-R as a strong predictor of PFS and OS with a hazard ratio of respectively 2.11 (95%CI: 1.36-3.26; $P<0.001$) and 1.94 (95%CI: 1.31-2.89; $P<0.001$). No other selected parameters, such as age, sex or response to Epo/G-CSF treatment, defined according to the IWG2006 criteria,[9] proved to be predictive of PFS or OS. By both separate and combined multivariate analysis, the percentage of Tregs and progenitor B cells remained significant predictors of PFS next to IPSS-R (Table 2). Using Harrell's c-concordance, we demonstrated an increase in the correct prediction of PFS from 73% to 83% when adding our newly identified prognostic markers to the IPSS-R.

The data presented here demonstrate that an immunosuppressive environment, characterized by a high number of Tregs and a low number of progenitor B cells, is associated with poorer survival in patients with very low, low and intermediate risk MDS. The multivariate analysis supports the significance of this finding, as the number of Tregs and progenitor B cells predict survival independently of the widely used IPSS-R.

While the presence of an immunosuppressive environment with an increased number of Tregs has been observed in high-risk MDS,[5] this study is the first to show by multivariate analyses the importance of Tregs as significant predictors of survival in low-risk MDS. The study, therefore, adds value to previous reports by demonstrating that, not only in high risk MDS, but also in lower risk MDS, Tregs have prognostic value, emphasizing the importance of Tregs in MDS.[5,10]

In contrast to the role of Tregs in MDS, less research has been conducted on the role and prognostic importance of progenitor B cells in MDS. While previous studies have shown there to be a decreased progenitor B-cell pool in patients with MDS in comparison to age-matched healthy controls, this study is the first to show by multivariate analysis the prognostic value of B-cell progenitors in low risk MDS.[8,11] Establishing a negative association between the percentage of progenitor B cells and survival, our results stand in line with previously published data reporting a reduced number of progenitor B cells in MDS.[8,11] Whereas we hypothesize that a reduction in the number of progenitor B cells is associated with an immunosuppressive environment, the reasons for and the pathophysiological consequences of a reduced progenitor B-cell pool in MDS remain to be elucidated.

Limitations of our study include the relatively small number of patients and hence a limited amount of cases used for the outcome analysis. In addition, we did not include

functional assays to assess the activity of the immune cells involved. This is currently under study. Finally, the predictive performance of the regression model needs to be validated on an external dataset to rebalance any over-optimism.

To conclude, this study provides evidence for the independent prognostic value of progenitor B cells and Tregs in patients with very low, low and intermediate risk MDS, hereby underlining the importance of immune surveillance in the prognosis of myelodysplastic syndromes.

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