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Clinical Significance of Microcytosis in Patients with Primary Myelofibrosis

Paolo Strati, MD, Naveen Pemmaraju, MD, Zeev Estrov, MD, Marylou Cardenas-Turanzas, MD, DrPH, Sherry Pierce, RN, Kate J. Newberry, PhD, Naval Daver, MD, Jorge Cortes, MD, Hagop Kantarjian, MD, and Srdan Verstovsek, MD, PhD

Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas

Abstract

Microcytosis is a relatively frequent finding in primary myelofibrosis (PMF); however its prognostic significance is unknown. We identified factors associated with microcytosis in PMF and measured its impact on outcomes. Among 725 patients with PMF, 140 (19%) showed microcytosis. In multivariate analysis, factors associated with microcytosis were absence of prior therapy, low iron, low transferrin saturation (satTF), and splenomegaly. Among 375 untreated patients, low satTF and splenomegaly were associated with microcytosis. Overall, microcytosis was associated with a higher risk of transformation to leukemia ($p=0.03$), but not shorter leukemia-free survival. Microcytosis in PMF may be related to dysregulation of iron homeostasis.

Keywords

primary myelofibrosis; microcytosis; iron homeostasis; transferrin saturation; leukemia

1.0 Introduction

Anemia is a common finding in primary myelofibrosis (PMF), with up to 38% of patients showing hemoglobin (HB) levels < 10 g/dL at the time of diagnosis.[1] It usually presents with normal or high mean corpuscular volume (MCV) related to ineffective erythropoiesis. [2] However, up to 28% of patients with PMF can present with microcytosis, even in the absence of severe anemia.[3]

In the general population microcytic anemia is typically associated with iron deficiency, secondary to poor oral intake, malabsorption or active bleeding, [4, 5] conditions that are not uncommon in patients with PMF.[6] Furthermore, iron homeostasis has been shown to be abnormal in patients with PMF.[7] Another common cause of microcytic anemia is chronic

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Correspondence: Srdan Verstovsek, MD, PhD, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 428, Houston TX 77030, Phone: (713) 745-3429 Fax: (713) 794-4297 sverstov@mdanderson.org.

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inflammation, which is a hallmark of PMF due to dysregulation of inflammatory cytokine signaling through the JAK-STAT pathway.[8, 9] Finally, microcytosis can be present even in patients with mild or no anemia, in the case of structural hemoglobinopathies, such as thalassemic trait.[10] Very rarely, in myeloid neoplasms, such as myelodysplastic syndromes[11] and occasionally PMF[12, 13], the neoplastic clone can modulate globin gene transcription, inducing similar conditions. Despite the divergent mechanisms that can cause low MCV, the only published study analyzing this subgroup in PMF (including 28 patients) did not find any prognostic significance for MCV.[3] In this study, we determined the factors associated with microcytosis in patients with PMF and analyzed its association with outcomes.

2.0 Methods

2.1 Patients and samples

We performed a retrospective chart review of 725 patients with a confirmed diagnosis of PMF who presented to The University of Texas MD Anderson Cancer Center (MDACC) between December 1990 and December 2013. PMF was diagnosed according to the World Health Organization criteria.[14] Dynamic International Prognostic Scoring System (DIPSS) and DIPSS-plus scores were assigned to each patient as previously described. [15–17] Demographic and clinical information at the time of presentation to MDACC, including medical history, results of physical examination, complete blood count and blood chemistry, and assessment of bone marrow aspiration/biopsy, were extracted from patient records. Information on iron metabolism profile that included iron levels (470 patients), ferritin levels (430 patients), total iron binding capacity (TIBC) and transferrin saturation (satTF) (461 patients) were collected. Microcytosis was defined as MCV < 82 femtoliters (fL), according to the MDACC reference range. MDACC reference ranges were also used for iron metabolism parameters. This study was based on a chart review protocol that was approved by the Institutional Review Board of MDACC and conducted in accordance with our institutional guidelines and the principles of the Declaration of Helsinki.

2.2 Study outcomes

In addition to identifying clinical and demographic factors associated with microcytosis in patients with PMF, we also identified factors associated with outcomes among PMF patients. Time to event variables were defined as time to first therapy (TTFT), progression-free survival (PFS), leukemia-free survival (LFS), and overall survival (OS). TTFT was defined as date of presentation at MDACC to date of first therapy. Patients who did not receive treatment were censored at the date of death or last follow-up. PFS was defined as time from first therapy to any event (including disease relapse or disease progression requiring therapy, leukemia onset and death). Patients were censored at date of last follow-up. OS was defined as time from presentation to date of death or last follow-up. LFS was defined as time from presentation to leukemia onset or date of last follow-up.

2.3 Statistical analysis

Categorical variables were compared using the χ^2 or Fisher exact tests. Logistic regression with a backward method was used to determine factors associated with microcytosis. Only

factors that were significant on univariate analysis were included. Time-to-event outcomes were analyzed using the method of Kaplan and Meier, and comparisons were made using the log-rank test. We constructed a Cox proportional hazard regression model, using a backward method. Only factors significant on univariate analysis were used. A p-value of <0.05 (two-tailed) was considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics 22 for Windows (SPSS Inc., Chicago, Illinois).

3.0 Results

3.1 Baseline characteristics

Seven hundred and twenty-five patients with a confirmed diagnosis of PMF presented to our institution between December 1990 and December 2013. Hundred-forty patients (19%) had an MCV lower than 82 fL, which we defined as microcytosis based on the reference range used at MDACC. Of them, none had clinical signs or symptoms of active bleeding. Among microcytic patients, 83/140 (59%) had hemoglobin (HB) levels ≤ 10 g/dL, 1/71 (1%) had low ferritin level, 5/90 (6%) had low TIBC, 44/90 (49%) had low satTF and 120 (86%) had never been previously transfused. Among 46 patients with microcytosis and normal-high satTF, hemoglobin electrophoresis was performed in only 5 cases and all were negative for beta-thalassemia trait. Unfortunately, genotyping for alpha-thalassemia was not performed on any of these patients. Baseline characteristics at the time of presentation are shown in Table 1.

3.2 Factors associated with microcytosis

To identify factors associated with microcytosis, we compared proportions of patients with and without microcytosis in each subgroup. At the time of presentation, 140 patients had an MCV < 82 fL (median, 78 fL [range, 54–81 fL]). Five-hundred and eighty-five patients had normal or high MCV (median, 88 fL [range, 82–131 fL]). Median time from diagnosis of PMF to MCV determination was 3 months and did not differ significantly between the 2 groups ($p=0.35$). On univariate analysis, factors associated with microcytosis were absence of previous therapy (not including transfusion; $p<0.001$), age < 65 years ($p=0.05$), white blood cell count (WBC) > 25,000/uL, peripheral blood blasts $\geq 1\%$ ($p=0.001$), bone marrow (BM) blasts > 5% ($p=0.02$), iron < 37 mcg/dL ($p<0.001$), satTF < 15% ($p<0.001$), spleen ≥ 10 cm ($p<0.001$), favorable chromosome banding analysis (CBA; $p=0.006$), JAK2 mutation ($p=0.05$), presence of constitutional symptoms ($p=0.05$), and absence of transfusion dependency ($p<0.001$). In multivariate analysis, factors independently associated with microcytosis included absence of prior therapy (OR 3.3, 95% CI=1.5–6.8; $p=0.003$), iron < 37 mcg/dL (OR 3.3, 95% CI=1.1–11, $p=0.05$), satTF < 15% (OR 4.6, 95% CI=1.6–13, $p=0.005$), and spleen > 10 cm (OR 3.9, 95% CI=1.4–6, $p=0.005$) (Table 2).

The association between absence of previous therapy and microcytosis suggests that treatment may have improved microcytosis. Therefore, to determine which factors are associated with microcytosis in the absence of therapy we performed a subgroup analysis that included 375 patients who were untreated at the time of presentation. Among untreated patients, 91 (24%) had an MCV < 82 fL, and 284 (76%) had normal or high MCV. In univariate analysis, factors associated with microcytosis were WBC > 25,000/uL ($p=0.001$),

peripheral blood blasts 1% ($p=0.01$), iron < 37 mcg/dL ($p<0.001$), satTF $< 15\%$ ($p<0.001$), spleen > 10 cm ($p<0.001$), favorable CBA ($p=0.006$), presence of constitutional symptoms ($p=0.03$), and absence of transfusion-dependency ($p=0.01$) (Supplemental Table 1). In multivariate analysis, satTF $< 15\%$ (OR 4.5, 95% CI=1.3–15.9, $p<0.001$), and spleen > 10 cm (OR 2.5, 95% CI=1.1–5.6, $p<0.001$) were found to be independently associated with microcytosis (Table 3).

3.3 First-line therapy and progression-free survival

Of 375 patients who were untreated at the time of presentation, 151 (40%) received therapy at MD Anderson. The median follow-up time for these patients was 26 months (range, 1–174 months). The median time to first therapy was 47 months (95% CI, 31–63 months) and did not differ significantly among patients with and without microcytosis ($p=0.10$). Sixty-three (42%) patients received a JAK inhibitor (JAKi) as first-line therapy and 88 (58%) received other therapies. After a median follow up of 17 months (range, 1–125 months), 100 of 151 (66%) treated patients had disease progression or disease relapse requiring therapy or died. Median PFS was 21 months (95% CI=14–28 months) and did not differ significantly among patients with and without microcytosis ($p=0.94$).

3.4 Factors associated with transformation to AML and leukemia-free survival

After a median follow up of 25 months (range 1–223 months), the median LFS had not been reached and the 5 years-LFS was 83% for the 725 patients. Factors associated with a shorter LFS on univariate analysis were male gender ($p=0.04$), BM blasts $> 5\%$ ($p<0.001$), and MCV < 82 fL ($p=0.03$) (Figure 1 and Supplemental Table 2). In the multivariable model, only BM blasts $> 5\%$ (HR 6.6, 95% CI=3.3–13, $p<0.001$) were independently associated with shorter LFS (Table 4). Of 725 patients, 46 (6%) had disease that transformed to AML. On univariate analysis, factors associated with AML transformation were age < 65 years ($p=0.05$), BM blasts $> 5\%$ ($p<0.001$), and MCV < 82 fL ($p=0.01$). In the multivariate model, age < 65 years (OR 2, 95% CI=1.1–4.1, $p=0.03$), BM blasts $> 5\%$ (OR 4.8, 95% CI=2.3–10.1, $p<0.001$), and MCV < 82 fL (OR 2.1, 95% CI=1.1–4.1, $p=0.03$) were independently associated with transformation to AML (Table 5 and Supplemental Table 3). During the same observation time, 350 of 725 patients died (48%). Median OS for all patients was 42 months (95% CI=37–47 months) and did not differ significantly among patients with and without microcytosis ($p=0.85$).

4.0 Discussion

Microcytosis is a relatively frequent finding in patients with PMF, even in absence of severe anemia. [3] Several factors may contribute to the development of microcytosis in PMF patients; however, whether microcytosis is associated with outcomes in PMF has not been well-studied. Here, we report a relatively large series of PMF patients with microcytosis. To date only one other study of microcytosis in patients with PMF has been published.[3] In our study, 19% of patients were microcytic at the time of presentation. Among untreated patients its incidence was higher (24%), similar to the previously published series (28%), which included only untreated patients.

Microcytosis is a very common finding in patients with PV, secondary to relative iron deficiency driven by erythroid cell overgrowth. [18] However, it is not clear which factors determine microcytosis in PMF. On MVA, one of the factors associated with microcytosis was low satTF. This suggests an actual iron deficiency in patients with PMF and microcytosis. Despite this, only a small proportion of patients showed low ferritin levels or TIBC values, supporting the use of satTF rather than other parameters in the evaluation of iron deficiency in patients with PMF. In genetic conditions, such as iron refractory iron deficient anemia (related to a mutation in the *TMPRSS6* gene) or in cases of acquired anemia, such as anemia related to chronic diseases, hepcidin is overproduced, leading ultimately to absolute or relative iron deficiency, which is characterized by low satTF.[19] Heparin is a liver peptide that plays a key regulatory role in iron homeostasis. It binds to the iron channel ferroportin, inducing a reduction in iron enteral absorption and macrophagic recycling.[20, 21] In PMF, hepcidin levels are abnormally high and are associated with inferior survival, independent of classic prognostic scores and inflammatory cytokine levels. [7] Thus, the low satTF observed in our analysis might be secondary to either PMF-associated or inflammation-associated hepcidin overproduction. Unfortunately, hepcidin levels or inflammatory markers, such as interleukin-6 or C-reactive protein, were not routinely measured in the patients included in this study. However, it would be interesting to compare hepcidin levels in patients with PMF and microcytosis to those in patients with normal-high MCV and eventually screen them for mutations in genes involved in the hepcidin regulatory pathway.

A clinical factor associated with microcytosis in this study was splenomegaly. During extramedullary hematopoiesis, iron is primarily taken up by the spleen and diverted from the erythroid precursors in the bone marrow. [22] This has been confirmed by recent spectroscopic studies, showing a significantly higher iron content in spleens of patients with PMF than in spleens of healthy individuals.[23] This result is consistent with a hepcidin-driven mechanism, leading to accumulation of iron into splenic macrophages and subsequent relative iron deficiency with microcytosis. Given the availability of new compounds that inhibit the production of hepcidin, these findings have important implications for the treatment of patients with PMF and microcytosis, where these drugs may have a potential therapeutic role.[24] It is interesting to note that the absence of previous therapy was independently associated with microcytosis in multivariable analysis, suggesting that low MCV may be a surrogate for active and untreated disease.[25]

Moreover, in this study, patients with PMF and microcytosis had a significantly higher incidence of leukemic transformation (although the time to transformation, as measured by LFS, was only associated with the percentage of BM blasts). Classical prognostic factors, such as thrombocytopenia and unfavorable cytogenetics, did not associate with shorter LFS. It would be interesting to investigate whether these patients have genetic instability, leading to mutations that involve both the hepcidin pathway and the risk of transformation to AML. Abnormally high levels of hepcidin have been described in patients with acute leukemia, although their independence from inflammatory cytokines hasn't been shown in this setting. [26] Of interest, in our analysis classic prognostic factors, such as thrombocytopenia and unfavorable cytogenetics, did not associate with a shorter LFS. However, this may have

been influenced by the heterogeneity of administered therapy in earlier studies [17]. In fact, unlike previous studies, a significant proportion of patients (42%) in our study received frontline treatment with JAK inhibitors, which may have modified the association between classical prognostic factors and LFS.

In conclusion, microcytosis is a relatively common finding in PMF, possibly related to a dysregulation in iron homeostasis. It would be interesting to test whether patients with PMF and low MCV may have genetic instability, leading to hepcidin overproduction and a higher risk of transformation to AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

PMF	primary myelofibrosis
satTF	transferrin saturation
MCV	mean corpuscular volume
HB	hemoglobin
MDACC	MD Anderson Cancer Center
TTFT	time to first therapy
PFS	progression-free survival
LFS	leukemia-free survival

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Highlights

- Microcytosis is a relatively frequent finding in primary myelofibrosis (PMF)
- Microcytosis in PMF may be caused by dysregulation of iron homeostasis.
- Microcytosis in PMF is associated with a higher risk of transformation to AML.

Figure 1. Leukemia-free survival by MCV

Patients with microcytosis had a significantly shorter median LFS (154 months) than patients with normal MCV (median LFS not reached, $p=0.03$). Eleven percent of patients with microcytosis had disease transformation to AML, while 5% of those with normal MCV transformed. MCV, mean corpuscular volume; n, number; LFS, leukemia-free survival; NR, not reached

Table 1

Univariate analysis of baseline characteristics associated with microcytosis among all patients.

Characteristics (N=725)	MCV < 82 (n=140)	MCV ≥ 82 (n=585)	P-value
No prior therapy Prior therapy	91 (65%) 49 (35%)	284 (49%) 301 (51%)	<0.001
Males Females	93 (66%) 47 (34%)	377 (64%) 208 (36%)	0.70
Age < 65 Age ≥ 65 years	82 (59%) 58 (41%)	288 (49%) 297 (51%)	0.05
HB < 10 g/dL HB ≥ 10	57 (41%) 83 (59%)	256 (44%) 329 (56%)	0.57
WBC > 25,000 /uL WBC ≤ 25,000	34 (24%) 106 (76%)	87 (15%) 498 (85%)	0.01
PLT < 100,000/uL PLT ≥ 100,000	35 (25%) 105 (75%)	175 (30%) 410 (70%)	0.30
PB blasts ≥ 1% PB blasts < 1%	84 (60%) 56 (40%)	262 (45%) 323 (55%)	0.001
BM blasts > 5% BM blasts ≤ 5%	18/132 (14%) 114/132 (86%)	38/548 (7%) 510/548 (93%)	0.02
Iron < 37 ug/dL Iron ≥ 37	39/87 (45%) 48/87 (55%)	32/383 (8%) 351/383 (92%)	<0.001
Ferritin < 10 ng/mL Ferritin ≥ 10	1/71 (1%) 70/71 (99%)	2/359 (1%) 357/359 (99%)	0.42
TIBC < 450 ug/dL TIBC ≥ 450	5/90 (6%) 85/90 (94%)	8/371 (2%) 363/371 (98%)	0.14
SatTF < 15% SatTF ≥ 15%	44/90 (49%) 46/90 (51%)	44/371 (12%) 327/371 (88%)	<0.001
RS present No RS	11/26 (42%) 15/26 (58%)	55/119 (46%) 64/119 (54%)	0.83
Spleen > 10 cm Spleen ≤ 10	74 (53%) 66 (47%)	165 (28%) 420 (72%)	<0.001
Palpable liver No hepatomegaly	35 (25%) 105 (75%)	111 (19%) 474 (81%)	0.13
Favorable CBA Unfavorable CBA	108/128 (85%) 20/128 (15%)	390/535 (73%) 145/535 (27%)	0.006
JAK2 mutation No JAK2 mutation	73/93 (78%) 20/93 (22%)	268/395 (68%) 127/395 (32%)	0.05
Constitutional symptoms No symptoms	113 (81%) 27 (19%)	423 (72%) 161 (28%)	0.05
No transfusions Transfusion-dependent	120 (86%) 20 (14%)	417 (71%) 168 (29%)	<0.001
DIPSS low Intermediate-I Intermediate-II High	7 (5%) 20 (14%) 27 (19%) 86 (62%)	45 (8%) 111 (19%) 109 (18%) 320 (55%)	0.36
DIPSS plus low Intermediate-I Intermediate-II High	5 (4%) 18 (13%) 18 (13%) 99 (70%)	36 (6%) 87 (15%) 91 (16%) 371 (63%)	0.42

Abbreviations: MCV, mean corpuscular volume; OR, odds ratio; HB, hemoglobin; WBC, white blood cells; PLT, platelets; PB, peripheral blood; BM, bone marrow; TIBC, total iron binding capacity; satTF, transferrin saturation; RS, ringed sideroblasts; CBA, chromosome banding analysis.

Table 2

Multivariate logistic regression analysis of baseline characteristics associated with microcytosis among all patients.

Characteristics (N=725)	MCV < 82 (n=140)	MCV ≥ 82 (n=585)	P-value (multivariate)	OR [95%CI]
No prior therapy Prior therapy	91 (65%) 49 (35%)	284 (49%) 301 (51%)	0.003	3.3 [1.5–6.8]
Age < 65 Age ≥ 65 years	82 (59%) 58 (41%)	288 (49%) 297 (51%)	0.14	1.7
WBC > 25,000 /uL WBC ≤ 25,000	34 (24%) 106 (76%)	87 (15%) 498 (85%)	0.56	0.8
PB blasts ≥ 1% PB blasts < 1%	84 (60%) 56 (40%)	262 (45%) 323 (55%)	0.35	1.4
BM blasts > 5% BM blasts ≤ 5%	18/132 (14%) 114/132 (86%)	38/548 (7%) 510/548 (93%)	0.49	1.7
Iron < 37 ug/dL Iron ≥ 37	39/87 (45%) 48/87 (55%)	32/383 (8%) 351/383 (92%)	0.05	3.3 [1.1–11]
SatTF < 15% SatTF ≥ 15%	44/90 (49%) 46/90 (51%)	44/367 (12%) 327/367 (88%)	0.005	4.6 [1.6–13]
Spleen > 10 cm Spleen ≤ 10	74 (53%) 66 (47%)	165 (28%) 420 (72%)	0.005	2.9 [1.4–6]
Favorable CBA Unfavorable CBA	108/128 (85%) 20/128 (15%)	390/535 (73%) 145/535 (27%)	0.24	2
JAK2 mutation No JAK2 mutation	73/93 (78%) 20/93 (22%)	268/395 (68%) 127/395 (32%)	0.14	1.9
Constitutional symptoms No symptoms	113 (81%) 27 (19%)	423 (72%) 161 (28%)	0.50	1.3
No transfusions Transfusion-dependent	120 (86%) 20 (14%)	417 (71%) 168 (29%)	0.37	1.7

Hosmer and Lemeshow Goodness of Fit test: $\chi^2=7.7$, df=8, p=0.47.

Abbreviations: MCV, mean corpuscular volume; OR, odds ratio; HB, hemoglobin; WBC, white blood cells; PLT, platelets; PB, peripheral blood; BM, bone marrow; TIBC, total iron binding capacity; satTF, transferrin saturation; RS, ringed sideroblasts; CBA, chromosome banding analysis.

Table 3

Multivariate logistic regression analysis of factors associated with microcytosis among untreated patients.

Characteristics (N=375)	MCV < 82 (n=91)	MCV ≥ 82 (n=284)	P-value (multivariate)	OR [95% CI]
WBC > 25,000 /uL WBC ≤ 25,000	23 (25%) 68 (75%)	28 (10%) 256 (90%)	0.10	2.4
PB blasts ≥ 1% PB blasts < 1%	53 (58%) 28 (42%)	121 (43%) 163 (57%)	0.27	1.6
Iron < 37 ug/dL Iron ≥ 37	24/56 (43%) 32/56 (57%)	14/169 (8%) 155/169 (92%)	0.42	1.8
SatTF < 15% SatTF ≥ 15%	27/57 (47%) 30/57 (53%)	18/167 (11%) 149/167 (89%)	0.02	4.5 [1.3–15.9]
Spleen > 10 cm Spleen ≤ 10 cm	42 (46%) 49 (54%)	60 (21%) 224 (79%)	0.03	2.5 [1.1–5.6]
Favorable CBA Unfavorable CBA	75/84 (90%) 9/84 (10%)	195/263 (75%) 64/263 (25%)	0.32	2
Constitutional symptoms No symptoms	72 (79%) 19 (21%)	188 (66%) 95 (34%)	0.31	1.6
No transfusions Transfusion-dependent	82 (90%) 9 (10%)	223 (79%) 61 (21%)	0.48	1.7

Hosmer and Lemeshow Goodness of Fit test: $\chi^2=4.8$, df=7, p=0.69.

Abbreviations: MCV, mean corpuscular volume; OR, odd ratio; WBC, white blood cells; PB, peripheral blood; satTF, transferrin saturation; CBA, chromosome banding analysis

Table 4

Multivariate Cox proportional hazards analysis of factors associated with LFS and transformation to leukemia

Characteristics (N=725)	5 years-LFS	P-value (multivariate)	HR [95% CI]
Males Females	82% 97%	0.07	1.9
BM blasts > 5% BM blasts ≤ 5%	60% 95%	<0.001	6.6 [3.3–13]
MCV < 82 fL MCV ≥ 82	80% 92%	0.12	1.6

Abbreviations: LFS, leukemia-free survival; HR, hazard ratio; CI, confidence interval; BM, bone marrow; MCV, mean corpuscular volume

Table 5

Multivariate logistic regression analysis of factors associated with transformation to leukemia

Characteristics (N=725)	AML (n=46)	No AML (n=679)	P-value	OR [95% CI]
Age < 65 years Age ≥ 65 years	30 (65%) 16 (35%)	340 (50%) 339 (50%)	0.03	2 [1.1–4.1]
BM blasts > 5% BM blasts ≤ 5%	12/45 (27%) 33/45 (73%)	44/635 (7%) 591/635 (93%)	<0.001	4.8 [2.3–10.1]
MCV < 82 fL ≥ MCV 82	16 (35%) 30 (65%)	124 (18%) 555 (82%)	0.03	2.1 [1.1–4.1]

Hosmer and Lemeshow Goodness of Fit test: $\chi^2=1.2$, df=3, p=0.77.

Abbreviations: CI, confidence interval; BM, bone marrow; MCV, mean corpuscular volume; AML, acute myeloid leukemia, OR, odds ratio.