

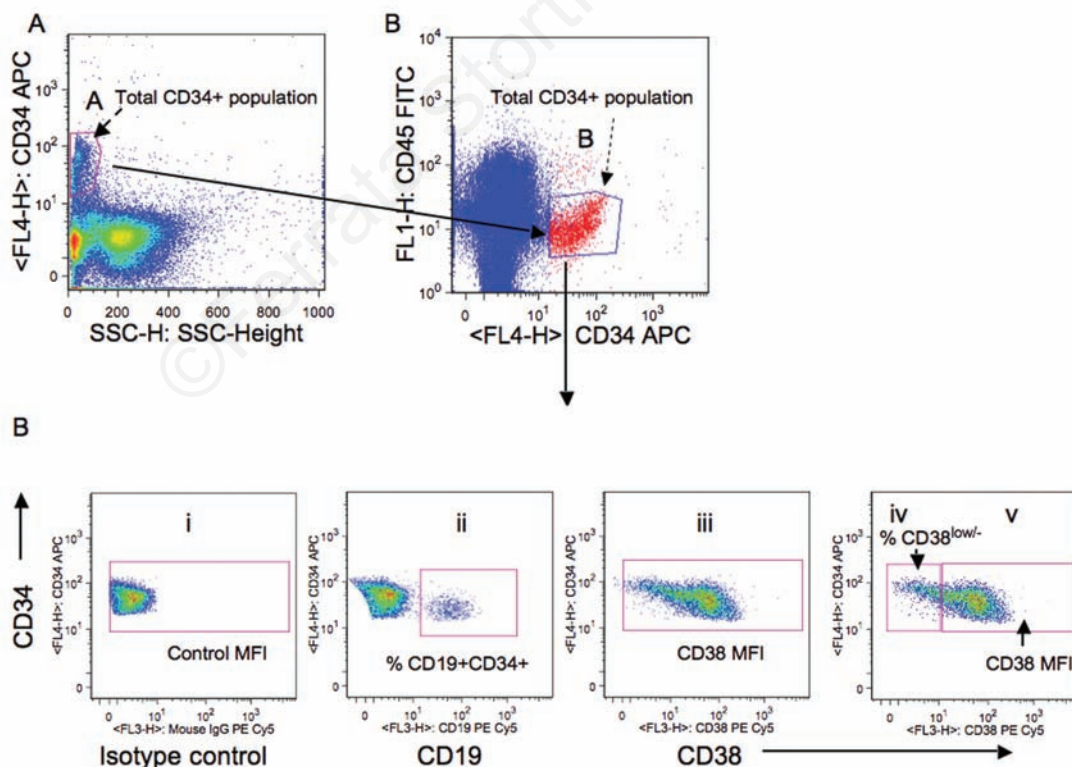
Reduced CD38 expression on CD34⁺ cells as a diagnostic test in myelodysplastic syndromes

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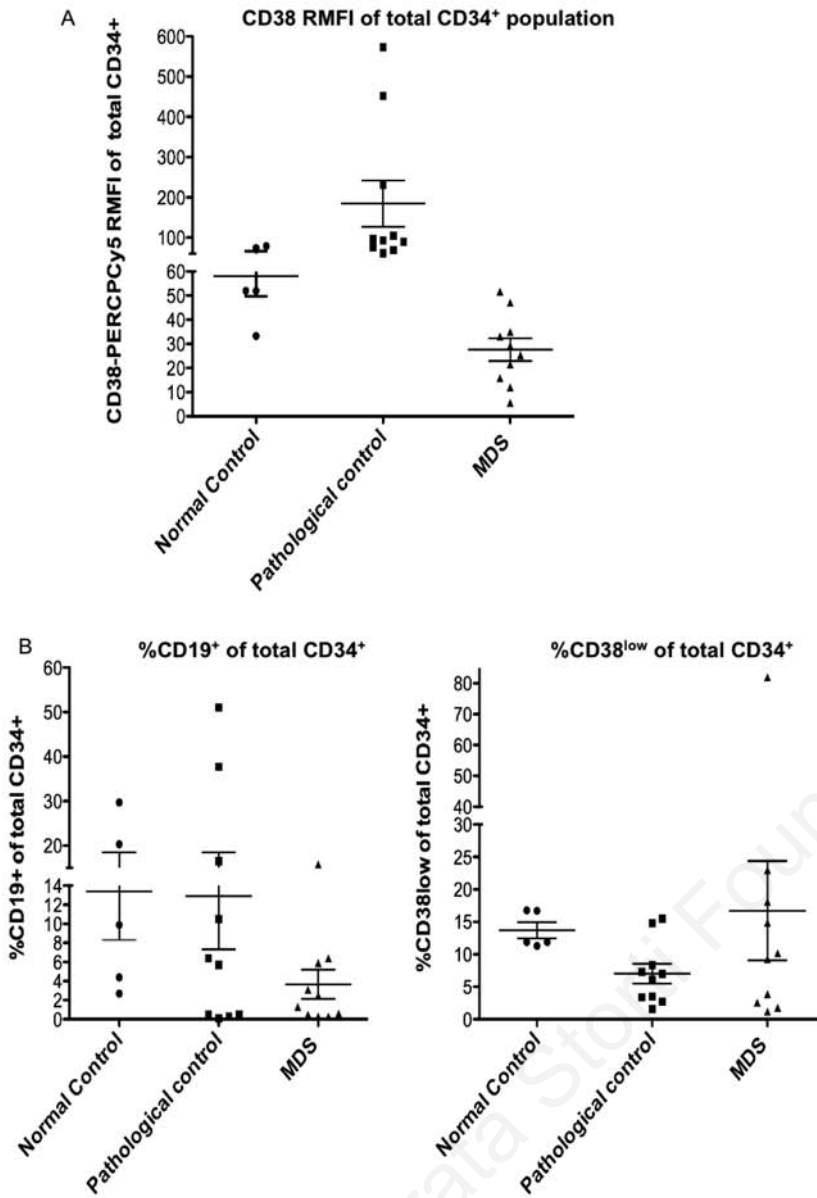
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Online Supplementary Table S1 and S2, refer directly to the corresponding PDFs.



Online Supplementary Figure S1. Outline of data analysis. (A) CD34⁺ cells were gated (Region A) on CD34 versus SSC plot ensuring that potential CD34⁺ B cell progenitors (lower side scatter) were included. (B) The reliability of this gating was verified by displaying these cells on a CD45 versus CD34 plot and further gating (Region B) was performed as necessary. This approach could be supplemented by initial FSC-SSC gating (not shown). (C) Percentage of CD34⁺ B cell progenitors (CD19⁺) was determined (ii). Average CD38 expression on the total CD34⁺ population (Region iii) was quantified by CD38-mean fluorescence intensity (MFI) then divided by MFI of isotype control (Region i) to give the more reproducible measurement of relative mean fluorescence intensity (RMFI). In order to determine the percentage of the more immature CD34⁺CD38^{low/-} cells in the total CD34⁺ population, the total CD34 population was subdivided into CD34⁺CD38^{low/-} subpopulation (Region iv) and CD34⁺CD38^{moderate/high} subpopulation (Region v). The latter subpopulation includes B cells progenitors (CD34⁺CD38^{high}) as well as common myeloid progenitors, granulocyte-macrophage progenitors and megakaryocyte-erythroid progenitors. In cohort 1, CD38 RMFI was also measured for CD34⁺CD38^{moderate/high} subpopulation (v).



Online Supplementary Figure S2. CD38 relative mean fluorescence intensity (RMFI) of CD34⁺ cells is reduced in a small cohort of MDS samples compared to controls (normal samples and controls). Graphs show - (A) CD38-RMFI values in CD34⁺ cells (B) percentages values for CD19⁺CD34⁺ cell population (left) and CD34⁺CD38^{low} (right) cells in samples from subjects with a normal blood counts and blood films who were having a hip replacement (normal control, closed circles, n=5); in samples from patients with a range of disorders (pathological controls, closed squares, n=10); patients with MDS (MDS, closed triangles, n=10). Samples are from Cohort 1 (Supplementary Table 1). CD34⁺ cells were defined by standard serial gating (CD34⁺SSC^{low} followed by CD34⁺CD45^{low}). (Supplementary Figure S1). CD38-RMFI = CD38PE-MFI divided by MFI of isotype control-PE staining. Horizontal bars are means and the SEM for each sample group is shown.

Supplementary Table 2: Patients in cohort 2

Full karyotype details are available on request.

Control samples

Sex	Age	Cytogenetics	Diagnosis
Female	73	46, XX	Anemia secondary to iron deficiency
Female	39	46, XX	Anemia due to folate deficiency and excessive alcohol intake
Male	41	46, XY	Pernicious anemia (B12 deficiency)
Male	57	46 XY	Anemia of chronic disease (inflammatory)
Female	78	46, XY	Anemia of chronic disease (inflammatory)
Female	40	46, XX	Anemia of chronic disease (inflammatory)
Male	48	46, XY	Anemia of chronic disease (renal and liver failure)
Female	64	46, XX	Anemia of chronic disease (inflammatory)
Female	76	46, XX	Anemia of chronic disease (chronic renal failure, diabetes mellitus)
10Male	80	46, XY	Anemia of chronic disease (inflammatory)
Male	74	46, XY	Anemia of chronic disease (diabetes mellitus, infection)
Male	55	46, XY	Anemia of chronic disease (inflammatory)
Female	67	46, XX	Anemia of chronic disease associated with rheumatoid arthritis
Female	33	46, XX	Anemia of chronic disease associated with lung carcinoma (no bone marrow involvement)
Female	57	46, XX	Anemia of chronic disease associated with bladder carcinoma (no bone marrow involvement)
Male	17	Not available	Anemia of chronic disease associated with Hodgkin's lymphoma
Male	67	46, XY	Anemia of chronic disease associated with systemic lupus erythematosus
Female	36	46, XX	Anemia of chronic disease associated with systemic lupus erythematosus
Male	41	46, XY	Pancytopenia secondary to sepsis
20Female	89	46, XX	Transient myeloid maturation arrest
Male	77	46, XY	Anemia due to chronic renal failure
Female	50	46, XX	Anemia due to chronic renal failure
Female	40	46, XX	Autoimmune thrombocytopenia
Female	69	46, XX	Autoimmune thrombocytopenia
Male	62	46, XY	Immune thrombocytopenia
Male	62	46, XY	Mild Immune thrombocytopenia
Male	25	46, XY	Immune thrombocytopenia
Female	37	Not available	Immune thrombocytopenia associated with systemic lupus erythematosus
Female	33	46, XX	Autoimmune neutropenia
Male	67	46, XY	Mild pancytopenia – immune mediated
30Male	67	46, XY	Immune neutropenia and thrombocytopenia
Male	64	46, XY	Autoimmune hemolytic anemia associated with B-cell non-Hodgkin's lymphoma
Male	19	46, XY	Neutropenia and autoimmune hemolytic anemia associated with CHARGE syndrome
Male	18	46, XY	Pancytopenia due to hemophagocytic syndrome
Female	26	46, XX	Pancytopenia due to hemophagocytic syndrome post liver

			transplant
Female	38	46, XX	Thrombocytopenia/splenomegaly due to Non Hodgkin's lymphoma
Male	35	46, XY	Pancytopenia due to splenomegaly due to Non-Hodgkin's lymphoma
Male	25	46, XY	Pancytopenia associated with T-cell Non-Hodgkin's lymphoma
Male	59	46, XY	Pancytopenia due to angioimmunoblastic Non-Hodgkin's lymphoma
40Male	23	Not available	Cytopenia associated with Hodgkin's Lymphoma
Female	46	Not available	Cytopenia associated with Diffuse large B-cell lymphoma
Female	83	46, XX	Cytopenia due to marrow infiltration with breast carcinoma
Female	74	46, XX	Splenic rupture with thrombocytopenia
Female	74	46, XX	Pancytopenia post CHOP chemotherapy for B-Non Hodgkin's Lymphoma
Male	38	46, XY	Pancytopenic post allograft for CML (molecular remission)
Male	17	46, XY	Routine molecular monitoring for acute promyelocytic leukemia in molecular remission
Male	34	46, XY	Pancytopenia from chemotherapy for acute promyelocytic leukemia in molecular remission
Female	34	46, XX	B-acute lymphoblastic leukaemia - complete remission
Female	45	46, XX	AML -inversion(16) in molecular remission
50Female	73	46, XX	Consumptive thrombocytopenia secondary due to hepatitis C infection.
Male	37	46, XY	Pancytopenia secondary to cirrhosis from hepatitis B infection
Female	26	46, XX	Pancytopenia associated with viral infection
Male	73	46, XY	Pancytopenia associated with chronic liver disease
Female	44	46, XX	Pancytopenia in a patient post liver transplant
Female	19	46, XX	Pancytopenia associated with liver disease
Female	49	46, XX	Severe Aplastic Anemia (46, XX cytogenetics)
Male	43	46, XY	Non severe aplastic anemia (46, XY cytogenetics)
Male	44	46, XY	Relative polycythaemia
Female	42	46, XX	Reactive thrombocytosis
Female	65	46, XX	Eosinophilia

MDS (<5% blasts)

Male	66	46, XY	RA hypoplastic
Female	28	monosomy 7	RA
Female	89	46, XX	RA
Male	67	46, XY	RA
Male	71	-Y	RA
Male	64	46, XY	RA
Male	75	46, XY	RA
Male	60	trisomy 8	RA
Female	73	Not available	RA
Female	82	46, XX	RA
Female	87	46, XX	RA
Female	72	trisomy 8	RA
Female	57	deletion 5q	RA
Female	68	46, XX	RA
Male	71	46, XY	RA

Female	86	46, XX	RA
Male	70	45,XY,-21	RA
Male	67	46, XY	RA
Male	61	addition 14	RA
Female	81	46, XX	RA
Male	81	addition15	RA
Male	61	46, XY	RARS
Male	64	46, XY	RARS
Male	72	deletion 20q	RARS
Female	54	46, XX	RARS
Female	71	deletion 2 addition 4	RARS
Female	79	deletion 5q	RARS
Male	58	46, XY	RCMD
Female	79	complex	RCMD
Female	82	deletion 5q, monosomy 7	RCMD
Male	72	Not available	RCMD
Female	28	monosomy 7	RCMD
Female	78	deletion 7q	RCMD
Male	57	monosomy 7	RCMD
Female	79	46, XX	RCMD
Male	58	deletion 5q	RCMD
Female	73	Not available	RCMD with PNH clone
Female	72	46, XX	RCMD-RS
Female	57	complex	RCMD
Male	73	46, XY	RCMD
Male	81	46, XY	RCMD
Male	60	deletion 20q	RCMD
Male	76	46, XY	RCMD
Male	72	Not available	RCMD
Female	69	complex	RCMD
Female	87	46, XX	RCMD
Female	80	complex	RCMD
Male	55	46, XY	RCMD
Female	40	deletion 20q	RCMD
Male	58	46, XY	RCMD
Male	65	monosomy 7	RCMD
Male	44	complex	RCMD
Male	66	Not available	RCMD
Male	75	46, XY	RCMD
Male	81	deletion 20q	RCMD
Male	59	46, XY	RCMD
Male	73	46, XY	RCMD
Male	69	46, XY	RCMD
Female	58	46, XX	MDS-U/MPD
Male	74	46, XY	MDS-U/MPD
Female	80	46, XX	MDS/MPD-U
Male	72	46, XY	MDS-U

MDS (>5% blasts)

Female	78	complex	RAEB-I
Male	77	trisomy 8	RAEB-I
Male	80	46, XY	RAEB-I
Female	79	t(9:14)	RAEB-I
Male	75	46, XY	RAEB-I
Male	74	46, XY	RAEB-I
Male	75	Not available	RAEB-I
Male	70	-Y	RAEB-I
Male	71	46, XY	RAEB-I
Male	79	trisomy 8	RAEB-I
Male	78	46, XY	RAEB-II
Female	63	deletion 5q	RAEB-I
Male	53	deletion 5q	RAEB-I
Male	80	monosomy 5, 7	RAEB-I
Male	85	46, XY	RAEB-I
Male	57	46, XY	RAEB-I
Female	80	46, XX	RAEB-I
Male	60	Not available	RAEB-II
Male	79	46, XY	RAEB-II
Female	63	complex	RAEB-II
Male	76	46, XY	RAEB-II
Male	75	46, XY	RAEB-II
Male	72	46, XY	RAEB-II
Male	78	complex	RAEB-II
Male	75	Not available	RAEB-II
Female	76	46, XX	RAEB-II
Male	65	46, XY	RAEB-II

MDS transformed to AML

Female	70	complex	AML transformed from MDS
Male	76	46, XY	AML transformed from MDS
Male	76	deletion 5q	AML transformed from MDS
Male	57	46, XY	AML transformed from MDS
Female	58	46, XX	AML transformed from MDS

AML

Female	69	46, XX	AML
Male	50	monosomy 7	AML
Female	73	46, XX	AML
Male	37	near tetraploid karyotype	AML
Male	37	near tetraploid karyotype	Refractory AML post treatment
Female	36	complex	AML
Male	73	inversion 3	AML
Female	46	t(8;21)	AML
Male	44	46, XY	AML
Male	52	46, XY	AML
Female	50	Not available	AML
Male	45	monosomy 7	AML

Female	73	complex	AML
Female	55	46, XX	AML
Male	69	46, XY	AML
Male	69	46, XY	AML
Female	77	complex	AML
Female	43	46, XX	AML
Male	67	Not available	Refractory AML
Female	91	Not available	AML
Female	91	Not available	Refractory AML post treatment
Male	65	deletion 7q	AML
Male	62	46, XY	AML
Male	72	46, XY	Relapsed AML
Male	67	46, XY	Relapsed AML

Chronic Myelomonocytic Leukemia

Male	86	Not available	Chronic Myelomonocytic Leukemia
Female	56	Not available	Chronic Myelomonocytic Leukemia
Male	74	Not available	Chronic Myelomonocytic Leukemia
Female	80	Not available	Chronic Myelomonocytic Leukemia

Myeloproliferative disease (not CML)

Female	85	46, XY	Polycythaemia rubra vera
Male	61	trisomy 8	MPD
Female	74	JAK-2 positive	Mixed PRV/ET
Female	37	Not available	Essential thrombocytosis
Female	52	Not available	MPD
Female	83	Not available	Essential thrombocytosis
Female	74	Not available	Essential thrombocytosis