

Bone marrow oxidative stress and specific antioxidant signatures in myelodysplastic syndromes

Frederic Picou, Christine Vignon, Christelle Debeissat, Sebastien Lachot, Olivier Kosmider, Nathalie Gallay, Amelie Foucault, Marie-Helene Estienne, Noemie Ravalet, Marie C Bene, Jorge Domenech, Emmanuel Gyan, Michaela Fontenay, and Olivier Herault

Supplemental Table 1: Cohort description

Supplemental Table 2: Description of MDS patients (IPSS-R and AO-Score)

Supplemental Table 3: List of transcripts, primers and probes

Supplemental Table 4: Statistical analyses of ROS levels in bone marrow cells (Kruskal-Wallis followed by Dunn post hoc test versus healthy control)

Supplemental Figure 1: Flow cytometry gating strategy to quantify the ROS level in the bone marrow cell subpopulations. CD45/SSC gating separates hematopoietic cell populations. Lymphocytic cells show the highest CD45 fluorescence intensity and the lowest SSC. Monocytic cells express slightly lower but still high amounts of CD45 and are distinguished from lymphocytes by their higher SSC. Granulocytic cells express low CD45 and broad SSC. Nucleated erythroid cells are characterized by reduced/absent CD45 expression and low SSC. The earliest cells committed to each lineage occupy a position of low-medium SSC and CD45.

Supplemental Figure 2: Expression level of antioxidant transcripts in BM of healthy controls. Results are expressed as Δ Ct values vs. three housekeeping genes (*GAPDH*, *ACTB*, *B2M*), n=25. The most highly expressed transcripts are those with the lowest Δ Ct value. The dark area delineates unexpressed transcripts.

Supplemental Figure 3: The antioxidant score (AO-Score) is an IPSS-R independent biomarker. Low-grade MDS patients are in green and high-grade MDS are in orange. Regression line (in red) and R² Pearson correlation values are indicated, showing that the AO-Score is not correlated with IPSS-R.

Supplemental Table 1: Cohort description

| Patients | Number (Tours/Paris) | Tours: median age (range) | Paris: median age (range) | Median IPSS-R (Tours/Paris) | Median BM blastosis (range) |
|--------------------|---------------------------------|--------------------------------------|--------------------------------------|--|--|
| MDS-SLD | 10 (4/6) | 74 (47-96) | 77 (60-81) | 1.3 (1.3-1.3) | 0.7 (0-3) |
| MDS-SLD-RS | 13 (5/8) | 73 (59-88) | 81 (73-88) | 1.9 (2.0/1.8) | 0.8 (0-3) |
| MDS-MLD | 29 (21/8) | 75 (65-82) | 75 (57-87) | 1.8 (1.8/1.8) | 0.9 (0-5) |
| MDS-MLD-RS | 13 (10/3) | 80 (62-87) | 85 (78-93) | 2.3 (2.4/2.0) | 0.8 (0-4) |
| MDS-EB-1 | 13 (5/8) | 67 (50-82) | 70 (35-88) | 3.6 (3.0/4.0) | 6.2 (5-9) |
| MDS-EB-2 | 13 (7/6) | 67 (51-83) | 71 (44-87) | 5.0 (5.0/5.0) | 14.8 (11-19) |
| sAML | 6 (6/0) | 71 (57-77) | na | na | 50 (26-90) |
| healthy volunteers | 25 (25/0) | 72 (61-81) | na | na | na |
| Total | 122 (83/39) | 74 (51-96) | 76 (35-93) | na | na |

na: non applicable; sAML: AML with morphologic dysplastic related changes

Supplemental Table 2: Description of MDS patients (IPSS-R and AO-Score)

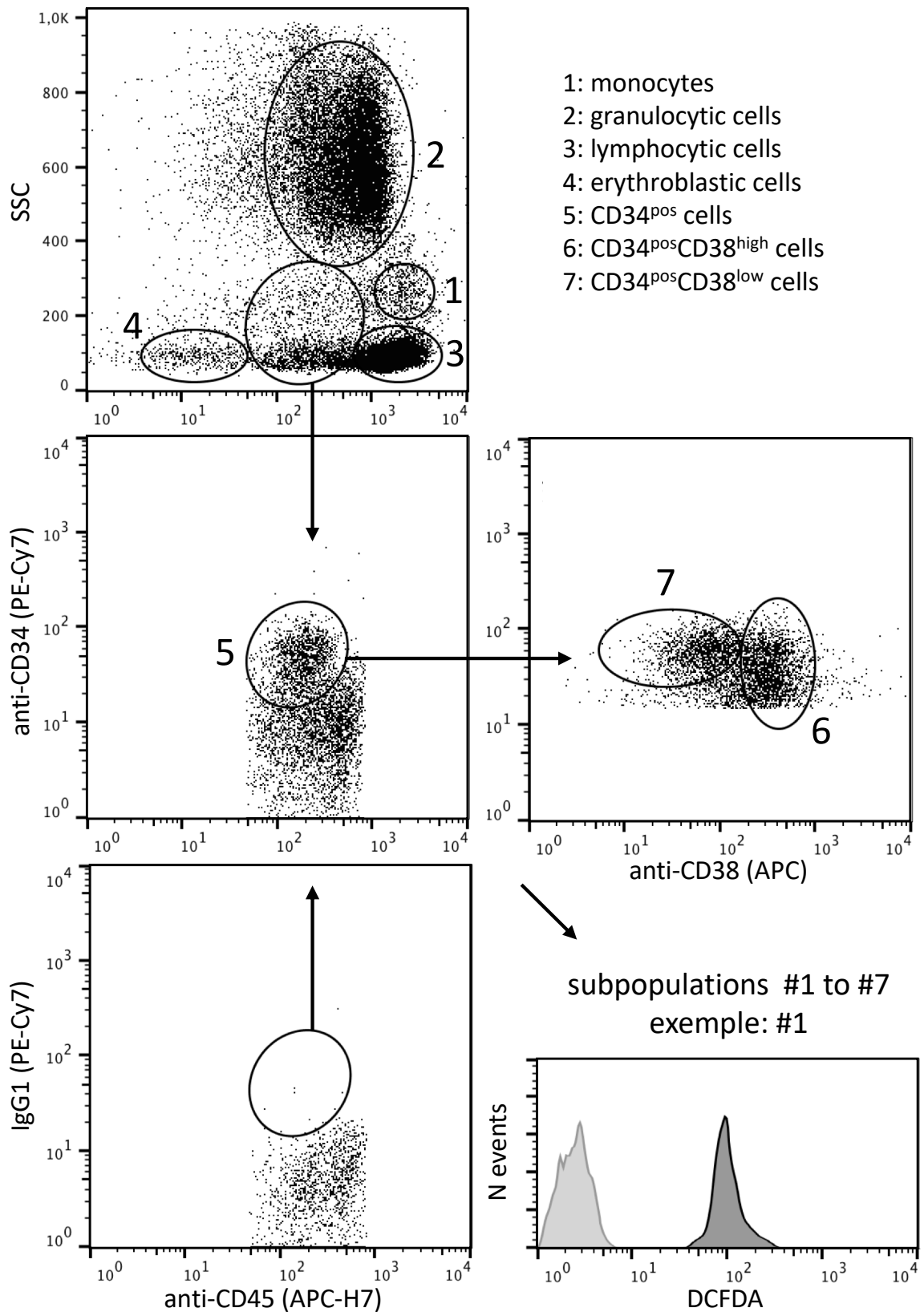
| MDS subtype | Patient (Tours: T Paris: P) | Karyotype | BM Blasts (%) | Hemoglobin (g/dL) | Platelets (10 ⁹ /L) | ANC (10 ⁹ /L) | R_IPSS Cyto | IPSS Risk Group | IPSS-R | AO-Score |
|-------------|-----------------------------------|-----------------|------------------|----------------------|-----------------------------------|--------------------------|-------------|--------------------|--------|----------|
| MDS-SLD | P1 | 46,XX | 3 | 8.8 | 55 | 2.3 | Good | Int | 3.5 | -1.71 |
| | P2 | 46, XX | 1 | 11.0 | 221 | 3.9 | Good | Very low | 1 | 0.08 |
| | P3 | 46,XY | 1 | 13.0 | 106 | 0.5 | Good | Very low | 1,5 | -3.42 |
| | T1 | 46,X,+15,-Y | 0 | 7.9 | 197 | 2.7 | Int | Int | 3,5 | 18.98 |
| | T2 | 46,X,-Y,+15 | 0 | 10.7 | 119 | 4.0 | Int | Low | 2 | 1.64 |
| | T3 | 46,XX | 0 | 13.5 | 135 | 0.9 | Good | Very low | 1 | -2.12 |
| | T4 | 45,X,-Y | 0 | 9.2 | 357 | 6.2 | Very good | Very low | 1 | 1.83 |
| MDS-RS-SLD | P4 | 46,XY | 1 | 9.9 | 302 | 2.5 | Good | Low | 2 | 2.09 |
| | P5 | 46,XY | 1 | 8.2 | 165 | 2.5 | Good | Low | 2 | -4.61 |
| | P6 | 46,XY | 1 | 10.8 | 222 | 4.9 | Good | Very low | 1 | -3.24 |
| | P7 | 45,XY,-9 | 1 | 10.4 | 204 | 4.0 | Int | Low | 2 | -2.16 |
| | P8 | 45,XX,-9 | 0 | 7.3 | 295 | 3.4 | Int | Int | 3,5 | 3.51 |
| | P9 | 46,XY | 2 | 9.9 | 599 | 4.2 | Int | Low | 2 | 2.18 |
| | T5 | 47,XX,+8 | 0 | 7.7 | 160 | 0.9 | Int | Int | 3,5 | 1.98 |
| | T6 | 46,XX | 0 | 9.4 | 187 | 2.3 | Good | Low | 2 | 2.83 |
| | T7 | 46,XY | 0 | 8.7 | 173 | 16.1 | Good | Low | 2 | 3.19 |
| | T8 | 46,XX | 0 | 9.0 | 486 | 3.9 | Good | Low | 2 | -0.74 |
| T9 | 46,XX | 0 | 9.3 | 545 | 2.6 | Good | Low | 2 | 1.71 | |
| MDS-MLD | P10 | 46,XX | 3 | 12.2 | 219 | 4.1 | Good | Low | 2 | -0.64 |
| | P11 | 46,XX | 1 | 6.2 | 101 | 0.1 | Good | Int | 3 | 0.99 |
| | P12 | 46,XY | 2 | 10.3 | 146 | 1.3 | Good | Very low | 1 | -15.61 |
| | P13 | 46,XY | 1 | 9.8 | 122 | 2.5 | Good | Low | 2 | -3.15 |
| | P14 | 46,XY | 4 | 9.4 | 139 | 4.5 | Good | Low | 3 | -3.76 |
| | P15 | 45,X,-Y | 2 | 10.1 | 165 | 3.7 | Very good | Very low | 0 | -0.15 |
| | P16 | 46,XX | 1 | 6.8 | 176 | 2.3 | Good | Low | 2,5 | 0.92 |
| | T10 | 46XY | 3 | 12.1 | 139 | 2.8 | Good | Low | 2 | 1.17 |
| | T11 | 45,X,-X | 0 | 8.9 | 61 | 1.6 | Int | Int | 3,5 | 15.04 |
| | T12 | 46,XY | 0 | 13.4 | 21 | 8.6 | Good | Low | 2 | -1.06 |
| | T13 | 46,XY | 0 | 10.3 | 142 | 0.4 | Good | Very low | 1,5 | 1.03 |
| | T14 | 43,XY,-3,-7,-12 | 0 | 11.5 | 77 | 0.9 | Poor | Int | 3,5 | -0.18 |
| | T15 | 46,XY | 0 | 10.8 | 311 | 2.6 | Good | Very low | 1 | -0.25 |
| | T16 | 46,XY | 0 | 13.2 | 186 | 0.9 | Good | Very low | 1 | 0.79 |
| | T17 | 46,XX | 0 | 9.7 | 307 | 6.6 | Good | Low | 2 | 0.42 |
| | T18 | 46,XY | 0 | 11.7 | 206 | 0.5 | Good | Very low | 1,5 | -2.19 |
| | T19 | 46,XX | 0 | 10.1 | 308 | 2.7 | Good | Very low | 1 | 2.75 |
| | T20 | 46,XX | 0 | 7.2 | 69 | 1.7 | Good | Low | 3 | 0.22 |
| | T21 | 47,XX,+8 | 0 | 8.8 | 134 | 2.8 | Int | Low | 3 | 0.1 |
| | T22 | 45,X,-Y | 0 | 13.7 | 129 | 1.9 | Very good | Very low | 0 | 1.85 |
| T23 | 46,XX | 0 | 10.4 | 32 | 0.3 | Good | Low | 2,5 | -1.07 | |
| T24 | 46,XY | 0 | 14.7 | 71 | 0.6 | Good | Low | 2 | 1.23 | |
| T25 | 46,XX | 0 | 8.6 | 86 | 3.6 | Good | Low | 2,5 | 1.98 | |
| T26 | 47,XY,+8 | 0 | 11.7 | 104 | 2.6 | Int | Low | 2 | -2.62 | |
| T27 | 46,XY | 0 | 11.5 | 81 | 14.0 | Good | Very low | 1,5 | 1.2 | |
| T28 | 46,XY,del(20q) | 0 | 9.6 | 155 | 2.0 | Good | Low | 2 | 0.87 | |
| T29 | 46,XX | 0 | 13.8 | 99 | 4.0 | Good | Very low | 1,5 | 2.29 | |
| MDS-RS-MLD | P17 | 46,XX | 1 | 7.6 | 128 | 2.6 | Good | Low | 2,5 | -1.84 |
| | P18 | 46,XY | 2 | 9.7 | 277 | 3.5 | Good | Low | 2 | -1.29 |
| | P19 | 46,XX | 1 | 8.9 | 274 | 1.2 | Good | Low | 2 | -2.29 |
| | P20 | 46,XX | 4 | 10.0 | 159 | 3.5 | Good | Low | 2 | -0.18 |
| | T30 | 46,X,-Y,+7 | 0 | 9.6 | 142 | 18.3 | Int | Low | 3 | 0.34 |
| | T31 | 46,XX | 0 | 8.9 | 436 | 5.0 | Good | Low | 2 | 2.21 |
| | T32 | 45,XX,-21 | 0 | 8.0 | 92 | 0.7 | Int | Int | 4 | 4.88 |
| | T33 | 46,XX | 0 | 8.7 | 82 | 2.2 | Good | Low | 2,5 | 0.21 |
| | T34 | 46,XX | 0 | 9.6 | 244 | 2.1 | Good | Low | 2 | 0.85 |
| | T35 | 46,XY | 0 | 9.8 | 463 | 2.8 | Good | Low | 2 | 2.35 |
| | T36 | 46,XX,del(12p) | 3 | 8.3 | 176 | 5.5 | Good | Low | 3 | 0.35 |
| | T37 | 46,XY,del(20q) | 0 | 10.2 | 56 | 9.7 | Good | Very low | 1,5 | 1.12 |
| | T38 | 46,XX | 0 | 10.3 | 224 | 2.4 | Good | Very low | 1 | 1.12 |
| T39 | 46,XY | 0 | 6.9 | 164 | 2.4 | Good | Low | 2,5 | 2.04 | |
| T40 | 46,XY | 0 | 6.5 | 374 | 1.1 | Good | Low | 2,5 | 4.42 | |
| MDS-EB-1 | P21 | 46,XX | 5 | 8.1 | 193 | 1.7 | Good | Int | 4 | -4.15 |
| | P22 | 46,XY,del(20q) | 9 | 8.3 | 63 | 1.9 | Good | Int | 4,5 | -56.67 |
| | P23 | 46, XY | 6 | 10.5 | 23 | 2.3 | Good | Int | 4 | -6.68 |
| | P24 | 46,XY | 5 | 10.7 | 135 | 1.0 | Good | Low | 3 | -10.67 |
| | P25 | 47,XY,+8 | 5 | 8.5 | 253 | 2.0 | Int | High | 5 | -4.48 |
| | T41 | 46,XY | 9 | 10.2 | 212 | 1.2 | Good | Low | 3 | -22.35 |
| | T42 | 46,XX | 6 | 7.6 | 19 | 1.6 | Good | High | 5,5 | -1.74 |
| | T43 | 46,XY | 5 | 13.2 | 122 | 1.6 | Good | Low | 3 | -0.92 |
| T44 | 46,XX,del(11q) | 6 | 12.6 | 29 | 1.8 | Very good | Low | 3 | -0.49 | |
| MDS-EB-2 | P26 | 46,XX | 15 | 10.5 | 121 | 2.4 | Good | Int | 4 | -11.33 |
| | P27 | 47,XX,+8 | 17 | 11.7 | 13 | 5.8 | Int | High | 6 | -3.13 |
| | T45 | 46,XY | 19 | 8.5 | 22 | 1.1 | Good | High | 6 | -5.75 |
| | T46 | 46,XX | 10 | 10.2 | 67 | 0.4 | Good | Int | 4 | -5.36 |
| | T47 | 46,XX | 14 | 8.3 | 52 | 11.2 | Good | High | 5,5 | -0.05 |
| | T48 | 46,XX | 16 | 8.7 | 587 | 0.5 | Good | High | 5,5 | -9.47 |

Supplemental Table 3: List of transcripts, primers and probes

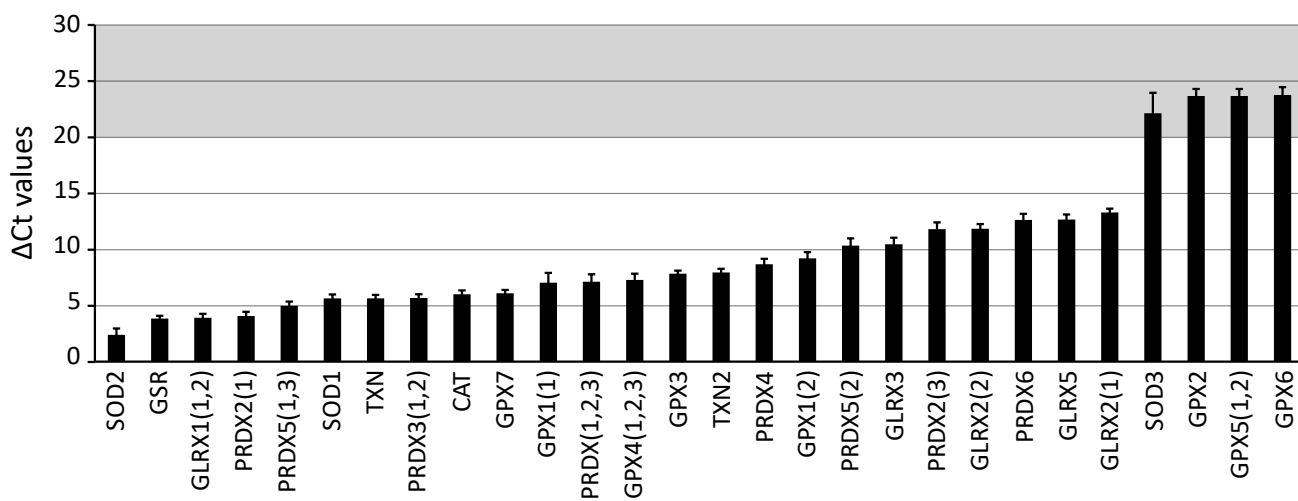
| Transcripts | Primer (forward) | Primer (reverse) | Probe |
|--------------|----------------------------|-----------------------------|-----------|
| SOD1 | gcatcatcaatttcgagcag | caggccttcagtcagtcctt | cttcccca |
| SOD2 | tccactgcaaggaacaacag | taagcgtgtcccacacat | ctgctggg |
| SOD3 | ctctcttttcaggagagaaaagctc | aacacagtagcgccagcat | aggagctg |
| CAT | cgcagttcggttctccac | gggtcccgaactgtgtca | ctccagca |
| TXN | ttacagccgctgtcaga | ggcttcctgaaaagcagtcctt | ggctgctg |
| TXN2 | gagacaccagtggttgga | gcttggccaccatcttctc | ctggggcc |
| GLRX | ggcttctggaattgtcgat | tgcatccgctatacaatctt | cagccacc |
| GLRX2(1) | gtggcactcgctggaatc | cgctcgtaaattctcaaagat | ctccatcc |
| GLRX2(2) | gttggtttggagcaggag | caaagatgatgatgtattgctct | ggcggcgg |
| GLRX3 | tcctcaagaaccacgctgt | tgagaagatatcaaaactgctaaactg | tggtgga |
| GLRX5 | gtgataactggggcgtgtt | actcaggcatgcacagca | ctccagca |
| GPX1(1) | caaccagtttggcatcag | gttcacctcgacttctcg | ccaccacc |
| GPX1(2) | ccctgtttgtggttagaacg | gagagaagggcagctagaacc | ctcctct |
| GPX2 | gtcctggcttccctg | tgctcaggatctcctcattctg | caggagaa |
| GPX3 | cagagatccttctaccctcaa | cccttctcaaagagctgga | agggtggag |
| GPX4 | tacggaccatggaggag | ccacacactgtggagctagaa | ctgcccga |
| GPX7 | ccatctgcttcaagtacc | ttcatctggggctactagg | ctccttcc |
| GSR | tgccagcttaggaataaccag | cctgcaccaacaatgacg | gctggaag |
| PRDX1(1,2,3) | cactgacaacatggggaagt | ttgctcttttgacatcagg | ccagccag |
| PRDX2(1) | gccttccagtacacagcagag | gttgggcttaatcgtgtcact | cttcccca |
| PRDX2(3) | gcaactcagatgcaactctatctact | tgaactggagtttccatcttcat | cagcctcc |
| PRDX3(1,2) | ctggacaccggattctccta | gggtgatctactgattaccttctg | ctgcttcc |
| PRDX4 | gcacctaagcaaagcgaaga | aaattctccatcgatcacagc | actgggaa |
| PRDX5(1,3) | tcctggctgatcccactg | atgccatcctgtaccaccat | ctccttcc |
| PRDX5(2) | caccttgatgttccaa | ggacaccagcgaatcatctagt | ctccttcc |
| PRDX6 | caatagacagtgttgggaccatc | tttctgtggctcttcacaa | gctccagg |
| GAPDH | agccacatcgctcagacac | gccaatacgaaccaatcc | cttcccca |
| ACTB | attggcaatgagcggctc | cgtaggatccacaggact | gctggaag |
| B2M | ttctggcctggaggctatc | tcaggaaattgactttccattc | ccagccgc |

Supplemental Table 4: Statistical analyses of ROS levels in bone marrow cells (Kruskal-Wallis followed by Dunn post hoc test vs. healthy controls)

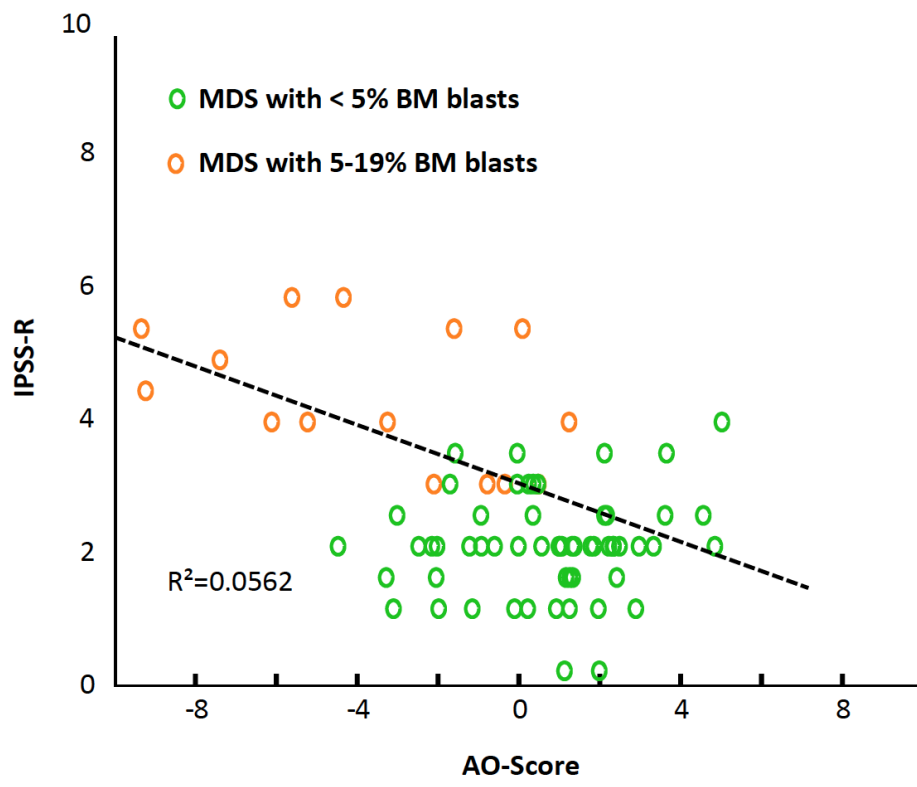
| Disease | <i>P</i> -value (vs . healthy controls) |
|------------------------------|---|
| CD34pos cells | |
| MDS-SLD | 0.0286 |
| MDS-SLD-RS | 0.0468 |
| MDS-MLD | 0.0013 |
| MDS-MLD-RS | 0.0483 |
| MDS-EB-1 | 0.0193 |
| MDS-EB-2 | 0.0352 |
| sAML | 0.0009 |
| CD34pos CD38low cells | |
| MDS-SLD | 0.0352 |
| MDS-SLD-RS | 0.0392 |
| MDS-MLD | 0.0419 |
| MDS-MLD-RS | 0.0265 |
| MDS-EB-1 | 0.0452 |
| MDS-EB-2 | 0.0422 |
| sAML | 0.0065 |



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3