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Surface marker abnormalities in myelodysplastic syndromes

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ABSTRACT

Background and Objective. **The myelodysplastic syndromes (MDS) are clonal stem cell disorders associated with a variety of abnormalities of mature and maturing cells, including surface antigen abnormalities. Granulocytes and monocytes function as members of the immune system. Surface antigens serve as biological sensors allowing various cells to interact with different stimuli. Abnormalities of surface antigens may be associated with defective cell function and may indicate a more severe or more advanced stage of the disease.**

Information Sources. **The author has a great interest in bone marrow changes in MDS and has several previous publications in this field. In addition, relevant articles published since 1966 were retrieved using Medline® of English literature and were included.**

State of the Art and Perspectives. **Several surface antigens in MDS have shown abnormal expression either in the intensity of fluorescence or the percentage of positive cells. These abnormalities include increased, decreased or lineage-aberrant expression. Abnormalities of several surface markers have prognostic significance. MDS patients with a low percentage of bone marrow cells expressing CD11b had a higher risk of evolution to acute myeloid leukemia and shorter survival compared to patients with more than 53% of marrow cells expressing CD11b (29 weeks versus 160 weeks). On the other hand, an increased percentage of bone marrow cells expressing early or immature markers, such as CD 13, CD33, CD34 and HLA-DR, has been associated with a worse outcome and with progression to a higher risk MDS or to acute myeloid leukemia. However, there are numerous discrepancies and inconsistencies in the literature when reviewing surface marker changes in MDS. These discrepancies may be related, at least in part, to the presence of an intracellular storage compartment of numerous surface antigens in the granulocytes and monocytes. Because of these storage pools, the techniques of preparing more mature granulocytes and monocytes, such as density gradient separation, and the interpretation of results must be carefully evaluated. Furthermore, various methods have been used to express abnormal results including percentage of positive or negative cells, fluorescent intensity (FI) of individual patients or a group of patients using a mean fluorescent channel (256 or 1024 channel mode), and finally the expression of FI as molecules of equivalent soluble fluorochromes or antibody binding capacities. Several mechanisms may be involved in the abnormal expression of surface antigens in MDS including defective granulopoiesis, defective intracellular storage pool, abnormal membrane of cytoplasmic granules, and the effect of high levels of marrow cytokines such as tumor necrosis factor alpha and transforming growth factor-β. Standardization of the methods of preparing and studying mature and maturing granulocytes and monocytes in MDS has to be achieved in order to produce comparable results, thus allowing surface marker studies to be utilized as diagnostic and prognostic tools in MDS.**
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Key words: myelodysplastic syndromes, surface antigens, flow cytometry, neutrophils, monocytes

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The myelodysplastic syndromes (MDS) are a group of clonal stem cell disorders characterized by ineffective hematopoiesis, peripheral cytopenias and increased risk of developing acute leukemia.[1,2] Based on the number of blasts, monocytes and ring sideroblasts in the peripheral blood (PB) and/or the bone marrow (BM), the French-American-British (FAB) Group has classified MDS into refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-t), or chronic myelomonocytic leukemia (CMML).[1] Furthermore, MDS may be classified into risk groups including low-, intermediate- and high-risk MDS using degree of cytopenias, percentage of BM blasts and cytogenetic findings.[3] Myelodysplastic cells usually display a variety of abnormalities detected by morphologic, cytochemical, immunocytochemical/surface marker, cytogenetic, molecular, and *in vitro* cell culture studies.[3-6] Surface antigens on mature cells such as monocytes and granulocytes provide the biological sensor which interacts with the surrounding media and thus allows for the appropriate response to different stimuli. Consequently, abnormalities in some of these surface antigens in MDS cells may produce a profound defect in the role of these cells in the immune system.[7,8] Several factors may influence surface antigen expression in MDS including defective granulopoiesis, defective

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intracellular storage pool, abnormal membrane of cytoplasmic granules, and the effect of high levels of marrow cytokines such as tumor necrosis factor-α and transforming growth factor-β.[5,6,9] Surface marker abnormalities in MDS have received little attention in several otherwise excellent reviews of the utilization of surface markers and flow cytometry in hematologic diseases.[10,11] Most of surface antigen alterations in MDS affect mature and maturing granulocytes and monocytes since, by definition, the percentage of blasts in the PB or the BM is less than 30%, and in over half of MDS patients is less than 5%.[12] There are obvious inconsistencies in the results of surface marker studies in MDS which may be related to one or more of the following factors:

a. *methodology;* some investigators have used visual evaluation of immunocytochemical reactions using fluorescent microscopy while others have utilized flow cytometry. These two methods may not produce identical results although some studies indicate very good correlation between both methods for certain antigens;[13]
b. *cell preparation*; due to the presence of intracellular storage pools of numerous antigens in neutrophils such as CD35, CD11b, CD11c and CD16, the results of density gradient (DG) separation may be different from those using the whole blood lysis (WL) technique.[14,15] This is compounded by the fact that some of these intracellular pools are defective in MDS.[5,8] In addition, cell pre-incubation at 37°C may affect the expression of some antigens such as CD35 while neutrophil suspension in serum-free medium can increase CD16 shedding from the surface of neutrophils;[16-18]
c. *the purity of the gate*; some investigators have utilized a CD45-side scatter gate which allows for better cell identification, particularly when studying blasts. Other investigators have gated using forward and side scatter properties or used no gate at all;[19]
d. *the definition of normal range;* although most investigators studying mature leukocytes have defined the normal range through studying a comparable group of healthy controls, other investigators have used ≥ 20% to define an abnormally increased expression of a certain antigen;[20]
e. *the expression of results:* various methods have been used to express abnormal results including percentage of positive or negative cells, fluorescent intensity (FI) of individual patients or a group of patients using a mean fluorescent channel (256 or 1024 channel mode), FI compared to a negative or a positive control material, and finally the expression of FI as molecules of equivalent soluble fluorochromes or antibody binding capacities;
f. *sensitivity of different antibodies and fluorescent labels*; phycoerythrin (PE)-labeled antibodies may be more sensitive in identifying weak expression of some antigens such as CD13, CD14, and CD33 on neutrophils than other fluorescent labels.[21]

Despite the lack of a homogeneous methodology to study mature and maturing cells in MDS, there is ample evidence of surface marker abnormalities on PB and BM cells in the majority of patients with MDS. Understanding the pattern of expression of surface antigens on normal maturing cells is an essential component for the optimum interpretation of surface marker abnormalities in MDS.

Complement receptors

CD35 (complement receptor 1, CR1, C3b and C4b receptor)

This antigen is normally expressed on erythrocytes, B-lymphocytes, a subset of T-lymphocytes, monocytes, eosinophils, neutrophils and follicular dendritic cells.[22] The density of CD35 molecules per cell is highest on neutrophils, monocytes and B-lymphocytes. CD35 appears relatively late during granulocyte development. Less than 1% of neutrophilic myelocytes, approximately 4% of neutrophilic metamyelocytes, 75% of neutrophilic bands, and virtually all segmented neutrophils express CD35.[22] This antigen is normally expressed before CD16.[23] In the resting state, PB neutrophils express only 5-10% of total cellular CD35 with the remainder being stored intracellularly on the membrane of the secretory granules which can be mobilized to the cell surface following activation.[22,24] As previously discussed, the expression of surface complement receptors is affected by the method of preparation including the anticoagulant used and the duration and temperature of incubation.[17] CD35 deficiency on PB neutrophils and monocytes, using the percentage of cells and FI, has been reported in 45-72% of MDS patients.[25-27] These studies have used the WL technique to avoid excessive manipulation of neutrophils. Another study, using DG for neutrophil and mononuclear cell separation, indicated a higher FI of CD35 on monocytes from low-risk but not from high-risk MDS patients compared to controls. This study showed no change in FI of granulocytes.[28] Moretti *et al.,*[8] using DG separation, confirmed both findings, i.e., the presence of a deficient population of neutrophils and increased FI of the entire neutrophilic population. This was seen only in high-grade MDS and correlated with defective *in vitro* neutrophilic functions such as locomotion and respiratory burst. Soluble CD35 was increased in sera from MDS patients but without being significantly correlated to surface CD35.[25] Bone marrow abnormalities of CD35 in MDS have not been studied. The prognostic significance of CD35 deficiency has not been studied.

CD21 (CR2, EBV receptor)

CR2 is expressed on B-lymphocytes and follicular dendritic cells.[22] Lymphocytes deficient in CD21 have been reported in three patients with MDS.[29]

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CD11b (CR3, receptor for C3bi)

Neutrophils, eosinophils, basophils, monocytes, a subset of lymphocytes, and natural killer (NK) cells normally express CD11b. Among these cells, granulocytes and monocytes have the highest antigen expression.[21,30] CD11b begins to be expressed at the early myelocyte stage, before the loss of CD33, and increases in intensity with maturation.[30,31] Similarly to CD35, intracellular CD11b is present on the membrane of the secretory granules. However, CD11b is also present on the membranes of the specific and gelatinase granules.[24] In normal BM and using the WL technique, the granulocytic gate contains < 20% CD11b-negative granulocytes.[32] Results of studying PB granulocytes and monocytes in MDS have been variable. Some investigators, using DG separation, indicated a normal percentage of cells expressing CD11b.[33] Yet, other investigators, using the WL technique, indicated deficiency of CD11b, by both FI and percentage of negative cells, in the majority of MDS cases.[7,25,34,35] In addition, neutrophils deficient in CD11b also displayed abnormal chemotaxis, superoxide release and aggregation. CD11b deficiency in PB occurred simultaneously with CD35 deficiency in 50% of cases.[25] On the other hand, some studies have indicated a higher FI in monocytes and granulocytes, particularly in high-grade MDS.[8,27,28,36] Most of these studies used DG separation for cell preparation. Moretti *et al*.[8] confirmed the presence of an increased number of CD11b-deficient neutrophils accompanied by a high FI of neutrophils. Bone marrow granulocytes, with the WL technique, showed CD11b deficiency in 41% of MDS patients. All these deficiencies were accompanied by a deficiency in CD16 (see below).[32] Blasts in the bone marrow in MDS have been consistently negative for CD11b.[32] The presence of less than 53% CD11b-positive cells among all marrow nucleated cells was reported in 54% of MDS, was associated with a higher risk of developing acute leukemia, and had no correlation with the percentage of BM blasts or FAB subtype.[19] Moreover, in a study of 84 patients with MDS, the presence of less than 70% CD11b-positive-neutrophils in PB was associated with poor survival.[7]

CD11c (CR4, receptor for C3bi, gp 150/95)

This antigen is normally expressed on monocytes, macrophages, granulocytes and a subset of lymphocytes. CD11c is expressed early in monocytic differentiation, before CD14 and CD11b, while in granulocytes it is detected at the myelocytic stage.[31,37] More than 90% of circulating neutrophils express CD11c.[33-35] In addition, circulating granulocytes and monocytes have significant intracellular stores. Therefore, activation of these cells is usually accompanied by an increased expression of surface CD11c.[15,37] Several studies on PB granulocytes and monocytes, using DG separation or the WL technique, showed no significant changes in MDS compared to controls.[25,27,28,33-35] Bone marrow cells have not been studied for CD11c expression in MDS.

Fc γ receptors

CD64 (FcγRI)

Peripheral blood monocytes, but not neutrophils, normally express CD64. In BM, myeloblasts and more mature granulocytic forms including a subset of neutrophilic bands express CD64 which is then completely lost in segmented neutrophils.[23,38] Monocytes from patients with MDS, both low- and high-risk groups, express CD64 with a stronger intensity compared to controls.[28] In addition, neutrophils in high-risk MDS patients may show persistent expression of CD64 on their surface.[27] The prognostic significance of CD64 changes in MDS has not been reported.

CD32 (FcγRII)

This antigen is normally present on PB neutrophils, monocytes, platelets and B lymphocytes. The majority of colony-forming units and all subtypes of granulocytic precursors express CD32.[38] Although one study showed no abnormality in CD32 expression on PB neutrophils and monocytes in MDS,[28] another study indicated a low intensity of CD32 expression on neutrophils in high-grade MDS with no reference to the prognostic implication of this finding.[27]

CD16 (FcγRIII)

This group includes a transmembrane-linked glycoprotein expressed on NK cells and macrophages termed CD16a, and a glycosyl-phosphatidyl-inositol (GPI)-linked glycoprotein termed CD16b which is preferentially expressed by neutrophils.[39,40] During granulopoiesis, CD16 appears late at the metamyelocyte stage.[30,31] PB monocytes are negative for CD16.[40] CD16 is located intracellularly on the membrane of the secretory granules, and as a result, its surface expression is upregulated following neutrophil stimulation.[18,24] Although some experiments suggested the loss of surface CD16 from neutrophils following stimulation,[16] this effect seemed to occur only in serum-free media and was not seen in the presence of plasma or serum.[18] The results of studying PB neutrophils for CD16 in MDS are variable. Some investigators indicated no abnormality in either the percentage of positive cells or the FI of CD16.[25,28,41] Others, using the WL technique, suggested a lower FI in the high-risk group of MDS patients,[27] or, with DG separation, a higher FI of all MDS patients.[36]

A recent report indicated that CD16 is deficient in PB neutrophils in 80% of the hypocellular subtype of MDS compared to a normal expression in normo- or hyper-cellular MDS subtypes.[42] CD16 deficiency in BM granulocytes has been reported in 64% of MDS patients.[32] Although myeloblasts from patients with acute myeloid leukemia (AML) express CD16 in 25% of cases, blasts in MDS have been consistently CD16 negative.[32,38]

No prognostic significance has been attached to abnormal expression of CD16.

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Adhesion-related antigens

CD62L (L-selectin)

This antigen is normally expressed on PB granulocytes, monocytes and a lymphocyte subpopulation, with the highest density of expression on basophils.[21] CD62L is maintained during granulopoiesis and monopoiesis while it is lost very early during erythropoiesis.[31] CD62L is released and shed from neutrophils following activation resulting in a decline in its surface expression.[15,43] Patients with low- and high-grade MDS show decreased FI of neutrophilic CD62L compared to controls. CD62L was lower in high-risk MDS compared to a low-risk group. The authors suggested that low CD62L expression may indicate *in vivo* activation of neutrophils or may indicate defective neutrophils in MDS.[27] The prognostic significance of these findings remains uncertain.

CD44

This antigen is normally present on granulocytes, T lymphocytes and to a lesser extent on monocytes and red blood cells.[44] Germinal center B cells are generally CD44 negative. CD44 is strongly expressed throughout granulopoiesis while it is downregulated with monocytic and erythroid maturation.[31] CD44 FI is reported to be increased in granulocytes from patients with high-risk MDS with an unreported clinical correlation.[28]

CD11a (leukocyte function antigen-1, LFA-1)/ CD18

CD11a is normally expressed on lymphocytes, granulocytes and monocytes while it is lost early during erythropoiesis.[31,45] CD18 is the β2 chain of leukocyte integrins that is associated with the α chains represented by CD11a, CD11b and CD11c. Several studies of PB neutrophils and monocytes in MDS indicated normal expression of CD11a.[25,28,33-35] One study, using FI, indicated a lower expression of CD11a on PB neutrophils in low- and high-risk MDS.[27] The results of CD18 are more variable. The expression of CD18 on PB neutrophils has been reported to be normal,[28,33,41] decreased,[27,35] or increased.[36] However, none of these studies discussed any clinical relevance.

CD54 (intercellular adhesion molecule-1, ICAM-1)

CD54 is normally present on bone marrow progenitors and is progressively lost during erythropoiesis at the stage of erythroblasts and during granulopoiesis at the stage of myelocytes. In addition, CD54 is strongly expressed by mature monocytes, and B cells while it is absent on terminally differentiated T cells.[46,47]

Neutrophils and monocytes from the peripheral blood of MDS patients did not express CD54 abnormally. Blasts from all patients with RAEB-t strongly expressed CD54 compared to those from only 34% of patients with *de novo* AML.[47]

Granulocytic and monocytic markers

CD13 (aminopeptidase N)

This antigen is strongly expressed on PB monocytes with a weaker expression on neutrophils, basophils and eosinophils.[21,48] The fluorescent intensity on monocytes, although high, is very variable.[21] However, some studies have considered CD13 as an immature marker which is expressed on <10% of BM mononuclear cells and is reported to disappear with neutrophil maturation.[19,48-52] The sensitivity of antibodies, particularly those labeled with PE, may explain the difference in these findings; PE-labeled anti-CD13 antibodies have allowed for more reliable detection of PB granulocytes and monocytes.[21,53] Moreover, CD13 is located intracellularly on the membrane of the neutrophilic secretory vesicles. This is why upregulation of surface CD13 accompanies neutrophil activation.[14,18,24] In fact, several studies have documented the greater expression of surface CD13 on PB neutrophils prepared by DG separation compared to the same specimens prepared by whole blood lysis.[14,15] Peripheral blood neutrophils in MDS displayed no abnormal CD13 expression.[25,26,54] As many as 90% of MDS patients show increased expression of CD13 in their marrow mononuclear cells suggesting a maturation shift towards immature forms.[20,49,50,55,56] Similar results were obtained using unfractionated bone marrow.[19] The increased expression of bone marrow CD13 has not been correlated with BM blast count[49,50] although the blasts are frequently positive for CD13.[32,57,58] Some studies have reported a much higher CD13 expression in high-risk groups than low-risk patients,[51,52,55] and in low-risk patients progressing to a higher-grade MDS.[49-51]

CD14 (endotoxin receptor)

Mature monocytes and some tissue macrophages normally express CD14. In addition, this antigen can be detected, although at a low intensity, on neutrophils and basophils using a more sensitive antibody labeling such as PE.[21,31] Approximately 30% of PB neutrophils weakly express CD14[48] while less than 10% of BM mononuclear cells are CD14 positive.[48,50,51] CD14 is GPI linked with an intracellular storage compartment on the membrane of the neutrophilic secretory vesicles.[24] Peripheral blood granulocytes, separated by DG, show aberrant increased expression of CD14 in 43% of patients.[59] Conversely, CD14 deficiency in PB monocytes has been reported in 32% of patients with MDS.[59] Bone marrow mononuclear cells from non-CMML patients show increased CD14 expression in approximately 50% of cases.[19,50,51] An unusual phenotype of BM granulocytes (CD14$^+$, CD66a$^+$) has been detected in occasional patients with MDS.[52] Although increased CD14 expression on BM mononuclear cells was more prominent in RAEB and RAEB-t, no independent prognostic significance has been reported.[19,50,51]

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CD15 (Lewis^x, 3-fucosyl-N-acetyllactosamine)

This antigen is normally present on granulocytes and monocytes. CD15 is expressed late in the myeloblast phase, is upregulated with maturation, and is slightly downregulated with terminal differentiation of granulocytes.[30,31] Intracellular storage compartments are present on the membranes of primary and secondary neutrophilic granules.[24,60] Stimulation of granulocytes with chemotactic peptides causes an increase in surface expression of CD15.[61] Several studies have indicated that there is no abnormal expression of surface CD15 on PB neutrophils in MDS.[25,28,36] However, one study, using two different CD15 antibodies, found deficient neutrophils in 26% of MDS patients and increased CD15 reactivity of monocytes in 42% of patients.[59] In BM, an increase of CD15-positive mononuclear cells was detected in 83% of MDS.[20] Blasts in BM have been consistently CD15 negative.[32] In addition to surface abnormalities, the intracellular component of CD15 has been found to be deficient in approximately 50% of MDS patients.[5] The anomalous increased CD15 positivity of BM mononuclear cells had no influence on prognosis.[20]

CD33 (gp67)

This antigen is restricted to hematopoietic cells and is normally present at high levels on monocytes, myeloid progenitors, myeloblasts, promyelocytes, myelocytes.[62,63] Although CD33 has been reported to be absent beyond the myelocytic stage, a recent study using PE-labeled antibodies reported a low level of expression on neutrophils, eosinophils and basophils.[21] The range of mononuclear cells which are CD33-positive in normal BM varies in different studies from 5-45%.[19,49,50,56,63] Some studies on PB neutrophils in MDS found no abnormality in CD33 expression while others found an increased number of CD33-positive neutrophils, particularly in RAEB and RAEB-t.[25,36,54] Depending on different definitions of normal ranges, 18-54% of MDS patients show increased expression of CD33 in their marrow cells suggesting a maturation shift towards immature forms.[19,20,50,56] Higher BM expression was most frequently detected in high-risk MDS patients.[51,55] Although the increased CD33 expression did not correlate with BM blast count,[49,50] it did correlate with the frequency of clonal cytogenetic abnormalities[49] and with a higher risk of disease progression.[20,52,51] However, the latter proved to be of no value using multivariate analysis.[20]

CD65

Granulocytes and monocytes normally express CD65.[40] One study reported no abnormal expression of CD65 on PB neutrophils or monocytes in MDS.[25]

CD66a (biliary glycoprotein, NCA-160)

CD66a is normally present on granulocytes, some tissue macrophages and bile canaliculi.[40] CD66a is expressed on myelocytes and more mature granulocyte forms while monocytes are CD66a negative.[52] CD66a is also present on the membrane of the neutrophilic specific granules, and surface expression may be enhanced following stimulation.[24,40] Bone marrow mononuclear cells from high-risk MDS patients have been frequently associated with increased immature granulocytic forms ($CD13^+$, $CD66a^-$) with occasional cases showing an aberrant immunophenotype ($CD14^+$, $CD66a^+$).[52] No prognostic significance of these findings was reported.

My8

This monoclonal antibody reacts with a myelomonocytic antigen normally expressed on monocytes and granulocytes at the promyelocytic stage and then increasing with neutrophil maturation. Approximately 75% of PB granulocytes and monocytes and approximately 35% of BM mononuclear cells express this antigen.[48] The vast majority of MDS patients have shown no abnormality in My8 reactivity in either PB or BM cells.[50,54] This antibody is not currently available for further studies.

NAT-9

This monoclonal antibody identifies an antigen normally expressed on more mature granulocytic cells from promyelocytes onwards and is not expressed on monocytes.[64] This antibody reacts with 45-65% of marrow mononuclear cells.[51] All MDS subtypes, except RARS have been associated with decreased BM expression of NAT-9 indicating increased immature forms. The clinical implications of this are not known.[51]

GPI-Anchored Antigens

Abnormalities in CD14 and CD16b expression have been described above.

CD24

This antigen is normally expressed on terminally differentiated granulocytes and precursor B lymphocytes and is downregulated with B-cell maturation.[65] Virtually all PB neutrophils express CD24.[53] One study reported no abnormal CD24 expression in PB granulocytes in MDS.[28]

CD66b (CD67, NCA-95)

CD66b is normally present on terminally differentiated granulocytes. Its expression has not been detected on non-hematopoietic cells.[40] Approximately 95% of PB neutrophils express CD66b.[53] CD66b is intracellularly located on the membrane of neutrophilic secondary granules, and surface expression is upregulated on stimulated or centrifuged neutrophils.[15,24,40] One study, using DG separation, indicated higher FI of CD66b expression on PB neutrophils from MDS patients than on those from controls with no significant difference between high- and low-risk groups.[28] Another study, using >1% CD66b-negative cells to define abnormal patients, reported CD66b deficiency (together with CD16) in PB neutrophils in 80% of patients with a hypocellular subtype of MDS despite

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a normal expression in hypercellular MDS.[42] The prognostic implications of these findings have not been studied.

CD87 (urokinase-type plasminogen activator receptor, u-PAR)

CD87 is normally expressed on monocytes and granulocytes while no expression can be detected on resting T and B cells, or red blood cells.[39] The intracellular pool is located on the membranes of the neutrophilic specific, gelatinase, and secretory granules and, as expected, surface expression is upregulated with neutrophilic stimulation.[24] The intensity of CD87 (M5 antigen) expression on granulocytes, with DG separation, is increased in high risk MDS while monocytes are associated with increased CD87 expression in both low- and high-risk MDS.[28] Despite these findings, all MDS patients studied were free of infection and the significance of increased CD87 expression remains uncertain.[28]

Stem cell antigens

CD34

Hematopoietic stem cells and endothelial cells normally express CD34. This antigen is expressed on 1-5% of normal bone marrow cells and 0.1-0.5% of PB cells.[13,66] CD34 antigen is capable of standing tissue fixation and processing. This is why there has been an excellent correlation between studies using flow cytometry on fresh tissue and those using immunohistochemistry on fixed tissue.[13] Peripheral blood granulocytes and monocytes of MDS patients had no abnormality when studied for CD34 surface expression.[25] However, CD34 (using $\geq$ 20% to define positivity) was increased in BM mononuclear cells in 34% of mostly high-grade MDS.[20] Blasts in the BM in MDS are frequently CD34 positive.[32,57,58] However, there was no correlation between CD34 positivity and blast cell count suggesting anomalous persistence of CD34 expression on maturing cells.[20] Increased CD34-positive cells (>1% of mononuclear cells) in PB has been associated with poor survival and higher risk of acute leukemic transformation.[67] Similarly, an increase in BM CD34-positive cells, as detected by both flow cytometry and immunohistochemistry, has been correlated with poor survival and increased risk of acute leukemic transformation.[20,58,68,69]

Cytokine receptors

CD116 (GM-CSF receptor)

Approximately 97% of neutrophils and 98% of monocytes in the PB of healthy individuals express CD116. The density of surface CD116 on monocytes is four times greater than that on neutrophils.[70] In normal BM, monocytic precursors express a high level of CD116 while granulocytic precursors express an intermediate level. The percentage of granulocytes and monocytes which are negative for CD116 is increased in PB and BM from MDS patients. This is accompanied by decreased antigenic density on the cell surface, as detected by FI, particularly in high-risk MDS.[70] The clinical significance of these findings has not been reported.

CD117 (C-kit, stem cell factor receptor)

This antigen is expressed on less than 1% of all marrow cells of healthy individuals including hematopoietic precursors and immature myeloid cells which are consistently negative for CD10 and CD15.[71] CD117 antigen has shown normal to increased expression on marrow progenitor cells in MDS patients.[72] The blasts from the majority of MDS cases are CD117 negative.[57] No studies to elucidate the significance of these findings have been performed.

Apoptosis-related surface markers

CD95 (APO-1, Fas antigen)

CD95 is normally expressed on PB neutrophils, monocytes, eosinophils, and memory T cells but is virtually absent on B lymphocytes, NK cells and virgin T cells.[39,73-75] Approximately 50% of normal BM mononuclear cells express CD95.[76] The intensity of CD95 expression in normal BM is higher on monocytes, followed by nucleated red blood cells, granulocytes, lymphocytes and blasts.[77] CD95 is usually absent on CD34-positive cells.[78] Fas expression is upregulated in BM in MDS with approximately 89% of BM mononuclear cells expressing CD95.[76] This upregulation is particularly expressed on CD34$^-$, CD33$^-$ and glycophorin-positive cells.[78] There has been no correlation between CD95 expression on a certain population of BM cells and the associated PB count i.e., on CD34-positive cells and the Bournemouth score, on CD33-positive cells and neutropenia, and on glycophorin-positive cells and the degree of anemia.[78] However, patients with low expression of CD95 on CD34-positive cells had more BM blasts.[78]

Fas-Ligand (Fas-L)

This is usually expressed on approximately 20% of normal BM mononuclear cells.

One study indicated that expression of Fas-L increased in MDS, occurring in approximately 40% of BM cells from such patients.[76]

Surface phosphatidylserine (PS)

The expression of PS, detected through binding to labeled Annexin-V, has been utilized as an early marker of apoptosis. PS expression in MDS was markedly increased compared to that in controls, confirming previous observations of markedly increased apoptosis in BM of MDS patients.[9]

There was no difference in PS expression on CD34-positive cells in MDS and normal bone marrow.[79] The high PS expression in MDS was correlated with unfavorable cytogenetics such as monosomy 5, monosomy 7, trisomy 8 or complex cytogenetic abnormalities.[79]

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CD45 isoforms

CD45RA is normally expressed on B cells, naive/resting T cells, and monocytes. During normal granulopoiesis, CD45RA is expressed on blasts and promyelocytes. However, this antigen is lost with further granulocytic maturation and is absent on PB neutrophils.[80] CD45RO is normally expressed on activated/memory T cells, a subset of B cells, monocytes and macrophages. Blasts from MDS patients have a higher expression of CD45RA than CD45RO (mean of 54.4 % vs 41.5%). The expression of CD45RO on blasts has the reverse pattern compared to that of CD45RA.[81] Increased BM memory T cells (CD4$^+$/CD45RO$^+$) and decreased naive T cells (CD4$^+$/CD45RA$^+$) have been reported in patients with RA and RAEB.[82]

HLA-DR

HLA-DR is normally expressed by early hematopoietic precursors. During the development of granulocytes and monocytes, HLA-DR is lost at the myeloblast stage while it is expressed throughout monocytic development.[83] Approximately 20% of BM lymphocytes express HLA-DR.[84] An increased percentage of HLA-DR-positive mononuclear BM cells has been reported in RAEB and RAEB-t while no significant changes have been found in patients with RA and RARS.[50,51] Hypocellular MDS is associated with a higher percentage of activated CD8 compared to that in normo- or hypercellular marrow and the overall activation is less than in aplastic anemia.[85] Using >10% of all marrow cells to define abnormal expression, Mittelman *et al.*[19] concluded that 70% of MDS patients have increased expression of this antigen. Blasts in BM have been consistently found to express HLA-DR.[32,57,58] Increased bone marrow HLA-DR expression was associated with increased risk of acute leukemic transformation.[19]

P-glycoprotein

P-glycoprotein (PGP) is a transmembrane glycoprotein encoded by the multidrug resistance gene. PGP is normally expressed by CD34-positive BM cells and gradually declines as these cells acquire the CD33 antigen.[86] Less than 1% of normal BM blasts are PGP-positive and all maturing granulocytes and erythroblasts are consistently negative. Circulating monocytes and 50-75% of circulating lymphocytes express PGP.[87] Approximately 50% of patients with MDS have increased PGP staining in BM blasts with a higher expression in high-risk MDS (approximately 60% of patients) compared to low-risk MDS (approximately 20% of patients).[87,88] Twenty-five to eighty percent of therapy-related MDS patients have shown increased PGP in BM blasts.[87,89] Increased PGP expression of BM blasts has been associated with an immature blast phenotype, a low blast peroxidase activity, a higher risk of acute leukemic transformation, and a worse response to anthracyclin-containing intensive chemotherapy.[87-89] Although one study suggested a relationship between increased PGP expression and abnormal karyotype, particularly of chromosome 7,[88] these findings could not be confirmed by other investigators.[87,89] However, the threshold used to define positive cases may have influenced the outcome since one study used more than 40% staining of BM blasts to define positive cases[88] while others used more than 5% blasts for the same purpose.[87,89]

Lymphocyte markers

T-cell markers

Most studies on PB of MDS patients indicate the common occurrence of lymphocytopenia accompanied by decreased CD2, CD3 and CD4 counts,[50,90-94] with only a small number of studies in disagreement.[82] Although the reported peripheral blood CD8 levels vary from decreased[49,91,93-95] to normal or increased,[50,92] the CD4:CD8 ratio has been consistently decreased or reversed; this ratio reversal is more pronounced in high-grade MDS.[91] The level of CD8-positive cells and the CD4:CD8 ratio have been inversely correlated with plasma levels of soluble interleukin 2 (IL-2) and the number of blood transfusions respectively.[50,96] Activated CD8 (DR$^+$) and activated cytotoxic lymphocytes (CD8$^+$/CD11a$^+$) cells are increased in PB in MDS.[82,85] Low CD3 and CD8 counts have been associated with acute leukemic transformation and poor survival[49,91,93] while low CD4 count has been predictive of increased risk of infection.[91] Anomalous CD3 expression on PB monocytes has been reported in a patient with CMML.[97]

The T-lymphocytes in BM of MDS patients displays limited changes including higher or lower CD3, lower CD4, decreased naive CD4, increased memory CD4, increased CD8/CD11a$^+$ cells and finally increased activated CD8, particularly in hypocellular MDS.[50,51,82,85]

B-cell markers

Using different methodologies to enumerate peripheral blood B-lymphocytes, such as B-cell associated surface antigens or surface immunoglobulin positivity, various results have been reported in MDS. Peripheral blood B-cells have been found to be normal,[82,93,98] decreased[50,94] or slightly increased.[95] As previously mentioned, deficiency in CD21, as identified by EBV receptors, was identified in some MDS patients.[29] The number of bone marrow B-lymphocytes has been reported to remain unchanged,[49,51,82] to be decreased[50] or to be increased[99] in MDS. Anomalous expression of CD20 on BM CD14-positive mononuclear cells has been reported in one case of CMML.[100] Increased bone marrow B-cells of >3% per 1000 nucleated BM cells in the marrow biopsy has been associated with poor survival.[99]

NK-cell markers

NK cells have been identified by a variety of anti-

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bodies, commonly those against CD16, CD56, and CD57. Peripheral blood NK cells in MDS cells have been reported to be normal,[85,91] increased[82] or decreased.[50,94] The absolute number of NK cells has been negatively correlated with the plasma level of soluble IL-2 receptor.[96] Despite reported quantitative and qualitative NK abnormalities in MDS, NK cells are polyclonal or, at most, oligoclonal with no monoclonality having been identified.[101]

The number of bone marrow NK cells has been found to be normal[49,85,99] or increased.[82] Blasts from PB and BM in high grade MDS may express CD56 which parallels a trend to poor survival and rearrangement of the mixed lineage leukemia gene.[102]

Erythroid-related antigens

Glycophorin A

This antigen is normally expressed at the erythroblast stage of erythroid development and is maintained throughout terminal red cell differentiation.[83] Surface glycophorin A mutation of the NN or NO variants has been identified on PB red cells in 43% of MDS patients with MN phenotype, but with no reported influence on prognosis.[103]

CD71 (transferrin receptor)

This antigen is normally expressed on erythroid committed stem cells, reaches its highest expression on early erythroblasts and is lost by the end of reticulocyte maturation.[83] Bone marrow erythroid precursors from MDS patients have a markedly decreased intensity of CD71 expression compared to those from normal marrow.[104]

Erythropoietin receptor (EPO-R)

One study indicated that EPO-R was normally expressed on BM mononuclear cells of MDS patients. However, the percentage of cells coexpressing EPO-R and CD34 in MDS patients was also found to be increased or decreased compared to that in control marrows. The response to EPO was independent of the percentage of these dually-positive cells.[72]

Platelet glycoproteins

CD42a (GPIX)

This antigen is exclusively expressed on platelets and megakaryocytes. Deficiency of CD42a on platelets in MDS results in an acquired Bernard-Soulier-type defect. Using flow cytometry, two populations of platelets were identified, one CD42a deficient and the other CD42a normal.[105]

Standardization and clinical application of surface marker studies in MDS

Methodology

The whole blood lysis technique should be the recommended method for studying mature or maturing granulocytes and monocytes in PB and BM in MDS. This procedure avoids excessive manipulation

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Table 1. Surface marker abnormalities of clinical significance in MDS.

Surface marker abnormalities of clinical impact

Antigen	*Cell type*	*Abnormality*	*Clinical impact*	*References*
CD11b	PB neutrophils	< 70% positive cells	worse survival	7
CD11b	BM-NC	< 53% positive cells	higher risk of AML	19
CD13	BM-NC, BM-MNC	increased expression	progression to HG MDS	50,51
CD33	BM-MNC	increased expression	progression to HG MDS	50,51
CD34	PB-MNC	> 1%	worse survival/higher risk of AML	67
CD34	BM-MNC	increased expression	worse survival/higher risk of AML	20,58,68,69
HLA-DR	BM-NC	> 10% positive cells	higher risk for AML	19
PGP	BM blasts	increased positive blasts	higher risk for AML/worse response to intensive chemotherapy	87,88
CD3	PB lymphocytes	decreased positive cells	worse survival/higher risk of AML	49,91,93
CD8	PB lymphocytes	decreased positive cells	worse survival/higher risk of AML	49,91,93
CD4	PB lymphocytes	decreased positive cells	higher risk of infection	91
CD19/CD22	BM-NC	> 3% positive cells	worse survival	99
CD56	PB/BM blasts	> 20% positive blasts	trend for worse survival	102

Surface markers associated with cytogenetic abnormalities

Antigen expression	*Associated cytogenetic abnormality*	
CD33 increased expression in BM-MNC	clonal cytogenetic abnormalities (non-specified)	49
PGP expression in >40% BM blasts	multiple, particularly of chromosome 7	88
PS increased expression in BM-NC	unfavorable cytogenetics (-5, -7, +8, complex)	79

Abbreviations: PB, peripheral blood; BM, bone marrow; NC, all nucleated cells; MNC, mononuclear cells; HG MDS, high-grade myelodysplastic syndromes; AML, acute myeloid leukemia; PS, phosphatidylserine; PGP, P-glycoprotein.

of cells which may induce mobilization of intracellular stores of numerous antigens to the cell surface and, therefore, may skew the results.

Diagnostic applications

Unless each laboratory establishes its own normal FI for each antigen on granulocytes and monocytes using its own instruments and preferred antibodies, the detection of negative cells could be an easier way in individual cases to diagnose MDS. Complement receptors, particularly CD35 and CD11b, are normally expressed by more than 95% of PB neutrophils and are the most common antigens to be deficient in MDS. Therefore, a deficiency in complement receptors may be easier to detect. The detection of such deficiencies in BM specimens may prove difficult. A BM with a left shift may have normal CD11b and decreased CD35, since the latter is a late-appearing antigen. We have been circumventing this problem by evaluating the BM aspirate material morphologically and by simultaneously studying other late-appearing antigens such as CD16b and CD87.

Clinical implications

Deficiency of complement receptors, such as CD11b, should alert the clinician to a higher likelihood of developing complications such as acute leukemia and of a worse survival. Similarly, increased percentage of bone marrow cells expressing early or immature markers such as CD 13, CD33, CD34 and HLA-DR may indicate a worse outcome and possible progression to a higher risk MDS or to acute leukemia. Moreover, abnormal lymphocytic percentages, such as low PB CD3, CD4 and CD8 or high BM CD19, have been correlated with poor survival and increased risk of both infections and acute leukemia. On planning anthracyclin-containing intensive chemotherapy, increased expression of BM PGP could denote a worse response to this regimen. The clinical significance of surface marker abnormalities in MDS is summarized in Table 1.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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