16q23-q24 (the same region as DHSt),[16] while others map to chromosome 2q35-q36, which may suggest the involvement of a heterodimer.[17]

Certain other conditions result in increased erythrocyte cation permeability, including sickle cell disease and the invasion of erythrocytes by the malaria parasite, *Plasmodium falciparum*.[18] The transporter of the cation leak in these conditions is not known; however, it is tempting to speculate that these leaks may also be conducted by disrupted or misfolded red cell proteins, as is the case for HSt. HSt represents a distinct group of disorders caused by mutations that convert band 3 or RhAG into cation conductors in the red cell membrane; the molecular bases of DHSt and FP remain elusive but may yet be found to result from mutations causing mis-folding in other multispanning membrane proteins. The latest report from Iolascon *et al.* identifies a new band 3 mutation resulting in HSt, accompanied by dyserythropoietic features, and raises the idea that tyrosine phosphorylation and associated signaling is altered in these cells. Further investigations of HSt at the molecular level should aid our understanding of the processes underlying the range of pathologies observed in this diverse group of conditions.

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Flow cytometry immunophenotyping for diagnosis of myelodysplastic syndrome

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The typical patient with myelodysplastic syndrome (MDS) presents with a normocytic or slightly macrocytic anemia that is refractory to treatment with folates and vitamin B12.[1] Many patients also have abnormal white blood cell and platelet counts, typically neutropenia and/or thrombocytopenia.

A rational approach to the diagnosis of myelodysplastic syndrome should be initially based on the exclusion of more common anemic disorders (Table 1). When anemia is frankly macrocytic, differential diagnosis should primarily include megaloblastic anemia. The real challenge is represented by the patient with normocytic anemia,

whose differential diagnosis should not only consider renal failure and chronic disease, but also less common conditions such as celiac disease.[2] Microcytic anemia is very rarely found in myelodysplastic syndromes and may be caused by somatic deletions of globin genes.

Examination of peripheral blood smear is mandatory in the initial work-up of any hematologic disorder, particularly of a myelodysplastic syndrome. This examination often reveals such morphological abnormalities as hypogranulated neutrophils with hyposegmented nuclei (pseudo-Pelger-Huët anomaly) and large platelets. Bone marrow aspiration is required for the assessment of dyserythropoiesis, dysgranulopoiesis, and dysmegakaryocytopoiesis, and for the enumeration of ringed sideroblasts and blast cells.[3]

While bone marrow biopsy may be avoided in elderly patients who are in any case only going to receive supportive care, it should be performed in the remaining patients due to its diagnostic and prognostic utility.[4] In fact, bone marrow biopsy may provide information about marrow cellularity, fibrosis and CD34$^+$ cell clusters. Hypoplastic MDS needs to be distinguished from both aplastic anemias and hypocellular acute myeloid leukemia.[5] Bone marrow fibrosis identifies a distinct subgroup of MDS with multilineage dysplasia, high transfusion requirement, and poor prognosis, while the presence of CD34$^+$ cell clusters is an independent risk factor for progression to acute leukemia.[4]

Cytogenetic abnormalities have a major role in the diagnosis of myelodysplastic syndrome and in risk assessment.[6,7] Fluorescence in situ hybridization (FISH) should complement conventional cytogenetics in particular cases. Specifically, FISH may improve the detection of deletion 5q31-q32 in patients with MDS without cytogenetic evidence of del(5q).[8,9]

The pathological hallmark of myelodysplastic syndrome is marrow dysplasia, which represents the basis of the World Health Organization (WHO) classification of these disorders.[10] This classification has been recently revised,[6,11] and provides clinicians with a very useful tool for defining the different subtypes of myelodysplastic syndrome. The combination of overt marrow dysplasia and clonal cytogenetic abnormality allows a conclusive diagnosis of MDS, but this is found in only a portion of MDS patients. In many instances, cytogenetics is not informative, and the diagnosis of MDS is based entirely and exclusively on morphological criteria. Diagnosis of MDS may be particularly difficult in patients with a normal karyotype or non-informative cytogenetics who do not have robust morphological markers, such as ringed sideroblasts or excess of blasts.

Flow cytometry immunophenotyping is a reliable method for quantitative and qualitative evaluation of hematopoietic cells, and not surprisingly has been evaluated as a potential diagnostic tool for myelodysplastic syndromes.[12-17] Despite many efforts, no one single simple immunophenotypic parameter has been proved to be diagnostic of MDS. Three articles in this issue of the journal provide additional observations in this field.

Ogata and co-workers[18] designed a flow cytometry protocol applicable in many laboratories, and verified its diagnostic utility in patients with low-risk myelodysplastic syndromes. The cardinal parameters were blasts, B-cell progenitors, myeloblast CD45 expression, and channel number of side scatter where the maximum number of granulocytes occurs. This protocol was able to discriminate between low-grade MDS without conventional markers (cytogenetic abnormalities, ringed sideroblasts) and nonclonal cytopenias with good specificity.

Goardon and coworkers[19] investigated whether reduced mean fluorescence intensity (MFI) of CD38 expression on CD34$^+$ cells could be used as a surrogate marker for abnormalities in the MDS CD34$^+$ compartment, and whether this may provide a single simple useful flow cytometric measurement diagnostic of MDS. They found that the examined immunophenotypic parameter diagnosed low-risk MDS with 95% sensitivity and 92% specificity, and concluded that it may be of value in the routine clinical diagnosis of MDS, especially in cases with a low blast count and normal karyotype.

The report by van de Loosdrecht *et al.*[20] describes the results of the first European LeukemiaNet (ELN) working conference on flow cytometry immunophenotyping in MDS. This article is a very comprehensive analysis of

Table 1. Diagnostic approach to a patient with myelodysplastic syndrome: standard and novel tools.

• The process starts with a differential diagnosis of cytopenia, most commonly of anemia.
• Morphological examination of peripheral blood and bone marrow smears according to the 2008 WHO criteria are the mainstay of MDS diagnosis.
• Bone marrow biopsy is important, as it provides clinically useful information on cellularity, fibrosis, and CD34$^+$ cells.
• Cytogenetic investigations have a major role not only in the diagnostic process, but also in risk assessment. The identification of a non-random chromosomal aberration makes diagnosis of MDS almost certain. FISH can be a useful complement to conventional cytogenetics in individual patients.
• Flow cytometry immunophenotyping may provide complementary information. The most common use is the assessment of blasts through immunophenotyping of CD34$^+$ cells: although discrepancies between this approach and morphological evaluation have been reported, the flow cytometry approach appears particularly useful for serial assessments in the individual patient. Pattern recognition strategies may complement morphological evaluation of dysgranulopoiesis. The development of monoclonal antibodies against mitochondrial ferritin might allow a reliable diagnosis of refractory anemia with ringed sideroblasts.
• Molecular studies might revolutionize the diagnostic process in the future. Based on the available evidence, the identification of somatic *TET2* mutations in circulating granulocytes allows a conclusive diagnosis of myeloid neoplasm. At present, this requires sequencing of whole gene. In addition, *TET2* mutations are found in 20-25% of MDS patients. However, other genes capable of determining clonal dominance may be identified in the future. Somatic mutations of *TET2* and other similar genes may eventually become a molecular marker of clonal myeloid neoplasm.

this topic, and provides detailed information on what is currently known in the field. The ENL group agreed that flow cytometry reports should always be descriptive in nature, with a statement that findings could be consistent with MDS. However, the group concluded that despite strong evidence for an impact of flow cytometry immunophenotyping in MDS, prospective validation of markers and immunophenotypic patterns are required against control patient groups, as well as further standardization in multi-center studies.

Standardization of flow cytometry in MDS may improve not only diagnosis of MDS, but also its prognostication.[21,22] However, it is not likely to revolutionize the approach to the MDS patient. This is more likely to happen with molecular markers such as mutant genes, as was the case with the myeloproliferative neoplasms. Indeed, recent papers report somatic mutations of *TET2* in about 20-25% of patients with MDS.[23,24] These mutations would cause clonal dominance of mutated stem cells, and predispose to the acquisition of additional mutations that determine the clinical phenotype. A new molecular era in the diagnosis of MDS might be starting.

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