Pathophysiology of Waldenström's macroglobulinemia

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Sixty-five years ago, Jan Waldenström described two patients presenting with oronasal bleeding and lymphadenopathy.[1] Both had manifestations of hyperviscosity syndrome, which was documented with an Ostwald viscometer. One had a cryoglobulin. Waldenström recognized that these patients had a disorder distinct from myeloma and that the cells found in their bone marrow were different from the plasma cells of myeloma. With the help of a colleague in Svedberg's laboratory, serum from these patients was examined in an analytical ultracentrifuge and found to contain a component with a rapid sedimentation coefficient (19S) and a calculated molecular weight of about one million. Waldenström postulated that this component was a preformed giant molecule rather than an aggregate of smaller subunits. The 19S molecule became known as macroglobulin.

Serum IgM is composed of mu (μ) heavy chains and κ or λ light chains and constitutes one of the five major classes of immunoglobulin in humans and many animal species. The serum concentration is normally about 1.5 mg/mL. The molecule contains approximately 12% carbohydrate and has a half-life in serum of 10 days. The molecular weight of the subunit is 185 kDa while that of the star-shaped pentamer (925 kDa) is similar to the figure Waldenström and his colleagues calculated in 1944. There are ten antigen binding sites per mole of IgM.[2] Multivalency enables the molecule to fix complement efficiently. IgM is the initial antibody formed after primary immunization.

Waldenström's macroglobulinemia (WM) is classified as an indolent form of B-cell non-Hodgkin's lymphoma known in the World Health Organization (WHO) classification as lymphoplasmacytic lymphoma. Infiltration with clonal lymphoplasmacytic cells, predominantly in the bone marrow, and an IgM monoclonal gammopathy are diagnostic findings.[3-5] The development of WM is preceded by an IgM monoclonal gammopathy of undetermined significance (MGUS) in most, perhaps all, patients.[6] The incidence of MGUS rises with increasing age. Transformation to overt WM or other malignant lymphoproliferative disorder occurs at a rate of 1.5% per year.

WM accounts for 1-2% of hematologic malignancies. The median age at presentation is 63 years and it is more common in males. Clinical manifestations are due to the monoclonal IgM protein, tissue infiltration by the lymphoplasmacytic cells, or both. Patients typically present with insidious weakness and mucous membrane bleeding. Visual disturbances and neurological complaints are common. Some patients have infections, dyspnea, and congestive heart failure. Physical examination may detect pallor, purpura, lymphadenopathy, hepatosplenomegaly (20%) and engorged retinal veins. The bone marrow contains variable numbers of pleomorphic lymphoid cells. Dutcher bodies may be seen as intracytoplasmic inclusions positive for periodic acid Schiff. Mast cell hyperplasia is common and may stimulate tumor cell proliferation and monoclonal IgM secretion. Blood smears show striking rouleau formation and occasional to frequent plasmacytoid lymphocytes which bear surface monoclonal IgM, usually κ, detected by flow cytometry. A typical churchspire peak is evident on serum protein electrophoresis and immunofixation electrophoresis can make the diagnosis by demonstrating the presence of monoclonal IgM, of which 80% with κ light chains. Multiple cytogenetic abnormalities have been described in WM but are not specific to this disease. Gene expression profiling has indicated that lymphoid cells of WM more closely resemble those of chronic lymphocytic leukemia than those of myeloma.[7] An International Prognostic Scoring System has been developed for WM to examine risk-adjusted therapy and assist with clinical trial comparisons.[8] Treatment of WM is beyond the scope of this editorial but an excellent review has recently appeared.[9]

The cell of origin

Genetic analysis of the V_H regions from patients with WM and IgM MGUS indicate that both develop from a post-germinal center cell that has undergone somatic hypermutation, possibly under the influence of antigen selection, but not isotype switching. Thus, WM and IgM MGUS arise from an IgM-expressing cell that transforms after cessation of somatic mutation, but without initiating switch events (Figure 1). Some unmutated monoclonal IgM can be found and may arise through a T-cell-independent mechanism.

The normal B-cell compartment is composed of cells that recirculate between primary and secondary lymphoid organs. The first B cells entering the recirculating pool from the bone marrow are "transitional B cells". In humans, these cells are $CD19^+$, $CD20^+$, $sIgD^+$, $sIgM^+$, $CD38^{++}$, $CD24^{++}$ and, as naïve B cells, express germline-encoded Ig variable region genes. Naïve B cells ($CD19^+$, $CD20^+$, $sIgD^+$, $sIgM^+$, $CD38^-$) are activated upon antigen encounter in association with antigen-specific T cells and dendritic cells. These B cells subsequently proliferate and differentiate in follicles and extrafollicular foci. Extrafollicular B-cell blasts give rise to short-lived plasma cells *in situ*. B-cell blasts within germinal centers ($CD19^+$, $CD20^+$, single isotype, $CD38^+$, $CD77^+$) acquire somatic mutations and are subject to selection and differentiation into centrocytes ($CD19^+$, $CD20^+$, single isotype, $CD38^+$, $CD77^-$) which will eventually give rise to conventional memory cells ($CD19^+$, $CD20^+$, single isotype, $CD38^-$, $CD27^+$) or cells of the plasma cell lineage such as plas-

mablasts ($CD19^+$, $CD20^-$, intracytoplasmic Ig^+, $CD38^{++}$, $CD27^{++}$, $CD138^{+/-}$) and plasma cells ($CD19^{+/-}$, $CD20^-$, intracytoplasmic Ig^+, $CD38^{++}$, $CD138^+$). The description of CD27 as a memory B-cell marker permitted the identification of an unswitched blood $CD19^+$ $CD20^+$ $sIgD^+$ $sIgM^+$ $CD27^+$ memory population in healthy individuals. This subset is thought to be related to splenic marginal zone B cells and seems to accumulate somatic hypermutation independently of T cells. Thus, cells expressing this phenotype are found already mutated in cord blood as well as in patients with X-linked hyper-IgM syndrome carrying mutations in the CD40 ligand that preclude germinal center formation, Ig isotype switching and conventional memory B-cell development.

The discovery that naïve B cells are the only peripheral B-cell subset expressing the ATP-binding cassette (ABC) B1 transporter enabled the finding of switched and unswitched memory B cells lacking the CD27 marker.[10] The origin of these cells, which have accumulated somatic mutations although at low level, is unclear. They could in fact represent a novel B-cell lineage that does not acquire CD27 expression upon antigenic stimulation, or derive from conventional marginal zone-like and/or post-germinal center memory B cells that have shed the CD27 molecule from their surface. CD27-negative memory B cells are expanded in patients with systemic autoimmune diseases such as systemic lupus erythematosus[11] and have been reported to be part of the malignant clonal spectrum of WM.[12] While the CD27-negative memory B-cell population in systemic lupus erythematosus seems to express IgG predominantly, in WM, these cells are characteristically IgM^+ and have lost sIgD expression. As in systemic lupus erythromatosus, they have undergone V_H gene somatic hypermutation. Shedding of surface CD27 might, in fact, be responsible for the described association between serum CD27 levels and tumor burden in patients with WM.[13] Furthermore, soluble CD27 stimulates expression of CD40L and APRIL on mast cells obtained from WM bone marrow.[14] These two tumor necrosis factor family members could then promote the activation and survival of surrounding B-cell clones and, therefore, contribute to disease pathogenesis.

Thus, WM could arise from a mature, memory-like B cell of either marginal zone or germinal center origin which does not undergo downstream switching but is able to acquire a lymphoplasmacytoid phenotype and

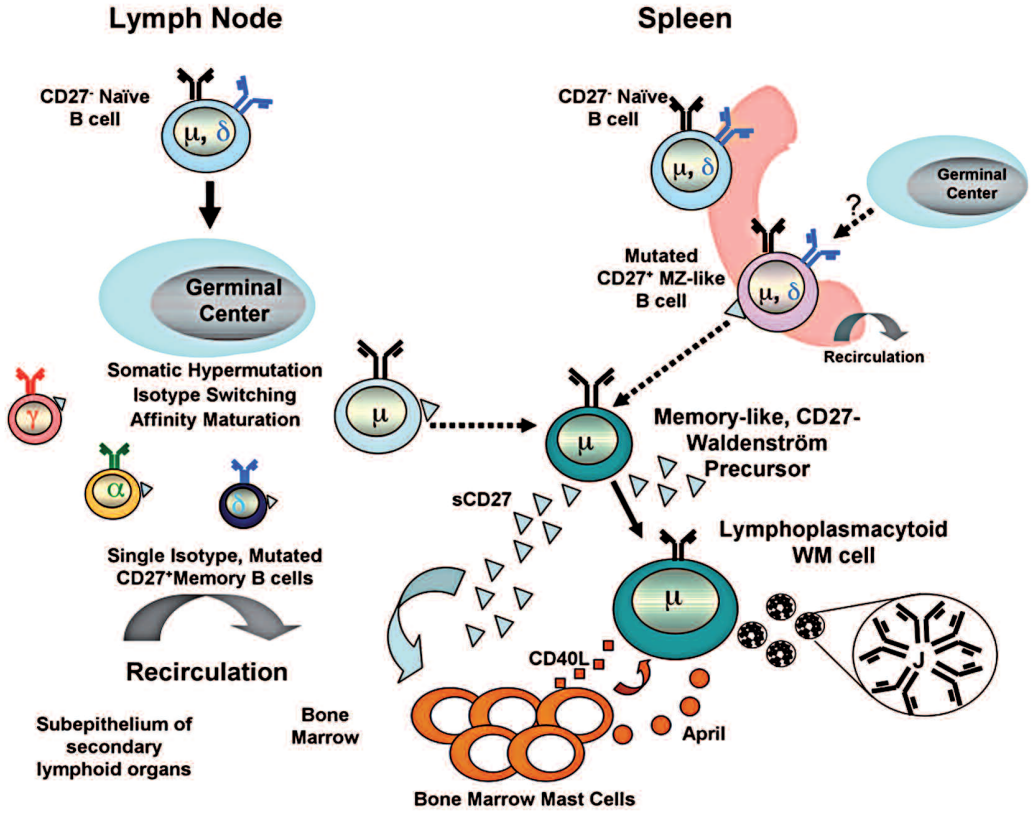


Figure 1. The WM cell could arise from a "memory-like", somatically mutated precursor that has lost classic memory markers such as CD27 due to shedding from the surface. Whether this memory precursor arises from a germinal center (single isotype memory B cell) or from a marginal zone-like, T-independent reaction (double isotype, IgM^+ IgD^+ B cell) remains to be determined, as both these cell types accumulate somatic mutations. Shedding of CD27 from the WM cell could have pathogenic implications. This molecule activates surrounding mast cells in the bone marrow to produce B cell activators and survival factors such as CD40L and APRIL that could drive the lymphoplasmacytoid differentiation of the WM clone. Some WM patients display decreased levels of IgA and IgG antibodies. Combined IgG and IgA deficiency could be an upstream event in WM, as these alterations can be found in relatives of WM patients. Whether this is due to a generalized defective switching process that sets the stage for malignant transformation of IgM-expressing clones remains to be elucidated. Revised and expanded from Stone *et al.*[21]

IgM secretory capacity. Whether this lymphoplasmacytoid differentiation is due to intrinsic alterations related to the malignant transformation event or is secondary to extrinsic factors, such as the expression of tumor necrosis factor family members on neighboring bone marrow mast cells, remains to be elucidated. Lymphoplasmacytoid WM cells retain expression of mature B-cell markers, such as CD20 and surface IgM, which are normally lost in healthy plasmablasts and plasma cells. Gene expression profiling further supports the understanding that these cells are closer in their transcriptome make up to malignant mature B cells (i.e. chronic lymphocytic leukemia) than to plasma cells (MM).[7] When compared to healthy mature B cells and chronic lymphocytic leukemia cells, WM cells over-express interleukin-6, a cytokine that promotes plasma cell differentiation.[7,15]

Hyperviscosity syndrome

Symptoms due to hyperviscosity occur in 20-30% of WM patients and may be the presenting manifestation. The symptoms and signs in patients with hyperviscosity syndrome include skin and mucosal bleeding, retinopathy with visual disturbances, and a variety of neurological disorders.[16,17] Cardiovascular manifestations are unusual. The fundoscopic exam is diagnostic, showing marked venous engorgement ("sausaging") in the retinal veins (Figure 2). Special ophthalmologic studies can detect lesser degrees of elevated viscosity.[18] Serum viscosity is normally 1.4 to 1.8 times that of water at 37°C. Hyperviscosity syndrome is unlikely unless the serum viscosity is above 4 centipoise (cp). As demonstrated originally by Waldenström, the Ostwald viscometer is a simple, reliable instrument for measuring and monitoring serum viscosity. The levels of viscosity that produce symptoms vary between patients. However, the viscosity level correlates closely with signs and symptoms in the same patient ("symptomatic threshold"), in part due to the range of intrinsic viscosity values of different Waldenström IgM proteins.[17] The treatment for hyperviscosity syndrome is plasmapheresis with or without concomitant chemotherapy. Plasmapheresis will usually reverse the signs and symptoms of the syndrome and is particularly effective in macroglobulinemia because 80% of IgM is intravascular. It is important to recognize hyperviscosity syndrome and treat it promptly. The diagnosis is made from fundoscopic examination of the eyes. Plasmapheresis and serial monitoring can be accomplished with the Ostwald tube.

It is usually not necessary that patients with hyperviscosity syndrome undergo plasmapheresis to the point at which their viscosity level is normal; it is usually sufficient that the levels are simply maintained below that patient's symptomatic threshold. Potential exceptions are patients with monoclonal macroglobulin antibodies that produce hemolytic anemia, cryoglobulinemia, neuropathy or other organ dysfunction. These patients may benefit from a more aggressive effort to maintain serum viscosity closer to normal.[17,19]

It has been reported that 30-70% of patients have a transient increase in IgM levels (flare) after rituximab therapy. Given the rise in IgM, plasmapheresis should be considered prior to rituximab if serum viscosity is greater than 3.5 cp or the IgM level greater than 5 g/dL. The use of combination chemotherapy regimens with rituximab may lessen the flare phenomenon.[17]

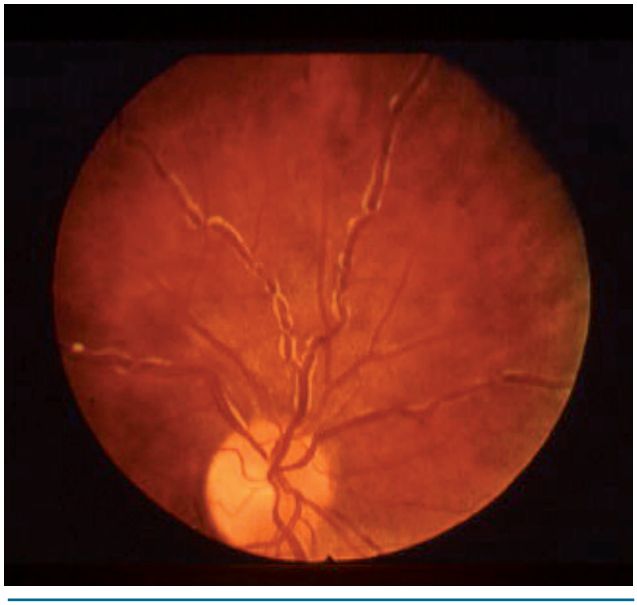
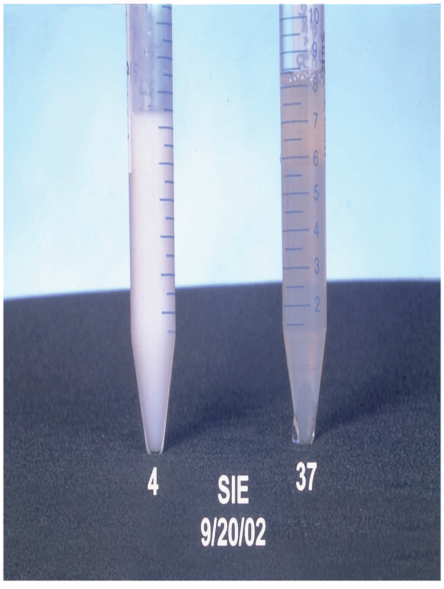


Figure 2. Fundoscopic appearance of a patient with WM and mixed cryoglobulinemia. Note the marked retinal venous engorgement and "sausaging." The white material at the edge of the veins may be cryoglobulin.[17]

Cryoglobulins

In his initial report, Waldenström noted that one of the two patients described had a demonstrated cryoprotein. Cryoglobulins are immunoglobulins that precipitate or gel at temperatures below 37°C and re-dissolve at 37°C (Figure 3).[20,21] The phase change is temperature-dependent and reversible. Single component cryoglobulins in WM patients are due to the temperature-sensitive insolubility of IgM and are usually concentration-dependent. Most mixed cryoglobulins are immune (antigen-antibody) complexes. Of 182 cryoglobulins analyzed at Baylor Sammons Cancer Center in Dallas, 69.8% were mixed IgM-IgG while 19.2% were single component, usually IgG.[17] In mixed cryoglobulins monoclonal IgM is an autoantibody to the Fc portion of polyclonal IgG. Mixed cryoglobulins are rheumatoid factor-positive and often present at a high titer. Primary binding between antigen and antibody is similar to that in other systems.[22] The cryoprecipitating phenomenon is caused by the immune complex, as separation of the reactants yields clear solutions. Cryoglobulins are important in the pathogenesis of symptoms and signs in patients. The manifestations may consist of weakness, purpura and arthralgias, acrocyanosis or other cold-sensitivity symptoms, cutaneous vasculitis, visual disturbances, and mucosal bleeding. Sometimes cerebral thrombosis, lymphadenopathy, hepatosplenomegaly, and renal disease, particularly proliferative glomerulonephritis may be present. Patients with cryoglobulinemia can, however, be asymptomatic. The thermal amplitude (the temperature at which the



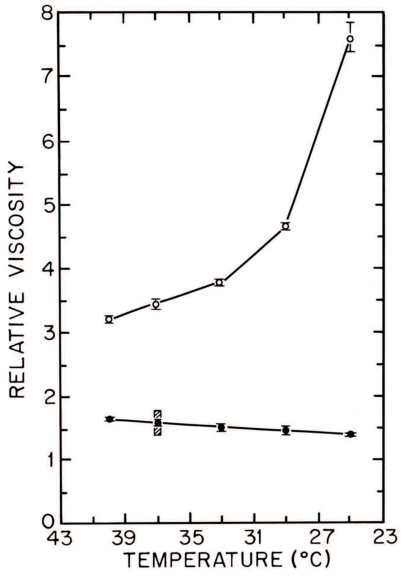


Figure 3. (A) Serum at 4°C and 37°C on September 20, 2002. The specimen was collected on March 30, 1970. This mixed cryoglobulin is from the patient whose fundus is shown in Figure 2.[20] (B) Temperature dependence of relative serum viscosity from a patient with macroglobulimemia and cryoglobulinemia. ○=patient's serum. ●=normal serum. Brackets indicate range of duplicate determinations. Bar indicates normal range (1.4-1.8) at 37°C.[22]

cryoglobulin forms) is probably more important than the cryocrit. The presence of cryoglobulinemia can significantly raise serum viscosity (Figure 3).[17,22] Hepatitis C viral RNA is sometimes found in mixed cryoglobulins. About 10% of patients with hepatitis C infection develop non-Hodgkin's lymphoma, especially WM. Cryoglobulinemia and lymphoma may improve after treatment with interferon. Hepatitis C infection is associated with a B-cell lymphoproliferative response of limited or overtly malignant degree, and thus appears significantly related to both mixed cryoglobulinemia and occasional development of WM or other non-Hodgkin's lymphomas.[17,20,21]

Chronic cold agglutinin disease

Cold agglutinins are anti-erythrocyte antibodies, usually IgM, that bind to the I or i red blood cell antigens at temperatures less than 37°C. The VH4-34 gene segment is necessary to encode anti-I specificity.[23] Many cold agglutinins have a high thermal amplitude so agglutination occurs in the 30-35 °C range and is clearly visible at room temperature. Cold agglutinins account for 30% of immunohemolytic anemias. Hemolysis is complement-mediated. Patients present with acrocyanosis, Raynaud's phenomenon, and/or hemolysis after exposure to cold. IgM in patients with cold agglutinin disease was the first monoclonal antibody described. The light chain type is almost always κ and cold agglutinin titers are elevated. Cold agglutinins are not generally cryoglobulins. Peripheral blood smears show clumping. Cold agglutinins are formed in approximately 50% of patients with *Mycoplasma* pneumonia and in some with viral infections. These can result in hemolytic anemia but the IgM antibodies in these patients are polyclonal and transient. By contrast, patients with cold agglutinin disease have monoclonal IgM and manifest hemoglobinemia and hemosiderinuria following cold exposure over the course of years. In some of these patients, the disease gradually evolves into typical WM.[24] Serum M-spikes tend to be lower than in other WM patients, partly related to the fact that the monoclonal IgM have antibody activity which causes hemolysis.[20] Thus individuals with cold agglutinin disease often present earlier than other WM patients.

Immunoglobulin M antibodies to neural antigens

Monoclonal IgM have been associated with peripheral neuropathy in patients with or without classic WM. The most frequently reported antigen recognized by these monoclonal IgM is myelin-associated glycoprotein (MAG). Other neural antigens have also been associated with polyneuropathies including several anti-gangliosides, sulfatide and tri-sulfated heparin disaccharide.[25] IgM binding to the above antigens does not overlap with rheumatoid factor-positive mixed cryoglobulins or cold agglutinins.[20]

Monoclonal macroglobulins with antibody activity that produce clinical manifestations (cold sensitivity with or without hemolytic anemia, polyneuropathy) are often recognizable (the patient tells you).[21] It is not clear, however, whether paraproteins from patients without autoimmune manifestations are functional antibodies. Clinical data support the role of chronic antigenic stimulation, which results in autoantibodies or antibodies to microbial antigens.[26,27] Foreign antigens such as bacterial lipopolysaccharide or hepatitis C virus may play a putative role in these disorders.[28] It has also been suggested that chronic antigenic stimulation may play a role in multiple myeloma.[29] It is likely that other antigens, not yet identified, are involved in selection of the malignant clone. Epitope-mediated antigen prediction technology may identify antigens reacting with paraprotein antibodies.[30] Identification of these antigens could provide insights into the process by which cells become malignant in WM.

Clinically significant manifestations produced by immune complexes certainly do occur in patients with autoreactive antibody syndromes. Such patients would be classified as having IgM MGUS were it not for the fact

the monoclonal IgM has antibody activity to the various respective autoantigens. Because of antigen-antibody interactions, patients with monoclonal autoimmune syndromes present at an earlier stage than those with typical WM who do not have evident antibody activity. Such monoclonal macroglobulin autoreactive antibodies, therefore, influence the clinical presentation and course of these patients.[21,28]

Familial clustering and hypogammaglobulinemia: novel clues to the pathogenesis of Waldenström's macroglobulinemia?

A familial predisposition to WM has been documented by several groups.[6,19,27] For example, a patient with Waldenström's macroglobulinemia for 37 years[17] had three children with IgM M-spikes and/or hypogammaglobulinemia. As many as 20% of WM patients have a family member with such immunoglobulin abnormalities. A study by Hunter *et al.*, reported in this issue of this journal, extends the hypogammaglobulinemia findings.[31] These investigators showed that approximately half their WM patients had hypogammaglobulinemia for the "uninvolved" immunoglobulins (IgG, IgA). These reduced levels of background normal immunoglobulins did not appear to predispose to bacterial infections, nor did they improve with effective treatment of the WM. The basis for hypogammaglobulinemia in these individuals is not clear but it is possible that the lower levels of IgG and IgA pre-date the development of WM. Thus, familial dysregulation of immunoglobulin production could be an event upstream of the malignant transformation in a way reminiscent of the role that chronic antigenic stimulation plays in MALT lymphoma. It will be of interest to see whether similarly low levels of IgG and IgA occur in the relatives of these patients. It will also be important to determine whether these individuals respond to antigenic challenge.

It has been known for decades that some patients with WM have reduced levels of the uninvolved immunoglobulins and impaired responses to immunization and pyogenic bacterial infections.[32] The data from Hunter *et al.*[31] show that low levels of background immunoglobulins in WM may reflect an underlying defect in these patients and provide a clue to pathogenesis. Further studies will help to unravel this "chicken-egg" dilemma.

In conclusion, there have been many advances in the understanding of WM in recent years.[5,19] Basic science has provided insights and a variety of novel agents are now available for the treatment of patients. With a disease as uncommon as WM, it is imperative that collaborators at institutions worldwide work together so that further progress can be made rapidly. International Workshops on WM as well as other meetings have contributed greatly to the dissemination of new and exciting information about this fascinating disease. Consequently, the outlook for WM patients has improved significantly.

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Iron overload in hematologic malignancies and outcome of allogeneic hematopoietic stem cell transplantation

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Iron overload is associated with organ toxicity and increased susceptibility to infection. Transfusional iron overload is a frequent complication of the therapy of hematologic malignancies, best exemplified in myelodysplastic syndrome (MDS). The risks of iron overload may be further increased in the context of hematopoietic stem cell transplantation (HSCT). Retrospective studies of patients who have undergone allogeneic HSCT have documented that pre-transplant red blood cell (RBC) transfusion-dependence and/or elevated serum ferritin levels (surrogate measures of iron overload) are associated with poorer post-transplant survival among patients with MDS and acute leukemias. A report by Alessandrino *et al.*,[1] published in this issue of this journal, adds to the debate by more precisely quantifying the impact of transfusional iron overload on allogeneic HSCT outcomes. In this perspective article, we review the literature related to iron overload, hematologic malignancies and allogeneic HSCT and suggest issues for future research.

Iron overload and hematologic malignancies

In benign hematologic disorders (e.g., thalassemia) iron overload due to increased iron absorption and hepcidin suppression, possibly from elevated growth differentiation factor 15 (GDF15) levels,[2] is common and results in toxicity and impaired survival. The toxicity of iron overload is related in part to intracellular generation of free radicals, resulting in oxidative damage and organ dysfunction (e.g. hepatotoxicity, cardiotoxicity, endocrine dysfunction), and in part to increased susceptibility to infection resulting from suppression of host immune responses, and from iron's role as an essential cofactor for pathogen growth.[3,4]

In hematologic malignancies, iron overload is a concern in those disorders associated with a relatively prolonged clinical course, ineffective erythropoiesis, increased iron absorption (possibly from elevated GDF15), and/or the need for multiple RBC transfusions. Although these features are best represented in MDS, in principle any hematologic malignancy associated with multiple RBC transfusions may result in clinically significant iron overload.

While there is some evidence that iron overload is of clinical relevance in acute leukemia,[5] the impact of transfusional iron overload has been best characterized in MDS. Malcovati *et al.* reported that RBC transfusion dependency was an independent negative prognostic factor for overall survival in MDS, and that increasing RBC transfusion load (total transfusions; transfusions per month) was associated with worse survival.[6] RBC transfusion dependency is now incorporated into a WHO-based prognostic staging system (WPSS) for MDS.[7] However, it remains uncertain whether impaired outcomes in MDS patients dependent on RBC transfusions are directly related to iron overload, or to worse MDS biology (e.g., increasing dyserythropoiesis). The association of RBC transfusion dependency with an increased risk of death or progression to acute leukemia suggests that more aggressive MDS biology may be a factor.[6] However, in this analysis, increasing iron overload (defined as a serum ferritin level >1000 ng/mL) was independently associated with worse survival even after discounting the known negative impact of RBC transfusion dependency, suggesting that iron overload may be an independent negative prognostic factor. Interestingly, the adverse impact of elevated ferritin levels was documented only in low-risk subtypes of MDS, such as refractory anemia, with or without ring sideroblasts, rather than in higher risk subtypes such as refractory anemia with multilineage dysplasia or refractory anemia with excess blasts. It is possible that aggressive disease biology trumps iron overload in patients with more advanced MDS. However, given the improved survival of MDS