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Abstract:
Waldenström Macroglobulinaemia (WM) is a rare, incurable, low-grade, B cell lymphoma. Symptomatic disease commonly results from marrow or organ infiltration and hyperviscosity secondary to IgM paraprotein, manifesting as anaemia, bleeding and neurological symptoms among others. The causes of the bleeding phenotype in WM remain obscure but are likely to involve several intersecting mechanisms. Evidence of defects in platelet function is lacking in the literature, but factors impacting platelet function and coagulation pathways such as hyperviscosity, vascular bed disturbances, abnormal haematopoiesis, acquired von Willebrand Factor (VWF) syndrome and cryoglobulinaemia may contribute to bleeding. Understanding the pathophysiological mechanisms behind bleeding are important, as common WM therapies including chemo-immunotherapy and Bruton's tyrosine kinase inhibitors, carry attendant bleeding risks. Furthermore, due to the relatively indolent nature of this lymphoma, most patients diagnosed with WM are often older and have one or more comorbidities requiring treatment with anticoagulant or antiplatelet drugs. It is thus important to understand the origin of the WM bleeding phenotype to better stratify patients according to their bleeding risk and thus enhance confidence in clinical decisions regarding treatment management. In this review, we detail the evidence for various contributing factors to the bleeding phenotype in WM and focus on current and emerging diagnostic tools that will aid evaluation and management of bleeding in these patients.

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Bleeding propensity in Waldenström Macroglobulinaemia: Potential causes and evaluation

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Abstract

Waldenström Macroglobulinaemia (WM) is a rare, incurable, low-grade, B cell lymphoma. Symptomatic disease commonly results from marrow or organ infiltration and hyperviscosity secondary to IgM paraprotein, manifesting as anaemia, bleeding and neurological symptoms among others. The causes of the bleeding phenotype in WM are complex and involve several intersecting mechanisms. Evidence of defects in platelet function is lacking in the literature, but factors impacting platelet function and coagulation pathways such as acquired von Willebrand Factor syndrome (AVWS), hyperviscosity, abnormal haematopoiesis, cryoglobulinaemia and amyloidosis may contribute to bleeding.

Understanding the pathophysiological mechanisms behind bleeding are important, as common WM therapies, including chemo-immunotherapy and Bruton's tyrosine kinase inhibitors, carry attendant bleeding risks. Furthermore, due to the relatively indolent nature of this lymphoma, most patients diagnosed with WM are often older and have one or more comorbidities, requiring treatment with anticoagulant or antiplatelet drugs. It is thus important to understand the origin of the WM bleeding phenotype, to better stratify patients according to their bleeding risk, and thus enhance confidence in clinical decisions regarding treatment management. In this review, we detail the evidence for various contributing factors to the bleeding phenotype in WM and focus on current and emerging diagnostic tools that will aid evaluation and management of bleeding in these patients.

Keywords

platelet

waldenström macroglobulinaemia

receptor

bleeding

Introduction

Waldenström Macroglobulinaemia (WM) is the clinical manifestation of lymphoplasmacytic lymphomas (LPL), which is a rare, low-grade, B cell lymphoma. WM is characterised by bone marrow infiltration with malignant cells and hypersecretion of immunoglobulin (Ig) M paraprotein. It constitutes less than 5% of all NHLs, with an incidence of ~0.3/100,000 cases/year.[1] Many patients are asymptomatic at the time of their initial diagnosis and do not require treatment.[2,3] However, approximately 30% of these patients are likely to need therapy within 2 years of diagnosis, and 80% within 10 years.[4] Indications for treatment are varied, but most commonly include constitutional symptoms, bone marrow or organ dysfunction, hyperviscosity and neuropathy. With an evolving treatment landscape in WM involving Bruton's tyrosine kinase (BTK) inhibitors, proteasome inhibitors and newer agents targeting BCL2 and CXCR4, understanding potential mechanisms of bleeding is important, as some of these therapies increase bleeding risk and necessitate the use of alternative agents in a personalised medicine approach.

Molecular basis of WM

WM patients carry one or more somatic genetic mutations within malignant lymphoplasmacytoid cells (Figure 1), which have been reported to be present in less mature lymphoid and hematopoietic progenitor cells in some cases.[5] Whether common WM mutations can be detected in megakaryocytes and platelets remains an open research question. Understanding the WM genomic landscape is important, not only because specific mutations can influence disease presentation, and treatment options,[3,6] but also because they can potentially affect platelet function (see below). The most common genetic defect in WM is a gain-of-function mutation within the myeloid differentiation factor (*MYD*) 88 gene resulting in a leucine-265 to proline (L265P) substitution within the cytoskeletal adaptor protein Myd88, detected in lymphoplasmacytoid cells

in over 90% of WM patients.[7-13] Of note, the MYD88^{L265P} transcript has also been detected in WM plasma cells, mature B lymphocytes, phenotypically normal B cell precursors and CD34^{+} haematopoietic precursor cells.[5] There are a number of other less common mutations now identified[14,15] and at least two distinct WM signature DNA methylation profiles specific for memory B cells or plasma cells.[16]

Through association with Toll-like receptors (TLR) and the Interleukin-1 receptor (IL-1R), Myd88 has an important role in coordinating innate immune cell responses. Cells expressing Myd88^{L265P} protein exhibit constitutive activation of TLR and IL-1R pathways, leading to nuclear translocation of transcription factor nuclear factor (NF) B and enhanced B cell proliferation and survival.[17,18] *MYD88^{L265P}* has also been detected in ~61% of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS) patients.[9,10,12,19] This mutation is likely to be an early oncogenic event in WM development, with IgM-MGUS acting as a precursor condition. However, additional genetic mutations likely contribute to WM onset.[14,20] Of note, Myd88 is expressed in platelets and megakaryocytes and is essential for appropriate TLR-driven platelet responses to viremia.[21] A link between Myd88 activation and platelet function will be discussed below.

Gain-of-function mutations in the gene encoding the C-X-C chemokine receptor (*CXCR*) 4 also occur in ~30% of WM patients.[22-24] CXCR4 engages with stromal derived factor (SDF) 1 to mediate the homing of cells to the bone marrow. Common activating mutations occur within serine-338 in the C-terminal region of *CXCR4*, which prevent CXCR4 internalisation following SDF-1 stimulation.[25] This leads to persistent CXCR4 activation and signalling via AKT, ERK and BTK pathways, and bone marrow myeloid cell migration, adhesion, proliferation and survival.[26,27]

The mutations in WM lymphoplasmacytoid cells, within genes involved in cell proliferation and survival, causes an over-proliferation of these cells, resulting in overproduction of IgM (Figure 1). Increased IgM contributes to the neurological and bleeding symptoms observed in WM, as correction of blood IgM levels often resolves symptoms.[24] WM malignant cell infiltration of the bone marrow leads to cytopenias, which can contribute to increased bleeding risk and predispose to infections. Whilst some of these mutations have been detected in lymphoid and hematopoietic precursors, it is not evident that the mutations arise in platelet-producing megakaryocytes or contribute to a bleeding phenotype. This review will explore the multifactorial nature of bleeding encountered in WM and explore considerations that may aid clinical decisions around therapy for these patients.

Waldenström Macroglobulinaemia patients can present with a bleeding phenotype

When Jan Waldenström first described WM in 1944, the symptoms in the two patients included oronasal bleeding.[28] Whilst bleeding is often a feature of the initial presentation of WM, bleeding symptoms usually resolve after therapeutic intervention, implying that WM disease aetiology does not directly affect platelet production and function. However, four studies have evaluated the frequency of bleeding in patients with WM before treatment intervention (Table 1). When considered together, approximately 17% of patients displayed bleeding symptoms,[2,29-31] with limited description of magnitude. Most other studies describe easy bruising and chronic oronasal bleeding as a common symptom of WM. It is worth noting that due to a paucity of large studies on this issue, evidence-based guidelines and standard of care management for treatment of

bleeding in these patients are lacking. Thus, reported treatment approaches are variable, with inconsistent reporting of key diagnostic information. Standardized reporting of laboratory findings and outcomes is also lacking.

Laboratory test abnormalities associated with bleeding in WM

The cause of the WM bleeding phenotype is unknown, but laboratory studies have identified several vascular and platelet abnormalities that often co-occur (Table 2). All of these sequelae are likely to contribute to bleeding symptoms in WM patients prior to initiation of treatment; however a number of these findings could occur as a result of IgM protein binding to and/or inhibition of coagulation factor(s) function.

Thrombocytopenia

Thrombocytopenia is a common occurrence in WM, often coincident with anaemia. One large WM study (n = 454) classified 18% of WM patients with thrombocytopenia.[32] Mechanisms by which thrombocytopenia arises are likely to be complex, potentially resulting from combinations that are autoimmune- and drug-mediated, as well as marrow infiltration which can cause overcrowding of hematopoietic stem cells and progenitors, resulting in disturbed megakaryopoiesis and thrombopoiesis.[33] Occasionally thrombocytopenia is secondary to peripheral platelet sequestration within the spleen (splenomegaly).[34] In isolation, thrombocytopenia rarely explains the observed bleeding, and the degree of bleeding is often out of step with the platelet count.

Acquired von Willebrand syndrome (AVWS)

Von Willebrand Factor (VWF) is a biorheological shear sensitive glycoprotein produced by the vascular endothelium and megakaryocytes. VWF plays a vital role in primary haemostasis by triggering GPIb-IX-V-mediated platelet activation and formation of adhesive bridge between platelets and vasculature at sites of endothelial injury. VWF is also a carrier protein for Factor VIII and contributes to fibrin clot formation.[35] Acquired von Willebrand syndrome (AVWS) is an uncommon disorder caused by a loss of high molecular weight VWF multimers, either by specific autoantibody-mediated destruction, absorption onto malignant cells, or increased fluid shear stress resulting in VWF multimer unfolding and proteolysis by ADAMTS-13.[36] In cancer, AVWS is reported in lymphoproliferative neoplasms and a number of myeloproliferative disorders.[37] AVWS occurs in 6% of WM patients, where incidence is strongly correlated with elevated IgM levels (30-60 g/L).[38] Symptoms include mucosal and GI bleeding, which generally resolve following WM therapy.[38] To mitigate bleeding, specific therapeutic approaches aim to increase VWF antigen levels (treatment with desmopressin and/or transfusion of FVIII/VWF concentrate), remove an offending autoantibody (plasmapheresis), or disturb destructive autoantibody functions, via transfusion of intravenous immunoglobulins (IVIg).[39,40] IVIg has been reported to successfully increase VWF/FVIII levels and reduce bleeding times in AVWS linked to IgG- but not IgM-MGUS.[41,42] Although the mechanism of action is unclear, these case reports suggest that IVIg could be a favourable therapeutic option for AVWS associated with WM.[39,41]

Hyperviscosity

Hyperviscosity, caused by the accumulation of large (~925-kDa), pentameric, positively-charged IgM paraprotein in the blood, is a classic manifestation of WM. The IgM proteins electrostatically interact with sialic acid-rich red blood cells, resulting in an agglutinating effect and contributing to increased viscosity.[43] Healthy individuals have around 1.5 g/L of IgM, of which 80% is intravascular,[44] and hyperviscosity emerges when IgM levels exceed 50-60 g/L.[44] Hyperviscosity causes the physical tearing of small venules from increased rheological drag,[46] and the suspected inhibitory coating of platelets by IgM protein, resulting in reduced platelet adhesion and aggregation.[47] The resulting symptoms include bleeding, vision problems and neurological symptoms, which occur in 13% of WM patients.[45] Acute management of hyperviscosity involves plasmapheresis, while longer term management with chemo-immunotherapy or targeted agents work by depleting the IgM producing cells in the marrow.

Haemostasis-inhibiting paraproteins

Circulating paraproteins have been reported to have VWF and Factor VIII-inhibitory activity in WM *in vivo*, caused by IgM[48-50] or IgG[51,52] antibodies. This has also been observed in other paraproteinemias, including multiple myeloma (MM), MGUS, lymphoma, chronic lymphocytic leukaemia (CLL), and amyloidosis.[53,54] Reports showed that the monoclonal IgM isolated from a WM patient demonstrated anti-platelet activity and immune thrombocytopenia (ITP) *in vivo,*[55,56] implying a derangement causing autoimmunity. Additionally, WM IgM cryoglobulins can suppress erythroid and granulocyte progenitor cells grown in culture *in vitro,*[57] and possibly megakaryocyte progenitor cell maturation, which could alter platelet quality and function.

Cryoglobulinaemia

Cryoglobulinaemia, where temperature-sensitive immunoglobulins form concentration-dependent insoluble aggregates that precipitate below 37°C, can occur in WM.[58] In WM, the cryoglobulins may form immune complexes, where monoclonal IgM antibodies bind to the Fc region of polyclonal IgG antibodies.[44] The symptoms of cryoglobulinaemia occur at varied cryoglobulin concentrations depending on the individual. These include purpura and mucosal bleeding, caused by the tearing of small blood vessels by the aggregates, and are observed in ~5% of WM patients.[44]

Amyloidosis

Amyloidosis can be associated with potentially life-threatening haemorrhage, by causing coagulation factor deficiency, hyperfibrinolysis, platelet dysfunction, angiopathy and/or vascular fragility.[59] Amyloidosis is characterised by the production of misfolded proteins, often monoclonal Ig light and/or heavy chains, which form insoluble amyloid fibrils, that accumulate and form plaques, leading to tissue and organ dysfunction. IgM-amyloidosis occurs in 7.5% of WM patients and is associated with a dramatic reduction in overall survival, from 12.1 to 2.5 years.[60] Bleeding occurs in 5-41% of amyloidosis patients, ranging from ecchymoses and purpura, to GI and post-procedural bleeding.[61-65] Bleeding results from increased vessel wall fragility from perivascular amyloid deposition, and/or from acquired FX deficiencies. Acquired FX deficiencies occur in 5-10% of amyloidosis patients and are caused by the absorption of FX and pentraxin-2 onto amyloid fibrils, particularly in

the spleen, resulting in direct FX removal from circulation and indirect FX internalisation by macrophages.[66] This could be corrected by splenectomy, chemotherapy treatment or autologous hematopoietic cell transplant.[66]

To summarise, WM patients display significantly elevated concentrations of serum IgM and one or more symptoms of blood hyperviscosity, cryoglobulinaemia, coagulation irregularities, thrombocytopenia, amyloidosis and bleeding. The factor(s) responsible for bleeding in untreated WM remains to be fully defined but bleeding is likely to be the result of a combination of all of the above observations.

Platelet dysfunction in WM

Platelets circulate throughout the vasculature and are the primary mediators of haemostasis (Figure 2). These are produced in the bone marrow by megakaryocytes, via controlled endomitosis. A healthy individual generally has a very stable platelet count; however the numbers of circulating platelets can range from 150-400 x 10^9 platelets/L. Thrombocytopenic individuals (<100 x 10^9 platelets/L) can have a heightened risk of bleeding, which significantly increases if the platelet count falls below 20 x 10^9 platelets/L. However, as mentioned earlier, bleeding can also occur in the absence of thrombocytopenia. Thus, the prediction of an imminent bleeding event should not rely solely on a low platelet count.[67-69] Besides the platelet count, platelet quality and functionality are critical components of an effective haemostatic response. This involves detection of injury-exposed collagen and other matrix proteins, and sensing alterations to local blood rheology. Platelets respond by adhering to a site of vascular injury and undergoing platelet activation. Platelet surface receptors coordinate this response[70] as well as the subsequent formation of a

thrombus (platelet aggregate or blood clot), which acts to seal the blood vessel, reduce blood loss and begin the process of wound repair (Figure 2).[71,72]

Platelet function is controlled by surface receptors and signalling pathway proteins

Levels of platelet receptors and their attendant surface densities mediate platelet responsiveness to molecular cues in the vasculature. Low receptor numbers and densities have been associated with bleeding in patients receiving mechanocirculatory support[73] and in trauma patients.[74] Further, a loss of platelet receptors, including glycoprotein (GP) Ib and GPVI,[75] and a diminution of platelet function prior to therapy[76] have been demonstrated in leukaemia patients. The molecular explanation for these losses remains undefined however may be linked to disturbances in bone marrow cellularity and megakaryocyte maturation.[77] As GPIb-IX-V and the collagen/fibrin receptor GPVI also contribute to efficient thrombus generation by binding thrombin and other coagulation proteins,[78-81] any alteration to normal levels of platelet adhesion receptors due to changes in megakaryocyte maturity could disrupt efficient thrombin generation at the platelet surface.

Bleeding has been observed in WM patients who do not have hyperviscosity, thrombocytopenia, cryoglobulinaemia or AVWS,[30,82] implying that there are other potential causes of bleeding in WM. It is possible that many WM patients do not display chronic bleeding symptoms but possess an underlying platelet lesion. When these patients experience vascular and haemostatic challenges such as surgery or trauma, or when the platelet lesion is present in combination with one or more common bleeding causes as outlined above, the platelet lesion can become more evident and unexplained bleeding complications ensue.[83,84] Studies specifically evaluating platelet function in WM would help

identify patients with reduced haemostatic capacity and enhanced bleeding risk, and this information could aid in clinical decisions regarding therapeutic approaches.

Standard WM therapies may accentuate the bleeding phenotype

WM is an incurable disease where the treatments aim to alleviate the symptoms and achieve prolonged remissions.[1-3] Treatment decisions in this regard are generally based on the symptoms, and diagnostic laboratory profile, and the availability of drugs and clinical trials.[85] For asymptomatic patients, a 'wait and watch' approach is routinely implemented.[3] Options for treatment of symptomatic WM patients include alkylating chemotherapy agents (bendamustine, cyclophosphamide), proteosome inhibitors (bortezomib, carfilzomib, ixazomib) or the first generation BTK inhibitor (ibrutinib), alone or in combination with rituximab. Newer therapeutic options include new irreversible, more selective BTK inhibitor oral therapeutic reagents (acalabrutinib, zanubrutinib) administered as a monotherapy, and emerging options include BCL2 antagonists (venetoclax).[85] Many of these treatments carry an attendant bleeding risk which can be enhanced in WM patients and will be discussed in this context below.

Rituximab

Rituximab is an anti-CD20 monoclonal antibody that specifically causes B cell depletion and thus acts to reduce IgM production.[86] Although rituximab is commonly used in combination with chemotherapy agents, if used as monotherapy, it can be associated with >25% rises in IgM (an

IgM flare), which can exacerbate hyperviscosity-related bleeding symptoms.[87] This risk is reduced when used in combination with other drugs. Alternatively, rituximab can be associated with acute thrombocytopenia, linked with cytokine release syndrome and complement activation.[88]

Alkylating chemotherapies

Alkylating chemotherapies, such as bendamustine and cyclophosphamide, have been used effectively as a frontline therapy in WM to kill rapidly dividing cells.[89] However, haematopoietic stem cells and their progenitor lineages are also sensitive to these therapies, resulting in cytopenias, particularly thrombocytopenia, and increasing the risk of bleeding.

BTK inhibitors

The B-cell receptor (BCR) signalling pathway is a central determinant of B-cell fate and function. This pathway is activated in WM, particularly in patients bearing the Myd88 mutation.[14] When an antigen binds to the BCR, this triggers BCR clustering and initiation of signal transduction via phosphorylation of BCR cytoplasmic tyrosine-based activation motifs (ITAMs). ITAM clustering enables recruitment of Src-family kinases, which serve to phosphorylate Syk and activate phosphoinositide 3-kinase (PI3K) δ. PI3K mediates the conversion of phosphatidylinositol 4,5 bisphosphate to phosphatidylinositol 3,4,5 triphosphate, which engages BTK. BTK phosphorylates phospholipase C (PLC) γ2, activating nuclear factor (NF) κB, nuclear factor of activated T cells (NFAT), and mitogen-activated protein (MAP) kinase pathways (Figure 3). These are all key elements of survival, development and cell proliferation pathways.

The clinical use of BTK inhibitors for the treatment of B-cell malignancies has grown remarkably, resulting in improved outcomes. At present, three different covalent irreversible BTKis are approved for clinical use. These inhibitors (ibrutinib, zanubrutinib, and acalabrutinib) all bind cysteine 481 within the ATP binding pocket of BTK with different avidities and selectivities, and serve to inhibit the phosphorylation of downstream kinases in the BCR signalling pathway, blocking B-cell activation. As many as 20 new BTK inhibitors are under development.[90] Nonetheless, bleeding remains a significant side effect that is associated with the use of these therapies.[91]

Platelet activation requires intra-platelet signalling, triggered by platelet receptor-ligand interactions, leading to enhanced platelet aggregation. A number of these pathways involve activation of BTK[92,93] (Figure 3), which is important in receptor signalling. Importantly there is redundancy in this pathway, as studies of genetically engineered mice or patients with X-linked agammaglobulinemia (XLA) with loss-of-function mutations to the *BTK* gene did not reveal any bleeding propensity, most likely due to compensatory signalling by Tec and other kinases.[94] BTK inhibitors interfere with BTK activity by irreversibly and covalently binding to the kinase domain,[95] preventing autophosphorylation of BTK, phosphorylation of PLCγ2, and of MAP kinases. Ibrutinib is less specific for BTK and can inhibit other tyrosine kinases such as TEC, ITK, TXK and EGFR, while the second-generation therapeutics zanubrutinib and acalabrutinib are more specific to BTK (Table 3). As BTK is critical for BCR signalling, there is a level of specificity achieved by targeting BTK. However, because of the broad kinase target spectrum of most tyrosine kinase inhibitors, they inevitably have off-target adverse events (Table 3). Platelets rely on tyrosine kinase activity for their activation. BTK is involved in platelet signalling pathways, mediated by GPVI, CLEC-2, GPIb and αIIbβ3, that enable platelet adhesion in flowing blood.[96] Platelet function has been shown to be inhibited in patients being treated with ibrutinib,[97,98] as well as a number of

other tyrosine kinase inhibitors.[99] Platelets from ibrutinib- but not zanubrutinib-treated patients showed reduced levels of GPIb-IX-V and αIIbβ3 and an ablation of platelet aggregate formation.[100]

Consistent with an off target effect of BTK inhibitors on platelets, a meta-analysis by Brown and colleagues found that ~40% of patients with B cell malignancies receiving ibrutinib experienced bleeding, with 4.4% experiencing major haemorrhage (> Grade III) (Table 3).[101] Reports of bleeding in WM patients as a result of ibrutinib treatment were slightly lower than calculated for all B cell malignancy patients,[96] with approximately 23% of WM patients on ibrutinib experienced bleeding (Table 4). Whilst differences in study design and definition of what constitutes a major or minor bleed may account for some of the disparity, it is likely that differences in disease pathogenesis may also contribute to incidence of bleeding across these different malignancies. Ibrutinib discontinuation reverses major toxicities observed in WM patients (bleeding, GI toxicity), despite causing IgM rebound in 73% and withdrawal symptoms (fever, body aches, night sweats, arthralgia, headaches) in 19%.[102,103]

Antiplatelet and anticoagulant therapies

WM affects an older demographic, many of whom have existing comorbidities. Treatments of these comorbidities can include antiplatelet or anticoagulant drugs, such as aspirin, clopidogrel, warfarin or direct oral anticoagulants, which carry an attendant bleeding risk. Treatment of WM patients with antiplatelet or anticoagulant therapies in combination with ibrutinib is common because of a significantly increased risk (~10%) of atrial fibrillation with ibrutinib.[24,104-110] The use of these therapeutics concomitantly elevates the bleeding risk. In one study of B cell

malignancy patients, major bleeding occurred in 3.7% of patients receiving ibrutinib monotherapy compared with 5.1% receiving ibrutinib in combination with antiplatelet reagents or anticoagulants.[101]

Taken together, WM treatments increase bleeding risk and treatments for WM comorbidities can enhance this risk. Therefore, it is important to strengthen our understanding of the molecular basis underlying the WM bleeding phenotype to accurately estimate bleeding risk, to adjust clinical management plans accordingly, minimise bleeding potential and improve patient quality of life.

Evaluating the bleeding phenotype in the diagnostic laboratory

Current approaches to WM patients with bleeding include coagulation testing, measurement of plasma viscosity, and specialised blood and platelet testing (Figure 4). Further platelet quality and function testing utilising research tools are emerging (Figure 4). However, these tests present several challenges which will be discussed below.

Laboratory tests assessing blood coagulation

Coagulation assays measuring PT/INR and aPTT are routinely performed.[15] It should be noted that these tests are influenced by several pre-analytical variables which can result in considerable intra- and inter-laboratory variation.[111] Variables include phlebotomy technique, anticoagulant volume based on patient haematocrit, sample mixing and centrifugation, sample transport conditions, delays in transport (over 4

hours), patient age, gender (females have increased levels of certain coagulation factors and anti-thrombin and reduced Protein S), physiological states (post-surgery, pregnancy) and drugs (anticoagulants, anti-platelets, anti-inflammatories).

VWF antigen and cofactor binding assays have value in the appropriate clinical context (bleeding phenotype).[15] VWF antigen assays measure VWF levels using a monoclonal antibody in a sandwich-based ELISA. The original VWF Ristocetin Cofactor binding assay (VWF:RCo) remains the gold standard method to measure VWF activity in plasma, by evaluating donor platelet agglutination following ristocetin-mediated VWF unfolding and binding to platelet-GPIbα, using LTA. Unfortunately, this method is insensitive at VWF levels below 20 U/dL, and subject to variation based on the source of ristocetin, variation in donor platelets and the presence of VWF A1 domain mutations resulting in poor ristocetin-VWF binding.[112] Newer versions of the VWF:RCo assay address sensitivity limitations, for example through a chemiluminescence-based method which directly evaluates VWF binding to magnetic particles coated with recombinant GPIbα in an active configuration (removing the requirement for ristocetin). Additionally, the VWF collagen-binding assay analyses VWF multimers by measuring the preferential binding of high-molecular weight VWF multimers to collagen, using an ELISA.[113] This assay has been shown to be more sensitive, reproducible and less variable than the VWF:RCo assay, improving discrimination between functional and non-function VWF.[114] VWF multimer analysis by gel electrophoresis is clinically informative but challenging to perform and not widely available as a diagnostic assay.

Thrombin generation assays measure the rate and ‘haemostatic potential’ of plasma for thrombin generation, via the cleavage of a quenched synthetic fluorogenic or chromogenic substrate. Assays require calibration, and although several commercial kits exist, limitations

including a lack of standardisation and reference values across laboratories have been highlighted and are being addressed.[115,116] Thrombin generation assays are becoming more prevalent, but will require additional clinical trials with well-defined endpoints to fully determine utility. To date, these assays have been used to monitor anticoagulant or antiplatelet therapy,[117] and abnormal measurements have been associated with bleeding in patients with rare inherited coagulation disorders such as haemophilia,[118] and the risk of venous thromboembolism recurrence.[119] Capturing the contribution of blood cells to thrombin potential however, remains a clear gap in clinical applications of this assay.[120] Platelets accelerate the initiation of thrombin production via provision of membranes bearing phosphatidylserine and receptors that interact with coagulation proteins (GPIb-IX-V complex, GPVI and IIb 3 amongst others),[72] as well as the release of granule contents. Platelets, erythrocytes, leukocytes and the endothelium are all likely to modulate thrombin potential *in vivo*. Further, in the setting of pathologies with high paraprotein levels such as WM, the degree to which high levels of plasma proteins interfere with normal production of thrombin remain to be determined.

Laboratory tests assessing platelet function

Both light transmission aggregometry (LTA) and the Platelet Function Analyser (PFA-100 and PFA-200)[121] are established diagnostic platelet function tests. Whole blood impedance aggregometry (Multiplate) is an emerging tool,[122] and together with the PFA-100/200, these techniques enable rapid screening of platelet responses to physiological agonists (ADP, thrombin, collagen). These tests are influenced by the same pre-analytical variables as the PT and aPTT tests and require platelet counts to be above 100 x 10^9/L, so are not suitable for thrombocytopenic

patients. The LTA also requires a high sample volume, and elevated levels of bilirubin and lipids increase plasma turbidity and affect LTA data. The PFA-100/200 has the advantage of incorporating fluid shear stress into the assay, and so provides a readout that is more physiological, but remains dependent on an aperture closure time and does not evaluate platelet secretion defects.[123] All of these tests lack sensitivity to changes in receptor levels and can discriminate only certain platelet function disorders.[124]

Laboratory tests assessing both blood coagulation and platelet function

Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are simple, automated, highly sensitive, emerging viscoelastic tests that provide a global assessment of haemostasis, and are widely utilised in massive transfusion and acute bleeding scenarios.[125] Parameters, including time to clot initiation, rate of clot formation, clot firmness and strength, and clot lysis time are quantified, and the contribution of platelets to clot parameters can also be gleaned.[126] Oncologic diseases can cause coagulopathic states that may be identifiable by TEG or ROTEM, however more work is required to evaluate the utility of these tests for assessing haemostasis beyond surgical bleeding.[125] Further, ROTEM and TEG are not yet standardised for evaluation of thrombocytopenic samples, which may be relevant to patients with haematological diseases, particularly those receiving treatment. There is no data to date specifically evaluating whole blood clotting using TEG or ROTEM in WM.

In summary, evaluating platelet function and coagulation in WM patients presents several challenges. First, none of the standardised platelet functional assays can evaluate platelets in thrombocytopenic WM patients. Second, evaluation of platelet receptor levels, which govern platelet function and are often diminished in haematological malignancies,[84] is not routinely evaluated by flow cytometry. This is due to lack of standardised routine protocols for platelet flow cytometry and lack of clear understanding of its clinical implications. Finally, none of these assays evaluate platelet function under conditions that replicate vascular shear rates found in flowing blood.

Future directions

In the research laboratory setting, several additional techniques can be used to evaluate platelet function. The bone marrow microenvironment is disrupted in WM, contributing to the initiation and propagation of WM lymphoplasmacytoid cells and likely disturbing megakaryocyte maturation and platelet production. Therefore, megakaryocyte and platelet flow cytometry can be used to evaluate the levels of receptors (αIIbβ3, GPIbα, GPVI), as well as extent of platelet activation (P-selectin, active IIb 3) on circulating platelets. Platelet flow cytometry has the advantage of remaining viable even when the platelet count is extremely low, and useful data can be gathered on chemically fixed samples, meaning samples can be stored for short periods. Levels of shed receptor ectodomains can be quantified by enzyme-linked immunosorbent assay (ELISA)[127] and microfluidic systems can be used to quantify platelet adhesion to immobilised substrates (collagen, fibrinogen, fibrin) under shear, providing direct readouts of platelet function under conditions that mimic a range of vascular rheological conditions.[128-130]

The generation of thrombin and fibrin as part of the coagulation pathway plays a crucial role in the securing of platelet aggregates across sites of blood vessel injury. Insufficient levels or defective coagulation factors can lead to formation of an unstable thrombus. Whole blood coagulation, evaluated by ROTEM or TEG, assess coagulation throughout all phases of clot formation, triggered via extrinsic or intrinsic coagulation pathways. Whilst these parameters have not been previously evaluated in WM patients, it would be of interest to compare samples from newly diagnosed individuals with those on different therapies and ascertain whether a platelet defect can be determined. Additionally, it might be of value to evaluate the effect of WM plasma, particularly from patients with high levels of IgM and hyperviscosity, in mixing experiments using healthy donor plasma-depleted blood to assess the effect of elevated IgM on whole blood coagulation.

Besides enhancing the availability of these tests, it will also be important to define the situations in which these tests will be most helpful. As information on the platelet lesion and more broadly, the haemostatic defects in WM emerge, existing diagnostic tools such as ROTEM or TEG may become more widely applied. Whilst platelet flow cytometry and testing for platelet activation markers using ELISAs remain distant from the diagnostic laboratory, these additional new approaches can potentially be developed and incorporated into diagnostic algorithms and may help guide therapy decisions in patients with compromised haemostatic pathways.

Finally, evaluation of WM-related genes within the megakaryocytic progenitor populations has not yet been explored. As platelets and megakaryocytes express both Myd88 and CXCR4,[131] the prevalence of WM-related gene mutations in the megakaryocytes and megakaryocyte progenitor populations should be evaluated. Platelet α-granules possess functional membrane-bound CXCR4 as well as SDF-1. Activating

CXCR4 mutations in megakaryocytes, like the *CXCR4*^{WHIM} mutation, prevent CXCR4 internalisation by platelets, following SDF-1 stimulation.[25] This may impair platelet aggregation, thromboxane A_2 production and dense granule secretion,[132] and could contribute to a bleeding phenotype.

Concluding remarks

With the advent of BTK inhibitors as efficacious and routine therapies for WM, it is important that patients are evaluated and monitored continually for bleeding propensity. By applying new approaches complemented by sensitive research-based techniques (flow cytometry, thrombin generation assays, platelet spreading assays), in combination with megakaryocyte-specific genetic approaches to evaluate common WM mutations, it may be possible to stratify WM patients for bleeding risk based on platelet functionality. One or more of these techniques could be integrated into routine testing for WM patients at diagnosis and then during treatment, particularly in patients who present with an elevated risk for bleeding.

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References:

1. Talaulikar D, Tam CS, Joshua D, et al. Treatment of patients with Waldenstrom macroglobulinaemia: clinical practice guidelines from the Myeloma Foundation of Australia Medical and Scientific Advisory Group. Intern Med J. 2017;47:35-49.
2. Garcia-Sanz R, Montoto S, Torrequebrada A, et al. Waldenstrom macroglobulinaemia: presenting features and outcome in a series with 217 cases. Br J Haematol. 2001;115:575-582.
3. Treon SP. How I treat Waldenström macroglobulinemia. Blood. 2015;126:721-732.
4. Bustoros M, Sklavenitis-Pistofidis R, Kapoor P, et al. Progression Risk Stratification of Asymptomatic Waldenstrom Macroglobulinemia. J Clin Oncol. 2019;37:1403-1411.
5. Rodriguez S, Celay J, Goicoechea I, et al. Preneoplastic somatic mutations including MYD88(L265P) in lymphoplasmacytic lymphoma. Sci Adv. 2022;8:eabl4644.
6. Treon SP, Meid K, Hunter ZR, et al. Phase I study of Ibrutinib and the CXCR4 antagonist Ulocuplumab in CXCR4 mutated Waldenstrom Macroglobulinemia. Blood. 2021.

7. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med. 2012;367:826-833.
8. Gachard N, Parrens M, Soubeyran I, et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia. 2013;27:183-189.
9. Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. Blood. 2013;121:2051-2058.
10. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. Blood. 2013;121:2522-2528.
11. Landgren O, Staudt L. MYD88 L265P somatic mutation in IgM MGUS. N Engl J Med. 2012;367:2255-2256; author reply 2256-2257.
12. Jimenez C, Sebastian E, Chillon MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenstrom's macroglobulinemia. Leukemia. 2013;27:1722-1728.
13. Ansell SM, Hodge LS, Secreto FJ, et al. Activation of TAK1 by MYD88 L265P drives malignant B-cell Growth in non-Hodgkin lymphoma. Blood Cancer J. 2014;4:e183.
14. Hunter ZR, Xu L, Tsakmaklis N, et al. Insights into the genomic landscape of MYD88 wild-type Waldenstrom macroglobulinemia. Blood Adv. 2018;2:2937-2946.

15. Maqbool MG, Tam CS, Morison IM, et al. A practical guide to laboratory investigations at diagnosis and follow up in Waldenstrom macroglobulinaemia: recommendations from the Medical and Scientific Advisory Group, Myeloma Australia, the Pathology Sub-committee of the Lymphoma and Related Diseases Registry and the Australasian Association of Clinical Biochemists Monoclonal Gammopathy Working Group. Pathology. 2020;52:167-178.
16. Roos-Weil D, Giacopelli B, Armand M, et al. Identification of 2 DNA methylation subtypes of Waldenström macroglobulinemia with plasma and memory B-cell features. Blood. 2020;136:585-595.
17. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia. Blood. 2013;122:1222-1232.
18. Deguine J, Barton GM. MyD88: a central player in innate immune signaling. F1000Prime Rep. 2014;6:97.
19. Landgren O, Tageja N. MYD88 and beyond: novel opportunities for diagnosis, prognosis and treatment in Waldenstrom's Macroglobulinemia. Leukemia. 2014;28:1799-1803.
20. Sewastianik T, Guerrera ML, Adler K, et al. Human MYD88L265P is insufficient by itself to drive neoplastic transformation in mature mouse B cells. Blood Adv. 2019;3:3360-3374.
21. Banerjee M, Huang Y, Joshi S, et al. Platelets endocytose viral particles and are activated via TLR (Toll-like receptor) signaling. Arterioscler Thromb Vasc Biol. 2020;40:1635-1650.

22. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood. 2014;123:1637-1646.

23. Poulain S, Roumier C, Venet-Caillault A, et al. Genomic landscape of CXCR4 mutations in Waldenstrom Macroglobulinemia. Clin Cancer Res. 2016;22:1480-1488.

24. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. N Engl J Med. 2015;372:1430-1440.

25. Dotta L, Tassone L, Badolato R. Clinical and genetic features of Warts, Hypogammaglobulinemia, Infections and Myelokathexis (WHIM) syndrome. Curr Mol Med. 2011;11:317-325.

26. Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. Leukemia. 2015;29:169-176.

27. Roccaro AM, Sacco A, Jimenez C, et al. C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. Blood. 2014;123:4120-4131.

28. Waldenström J. Incipient myelomatosis or «essential« hyperglobulinemia with fibrinogenopenia — a new syndrome? Acta Medica Scandinavica. 1944;117:216-247.

29. Gertz MA, Merlini G, Treon SP. Amyloidosis and Waldenstrom's macroglobulinemia. Hematology Am Soc Hematol Educ Program. 2004:257-282.

30. Merchionne F, Procaccio P, Dammacco F. Waldenstrom's macroglobulinemia. An overview of its clinical, biochemical, immunological and therapeutic features and our series of 121 patients collected in a single center. Crit Rev Oncol Hematol. 2011;80:87-99.
31. Zangari M, Elice F, Fink L, Tricot G. Hemostatic dysfunction in paraproteinemias and amyloidosis. Semin Thromb Hemost. 2007;33:339-349.
32. Buske C, Sadullah S, Kastritis E, et al. Treatment and outcome patterns in European patients with Waldenstrom's macroglobulinaemia: a large, observational, retrospective chart review. Lancet Haematol. 2018;5:e299-e309.
33. Kuter DJ. Managing thrombocytopenia associated with cancer chemotherapy. Oncology (Williston Park). 2015;29:282-294.
34. Kolikkat N, Moideen S, Khader A, Mohammed TP, Uvais NA. Waldenstrom's Macroglobulinemia: A case report. J Family Med Prim Care. 2020;9:1768-1771.
35. Gardiner EE, Andrews RK. Structure and function of platelet receptors initiating blood clotting. Adv Exp Med Biol. 2014;844:263-275.
36. Mital A. Acquired von Willebrand Syndrome. Adv Clin Exp Med. 2016;25:1337-1344.
37. Federici AB, Rand JH, Bucciarelli P, et al. Acquired von Willebrand syndrome: data from an international registry. Thromb Haemost. 2000;84:345-349.
38. Castillo JJ, Gustine JN, Meid K, et al. Low levels of von Willebrand markers associate with high serum IgM levels and improve with response to therapy, in patients with Waldenström macroglobulinaemia. Br J Haematol. 2019;184:1011-1014.
39. Franchini M, Mannucci PM. Acquired von Willebrand syndrome: focused for hematologists. Haematologica. 2020;105:2032-2037.

40. Boros P, Gondolesi G, Bromberg JS. High dose intravenous immunoglobulin treatment: mechanisms of action. Liver Transpl. 2005;11:1469-1480.

41. Abou-Ismail MY, Rodgers GM, Bray PF, Lim MY. Acquired von Willebrand syndrome in monoclonal gammopathy - A scoping review on hemostatic management. Res Prac Thromb Haemostas. 2021;5:356-365.

42. Dicke C, Schneppenheim S, Holstein K, et al. Distinct mechanisms account for acquired von Willebrand syndrome in plasma cell dyscrasias. Ann Hematol. 2016;95:945-957.

43. Javadi E, Deng Y, Karniadakis GE, Jamali S. In silico biophysics and hemorheology of blood hyperviscosity syndrome. Biophysical Journal. 2021;120:2723-2733.

44. Stone MJ. Waldenstrom's macroglobulinemia: hyperviscosity syndrome and cryoglobulinemia. Clin Lymphoma Myeloma. 2009;9:97-99.

45. Castillo JJ, Treon SP. Initial Evaluation of the Patient with Waldenstrom Macroglobulinemia. Hematol Oncol Clin North Am. 2018;32:811-820.

46. Gertz MA. Acute hyperviscosity: syndromes and management. Blood. 2018;132:1379-1385.

47. van Breugel HF, de Groot PG, Heethaar RM, Sixma JJ. Role of plasma viscosity in platelet adhesion. Blood. 1992;80:953-959.

48. Mayerhofer M, Haushofer A, Kyrle PA, et al. Mechanisms underlying acquired von Willebrand syndrome associated with an IgM paraprotein. Eur J Clin Invest. 2009;39:833-836.

49. McKelvey EM, Kwaan HC. An IgM circulating anticoagulant with factor VIII inhibitory activity. Ann Intern Med. 1972;77:571-575.

50. Castaldi PA, Penny R. A macroglobulin with inhibitory activity against coagulation factor VIII. Blood. 1970;35:370-376.

51. Mazurier C, Parquet-Gernez A, Descamps J, Bauters F, Goudemand M. Acquired von Willebrand's syndrome in the course of Waldenstrom's disease. Thromb Haemost. 1980;44:115-118.

52. Endo T, Yatomi Y, Amemiya N, et al. Antibody studies of factor VIII inhibitor in a case with Waldenstrom's macroglobulinemia. Am J Hematol. 2000;63:145-148.

53. Loftus LS, Arnold WN. Acquired hemophilia in a patient with myeloma. West J Med. 1994;160:173-176.

54. Taher A, Abiad R, Uthman I. Coexistence of lupus anticoagulant and acquired haemophilia in a patient with monoclonal gammopathy of unknown significance. Lupus. 2003;12:854-856.

55. Varticovski L, Pick AI, Schattner A, Shoenfeld Y. Anti-platelet and anti-DNA IgM in Waldenstrom macroglobulinemia and ITP. Am J Hematol. 1987;24:351-355.

56. Owen RG, Lubenko A, Savage J, Parapia LA, Jack AS, Morgan GJ. Autoimmune thrombocytopenia in Waldenström's macroglobulinemia. Am J Hematol. 2001;66:116-119.

57. Zago-Novaretti M, Khuri F, Miller KB, Berkman EM. Waldenstrom's macroglobulinemia with an IgM paraprotein that is both a cold agglutinin and a cryoglobulin and has a suppressive effect on progenitor cell growth. Transfusion. 1994;34:910-914.

58. Stone MJ, Pascual V. Pathophysiology of Waldenstrom's macroglobulinemia. Haematologica. 2010;95:359-364.

59. Nicol M, Siguret V, Vergaro G, et al. Thromboembolism and bleeding in systemic amyloidosis: a review. ESC Heart Fail. 2022;9:11-20.

60. Zanwar S, Abeykoon JP, Ansell SM, et al. Primary systemic amyloidosis in patients with Waldenstrom macroglobulinemia. Leukemia. 2019;33:790-794.

61. Sundaram S, Rathod R. Gastric Amyloidosis Causing Nonvariceal Upper Gastrointestinal Bleeding. ACG Case Rep J. 2019;6:3-4.

62. Osman K, Comenzo R, Rajkumar SV. Deep venous thrombosis and thalidomide therapy for multiple myeloma. N Engl J Med. 2001;344:1951-1952.

63. Mitrani LR, De Los Santos J, Driggin E, et al. Anticoagulation with warfarin compared to novel oral anticoagulants for atrial fibrillation in adults with transthyretin cardiac amyloidosis: comparison of thromboembolic events and major bleeding. Amyloid. 2021;28:30-34.

64. Gamba G, Montani N, Anesi E, et al. Abnormalities in thrombin-antithrombin pathway in AL amyloidosis. Amyloid. 1999;6:273-277.

65. Cowan AJ, Skinner M, Seldin DC, et al. Amyloidosis of the gastrointestinal tract: a 13-year, single-center, referral experience. Haematologica. 2013;98:141-146.

66. Patel G, Hari P, Szabo A, et al. Acquired factor X deficiency in light-chain (AL) amyloidosis is rare and associated with advanced disease. Hematol Oncol Stem Cell Ther. 2019;12:10-14.

67. Hicks SM, Coupland LA, Jahangiri A, Choi PY, Gardiner EE. Novel scientific approaches and future research directions in understanding ITP. Platelets. 2020;31:315-321.

68. Neunert C, Noroozi N, Norman G, et al. Severe bleeding events in adults and children with primary immune thrombocytopenia: a systematic review. J Thromb Haemost. 2015;13:457-464.

69. Vinholt PJ, Hvas AM, Nybo M. An overview of platelet indices and methods for evaluating platelet function in thrombocytopenic patients. Eur J Haematol. 2014;92:367-376.

70. Hansen CE, Qiu Y, McCarty OJT, Lam WA. Platelet mechanotransduction. Annual Review of Biomedical Engineering. 2018;20:253-275.

71. Ruggeri ZM. Platelet adhesion under flow. Microcirculation. 2009;16:58-83.

72. Andrews RK, Gardiner EE, Shen Y, Berndt MC. Platelet interactions in thrombosis. IUBMB Life. 2004;56:13-18.

73. Muthiah K, Connor D, Ly K, et al. Longitudinal changes in haemostatic parameters and reduced pulsatility contribute to non-surgical bleeding in patients with centrifugal continuous flow left ventricular assist devices. J Heart Lung Transpl. 2016;35:745-751.

74. Vulliamy P, Montague SJ, Gillespie S, et al. Loss of GPVI and GPIbα contributes to trauma-induced platelet dysfunction in severely injured patients. Blood Adv. 2020;4:2623-2630.

75. Qiao J, Schoenwaelder SM, Mason KD, et al. Low adhesion receptor levels on circulating platelets in patients with lymphoproliferative diseases prior to receiving Navitoclax (ABT-263). Blood. 2013;121:1479-1481.

76. Kamel S, Horton L, Ysebaert L, et al. Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation. Leukemia. 2015;29:783-787.

77. Thomas S, Krishnan A. Platelet heterogeneity in myeloproliferative neoplasms. Arterioscler Thromb Vasc Biol. 2021;41:2661-2670.

78. Kaplan ZS, Zarpellon A, Alwis I, et al. Thrombin-dependent intravascular leukocyte trafficking regulated by fibrin and the platelet receptors GPIb and PAR4. Nature Comm. 2015;6:7835.

79. Mammadova-Bach E, Ollivier V, Loyau S, et al. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. Blood. 2015;126:683-691.

80. Dumas JJ, Kumar R, Seehra J, Somers WS, Mosyak L. Crystal structure of the GPIbα-thrombin complex essential for platelet aggregation. Science. 2003;301:222-226.

81. Byzova TV, Plow EF. Networking in the hemostatic system. Integrin aIIbb3 binds prothrombin and influences its activation. J Biol Chem. 1997;272:27183-27188.

82. Haider S, Latif T, Hochhausler A, Lucas F, Abdel Karim N. Waldenstrom's macroglobulinemia and peripheral neuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes with a bleeding diathesis and rash. Case Rep Oncol Med. 2013;2013:890864.

83. Zangari M, Elice F, Tricot G, Fink L. Bleeding disorders associated with cancer dysproteinemias. Cancer Treat Res. 2009;148:295-304.

84. Luu S, Gardiner EE, Andrews RK. Bone marrow defects and platelet function: A focus on MDS and CLL. Cancers (Basel). 2018;10:147.

85. Castillo JJ, Advani RH, Branagan AR, et al. Consensus treatment recommendations from the tenth International Workshop for Waldenström Macroglobulinaemia. Lancet Haematol. 2020;7:e827-e837.

86. Salles G, Barrett M, Foa R, et al. Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. Adv Ther. 2017;34:2232-2273.

87. Ghobrial IM, Fonseca R, Greipp PR, et al. Initial immunoglobulin M 'flare' after rituximab therapy in patients diagnosed with Waldenstrom macroglobulinemia: an Eastern Cooperative Oncology Group Study. Cancer. 2004;101:2593-2598.

88. Ram R, Bonstein L, Gafter-Gvili A, Ben-Bassat I, Shpilberg O, Raanani P. Rituximab-associated acute thrombocytopenia: an under-diagnosed phenomenon. Am J Hematol. 2009;84:247-250.

89. Cheson BD, Leoni L. Bendamustine: mechanism of action and clinical data. Clin Adv Hematol Oncol. 2011;9:1-11.

90. Robak E, Robak T. Bruton's kinase inhibitors for the treatment of immunological diseases: current status and perspectives. J Clin Med. 2022;11.

91. von Hundelshausen P, Siess W. Bleeding by Bruton Tyrosine Kinase-Inhibitors: Dependency on Drug Type and Disease. Cancers (Basel). 2021;13.

92. Liu J, Fitzgerald ME, Berndt MC, Jackson CW, Gartner TK. Bruton tyrosine kinase is essential for botrocetin/VWF-induced signaling and GPIb-dependent thrombus formation *in vivo*. Blood. 2006;108:2596-2603.

93. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. Arterioscler Thromb Vasc Biol. 2010;30:2341-2349.

94. Atkinson BT, Ellmeier W, Watson SP. Tec regulates platelet activation by GPVI in the absence of Btk. Blood. 2003;102:3592-3599.

95. Burger JA, Buggy JJ. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765). Leuk Lymphoma. 2013;54:2385-2391.

96. Shatzel JJ, Olson SR, Tao DL, McCarty OJT, Danilov AV, DeLoughery TG. Ibrutinib-associated bleeding: pathogenesis, management and risk reduction strategies. J Thromb Haemost. 2017;15:835-847.

97. Levade M, David E, Garcia C, et al. Ibrutinib treatment affects collagen and von Willebrand Factor-dependent platelet functions. Blood. 2014:In press.

98. Bye AP, Unsworth AJ, Desborough MJ, et al. Severe platelet dysfunction in NHL patients receiving ibrutinib is absent in patients receiving acalabrutinib. Blood Adv. 2017;1:2610-2623.

99. Tullemans BME, Heemskerk JWM, Kuijpers MJE. Acquired platelet antagonism: off-target antiplatelet effects of malignancy treatment with tyrosine kinase inhibitors. J Thromb Haemost. 2018;16:1686-1699.

100. Dobie G, Kuriri FA, Omar MMA, et al. Ibrutinib, but not zanubrutinib, induces platelet receptor shedding of GPIb-IX-V complex and integrin aIIbb3 in mice and humans. Blood Adv. 2019;3:4298-4311.

101. Brown JR, Moslehi J, Ewer MS, et al. Incidence of and risk factors for major haemorrhage in patients treated with ibrutinib: An integrated analysis. British Journal of Haematology. 2019;184:558-569.

102. Castillo JJ, Gustine JN, Meid K, Dubeau T, Severns P, Treon SP. Ibrutinib withdrawal symptoms in patients with Waldenstrom macroglobulinemia. Haematologica. 2018;103:e307-e310.

103. Gustine JN, Meid K, Dubeau T, et al. Ibrutinib discontinuation in Waldenstrom macroglobulinemia: Etiologies, outcomes, and IgM rebound. Am J Hematol. 2018;93:511-517.

104. Gustine JN, Meid K, Dubeau TE, Treon SP, Castillo JJ. Atrial fibrillation associated with ibrutinib in Waldenstrom macroglobulinemia. Am J Hematol. 2016;91:E312-313.

105. Ali N, Malik F, Jafri SIM, Naglak M, Sundermeyer M, Pickens PV. Analysis of efficacy and tolerability of Bruton tyrosine kinase inhibitor ibrutinib in various B-cell malignancies in the general community: A single-center experience. Clin Lymphoma Myeloma Leuk. 2017;17S:S53-S61.

106. Dimopoulos MA, Tedeschi A, Trotman J, et al. Phase 3 Trial of Ibrutinib plus Rituximab in Waldenstrom's Macroglobulinemia. N Engl J Med. 2018;378:2399-2410.

107. Dimopoulos MA, Trotman J, Tedeschi A, et al. Ibrutinib for patients with rituximab-refractory Waldenstrom's macroglobulinaemia (iNNOVATE): an open-label substudy of an international, multicentre, phase 3 trial. Lancet Oncol. 2017;18:241-250.

108. Treon SP, Gustine J, Meid K, et al. Ibrutinib monotherapy in symptomatic, treatment-naive patients with Waldenstrom Macroglobulinemia. J Clin Oncol. 2018;36:2755-2761.

109. Fradley MG, Gliksman M, Emole J, et al. Rates and risk of atrial arrhythmias in patients treated with ibrutinib compared with cytotoxic chemotherapy. Am J Cardiol. 2019;124:539-544.

110. Abeykoon JP, Zanwar S, Ansell SM, et al. Ibrutinib monotherapy outside of clinical trial setting in Waldenstrom macroglobulinaemia: practice patterns, toxicities and outcomes. Br J Haematol. 2020;188:394-403.

111. Favaloro EJ, Funk DM, Lippi G. Pre-analytical Variables in Coagulation Testing Associated With Diagnostic Errors in Hemostasis. Laboratory Medicine. 2012;43:1-10.

112. Sharma R, Haberichter SL. New advances in the diagnosis of von Willebrand disease. Hematology. 2019;2019:596-600.

113. Favaloro EJ, Oliver S, Mohammed S, Vong R. Comparative assessment of von Willebrand factor multimers vs activity for von Willebrand disease using modern contemporary methodologies. Haemophilia. 2020;26:503-512.

114. Laporte P, Tuffigo M, Ryman A, et al. HemosIL VWF:GPIbR assay has a greater sensitivity than VWF:RCo technique to detect acquired von Willebrand syndrome in myeloproliferative neoplasms. Thromb Haemost. 2022. In press

115. Lim HY, Donnan G, Nandurkar H, Ho P. Global coagulation assays in hypercoagulable states. J Thromb Thrombolysis. 2022:In press.

116. Ninivaggi M, de Laat-Kremers R, Tripodi A, et al. Recommendations for the measurement of thrombin generation: Communication from the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies. J Thromb Haemost. 2021;19:1372-1378.

117. de Breet CPDM, Zwaveling S, Vries MJA, et al. Thrombin Generation as a Method to Identify the Risk of Bleeding in High Clinical-Risk Patients Using Dual Antiplatelet Therapy. Frontiers in Cardiovascular Medicine. 2021;8.

118. Beltrán-Miranda CP, Khan A, Jaloma-Cruz AR, Laffan MA. Thrombin generation and phenotypic correlation in haemophilia A. Haemophilia. 2005;11:326-334.

119. Tripodi A, Martinelli I, Chantarangkul V, Battaglioli T, Clerici M, Mannucci PM. The endogenous thrombin potential and the risk of venous thromboembolism. Thromb Res. 2007;121:353-359.

120. Wan J, Konings J, de Laat B, Hackeng TM, Roest M. Added value of blood cells in thrombin generation testing. Thromb Haemost. 2021;121:1574-1587.

121. Favaloro EJ, Bonar R. An update on quality control for the PFA-100/PFA-200. Platelets. 2018;29:622-627.

122. Vinholt PJ. The role of platelets in bleeding in patients with thrombocytopenia and hematological disease. Clinical Chemistry and Laboratory Medicine (CCLM). 2019;57:1808-1817.
123. Paniccia R, Priora R, Liotta AA, Abbate R. Platelet function tests: a comparative review. Vascular health and risk management. 2015;11:133-148.
124. Moenen FC, Vries MJ, Nelemans PJ, et al. Screening for platelet function disorders with Multiplate and platelet function analyzer. Platelets. 2019;30:81-87.
125. Walsh M, Kwaan H, McCauley R, et al. Viscoelastic testing in oncology patients (including for the diagnosis of fibrinolysis): Review of existing evidence, technology comparison, and clinical utility. Transfusion. 2020;60:S86-S100.
126. Kay AB, Morris DS, Collingridge DS, Majercik S. Platelet dysfunction on thromboelastogram is associated with severity of blunt traumatic brain injury. Am J Surg. 2019;218:1134-1137.
127. Al-Tamimi M, Arthur JF, Gardiner EE, Andrews RK. Focusing on plasma glycoprotein VI. Thromb Haemost. 2012;107:648-655.
128. Lui M, Gardiner EE, Arthur JF, et al. Novel stenotic microchannels to study thrombus formation in shear gradients: Influence of shear forces and human platelet-related factors. Int J Mol Sci. 2019;20.
129. Mangin PH, Gardiner EE, Nesbitt WS, et al. In vitro flow based systems to study platelet function and thrombus formation: Recommendations for standardization: Communication from the SSC on Biorheology of the ISTH. J Thromb Haemost. 2020;18:748-752.

130. de Witt SM, Swieringa F, Cavill R, et al. Identification of platelet function defects by multi-parameter assessment of thrombus formation. Nature communications. 2014;5:e4257.

131. Burkhart JM, Vaudel M, Gambaryan S, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood. 2012;120:e73-e82.

132. Chatterjee M, Rath D, Gawaz M. Role of chemokine receptors CXCR4 and CXCR7 for platelet function. Biochem Soc Trans. 2015;43:720-726.

133. Hivert B, Caron C, Petit S, et al. Clinical and prognostic implications of low or high level of von Willebrand factor in patients with Waldenstrom macroglobulinemia. Blood. 2012;120:3214-3221.

134. Gavriatopoulou M, Terpos E, Ntanasis-Stathopoulos I, et al. Elevated vWF antigen serum levels are associated with poor prognosis, and decreased circulating ADAMTS-13 antigen levels are associated with increased IgM levels and features of WM but not increased vWF levels in patients with symptomatic WM. Clin Lymphoma Myeloma Leuk. 2019;19:23-28.

135. Stockschlaeder M, Schneppenheim R, Budde U. Update on von Willebrand factor multimers: focus on high-molecular-weight multimers and their role in hemostasis. Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis. 2014;25:206-216.

136. Shahani T, Covens K, Lavend'homme R, et al. Human liver sinusoidal endothelial cells but not hepatocytes contain factor VIII. J Thromb Haemost. 2014;12:36-42.

137. Federici AB. The factor VIII/von Willebrand factor complex: basic and clinical issues. Haematologica. 2003;88:Erep02.

138. Saraya AK, Kasturi J, Kishan R. A Study of Haemostasis in Macroglobulinaemia. Acta Haematologica. 1972;47:33-42.

139. Kasturi J, Saraya AK. Platelet functions in dysproteinaemia. Acta Haematol. 1978;59:104-113.

140. Camera M, Brambilla M, Toschi V, Tremoli E. Tissue factor expression on platelets is a dynamic event. Blood. 2010;116:5076-5077.

141. Siddiqui FA, Desai H, Amirkhosravi A, Amaya M, Francis JL. The presence and release of tissue factor from human platelets. Platelets. 2002;13:247-253.

142. Estupiñán HY, Berglöf A, Zain R, Smith CIE. Comparative Analysis of BTK Inhibitors and Mechanisms Underlying Adverse Effects. Frontiers in Cell and Developmental Biology. 2021;9.

143. Kaptein A, de Bruin G, Emmelot-van Hoek M, et al. Potency and Selectivity of BTK Inhibitors in Clinical Development for B-Cell Malignancies. Blood. 2018;132:1871-1871.

144. Brown JR. Ibrutinib in chronic lymphocytic leukemia and B cell malignancies. Leuk Lymphoma. 2014;55:263-269.

Table 1: Studies reporting the frequency of bleeding in treatment-naïve WM patients.

Study	Sample size	% bleeding	Nature of bleeding
Perkins et. al. 1970	62	56	Not specified
Merlini et. al. 1999	215	7	Haemorrhagic manifestations

Garcia-Sanz et. al. 2001	217	23	Not specified
Merchione et. al. 2011	121	12.3	Epistaxis and gum bleeding

Table 2: Laboratory test abnormalities associated with bleeding in WM patients. Sample size indicates number of patients and percentage of cohort with clinically significant bleeding. VWF: von Willebrand Factor, AVWS: acquired von Willebrand syndrome, ADAMTS-13: a disintegrin and metalloprotease, TF: tissue factor, PT: prothrombin time, aPTT: activated partial thromboplastin time.

Reference	Sample size	Finding	Comments and observations that may explain bleeding symptoms
Hivert et. al. 2021[133]	43/72 (59%)	Reduced VWF levels	- Possibly due to IgM-mediated inhibition.[48] - Possibly due to specific autoantibody-mediated destruction, absorption onto malignant cells, altered blood rheology resulting in VWF multimer proteolysis by ADAMTS-13.[36] - Results in reduced VWF-GPIb-IX-V-mediated platelet activation and bleeding.
Gavriatopoulou et. al. 2019[134]	6/42 (14%)	Increased VWF levels	- Correlated with poor prognosis and low circulating ADAMTS-13 levels.[134] - Reflects greater engagement between WM lymphoplasmacytoid cells and endothelium.[133,134] - Could trigger thrombosis due to increased VWF-GPIb-IX-V-mediated platelet activation.
Hivert et. al.	10/72		- Could result in increased bleeding due to low numbers of VWF multimers.[135]

2021[133]	(14%)			
Castillo et. al. 2018[38]	49/320 (15%)	Reduced FVIII levels	-	FVIII is produced in the liver. Low FVIII levels possibly caused by liver dysfunction.[136]
			-	FVIII is bound to VWF in plasma. Low FVIII levels possibly coincide with AVWS.
			-	Possibly due to IgM-mediated inhibition of FVIII or VWF[48-50]
			-	Results in decelerated FX activation and bleeding.[137]
Saraya et. al. 1972[138]	3/3 (100%)	Reduced platelet adhesion	-	Reduced *in vivo* platelet adhesion to a wound.[138]
			-	Results in bleeding
Kasturi et. al. 1978[139]	4/4 (100%)			
Kasturi et. al. 1978[139]	4/4 (100%)	Reduced platelet activation	-	Platelets take up microparticles containing tissue factor. Platelet activation measured via release of tissue factor in response to ADP.[140,141]
			-	Results in bleeding
Saraya et. al. 1972[138]	2/3 (67%)	Reduced platelet aggregation	-	Reduced aggregation in response to ADP and adrenaline. Within two standard deviations for noradrenaline, thrombin and collagen.[138]
			-	Results in bleeding

Kasturi et. al. 1978[139]	3/4 (75%)

BTK inhibitor	Number of studies evaluated[142]	Total number of patients[142]	Type of haematological malignancies included[142]	Inhibited kinases: IC_{50} ± SD (nM)[143]	% Adverse events: Grade 1-2 (≥Grade 3)[142]
Ibrutinib	12	1263	TN + R/R CLL/SLL with P53 aberrations or del(17)p TN + pre-treated WM R/R MCL Pre-treated GvHD	BTK: 1.5 ± 0.2 TEC: 10 ± 12 ITK: 4.9 ± 1.2 TXK: 2.0 ± 0.3 EGFR: 5.3 ± 1.3	Bleeding: 35 (4.4)[144] AF: 12.4 (5.9) Hypertension: 19.6 (15.6) Rash: 15.8 (1.1) Diarrhoea: 47.3 (3.8)
Acalabrutinib	9	937	TN + R/R + ibrutinib-intolerant CLL/SLL TN + R/R WM R/R MCL	BTK: 5.1 ± 1.0 TEC: 126 ± 11 ITK: >1000 TXK: 368 ± 141 EGFR: >1000	Bleeding: 40.7 (2.5) AF: 4.3 (1.1) Hypertension: 8.6 (3.9) Rash: 16.1 (0.3) Diarrhoea: 37.7 (1.9)
Zanubrutinib	6	756	TN + R/R CLL/SLL with del(17)p TN + R/R + symptomatic WM R/R MCL	BTK: 0.5 ± 0.0 TEC: 44 ± 19 ITK: 50 ± 5	Bleeding: 39.2 (3.9) AF: 2.0 (0.7) Hypertension: 10.9 (2.8)

				TXK: 2.2 ± 0.6	Rash: 17.7 (0)
				EGFR: 21 ± 1	Diarrhoea: 20.8 (1.4)
			R/R CLL	BTK: 5.6 ± 1.0	Bleeding: 17.8 (1.8)
			TN + R/R WM	TEC: 77 ± 7	AF: 0 (0)
Tirabrutinib	5	178	R/R MCL	ITK: >1000	Hypertension: 0 (0)
			R/R B cell malignancies or NHLs	TXK: 116 ± 35	Rash: 24.7 (3.7)
				EGFR: >1000	Diarrhoea: 19.8 (1.1)

Table 3: Inhibited kinases and adverse events associated with various Bruton's tyrosine kinase inhibitors. BTK: Bruton's tyrosine kinase, TN: treatment-naïve, R/R: relapsed/refractory, CLL: chronic lymphocytic leukaemia, SLL: small lymphocytic lymphoma, WM: Waldenström Macroglobulinaemia, MCL: mantle cell lymphoma, NHL: non-Hodgkin lymphoma, GvHD: graft-versus-host disease, TEC: tyrosine kinase expressed in hepatocellular carcinoma, ITK: interleukin-2-inducible T-cell kinase, TXK: T and X cell expressed kinase, EGFR: epidermal growth factor receptor, AF: atrial fibrillation, SD: standard deviation.

Reference	Sample size	Number of WM patients	Study type	n minor bleeding (% Grade 1-2)	n major bleeding (% Grade 3-5)
Abeykoon et al. 2019	80	80 (100%)	Non-clinical trial (84% previously-treated, 16% treatment naïve)	3 (4%) haemorrhage, 1 (1%) petechiae, 2 (3%) haematuria	1 (1%)
Ali et. al. 2017	45	8 (18%)	Retrospective observational cohort analysis Previously-treated	14 (30.5%)	0 (0%)
Dimopoulos et al. 2017	31	31 (100%)	Previously-treated	12 (39%) bleeding 7 (23%) bruising	0 (0%)
Dimopoulos et al. 2018	75	75 (100%)	Phase 3 Trial of ibrutinib plus rituximab	51% in ibrutinib + rituximab *cf* 31% placebo + rituximab	4% incidence in each arm
Treon et. al. 2018	30	30 (100%)	Treatment-naïve	2 (7%) bruising, 1 (3) post-procedural haemorrhage	1 (3%)
Treon et. al. 2015	63	63 (100%)	Previously-treated	2 (3%)	0 (0%)

Table 4: Reports of bleeding frequency in Waldenström Macroglobulinaemia patients treated with ibrutinib. n: number of individuals; minor bleeding consisted of Grade 1-2 bleeds; major bleeding comprised Grade 3-5 bleeds.

Figures:

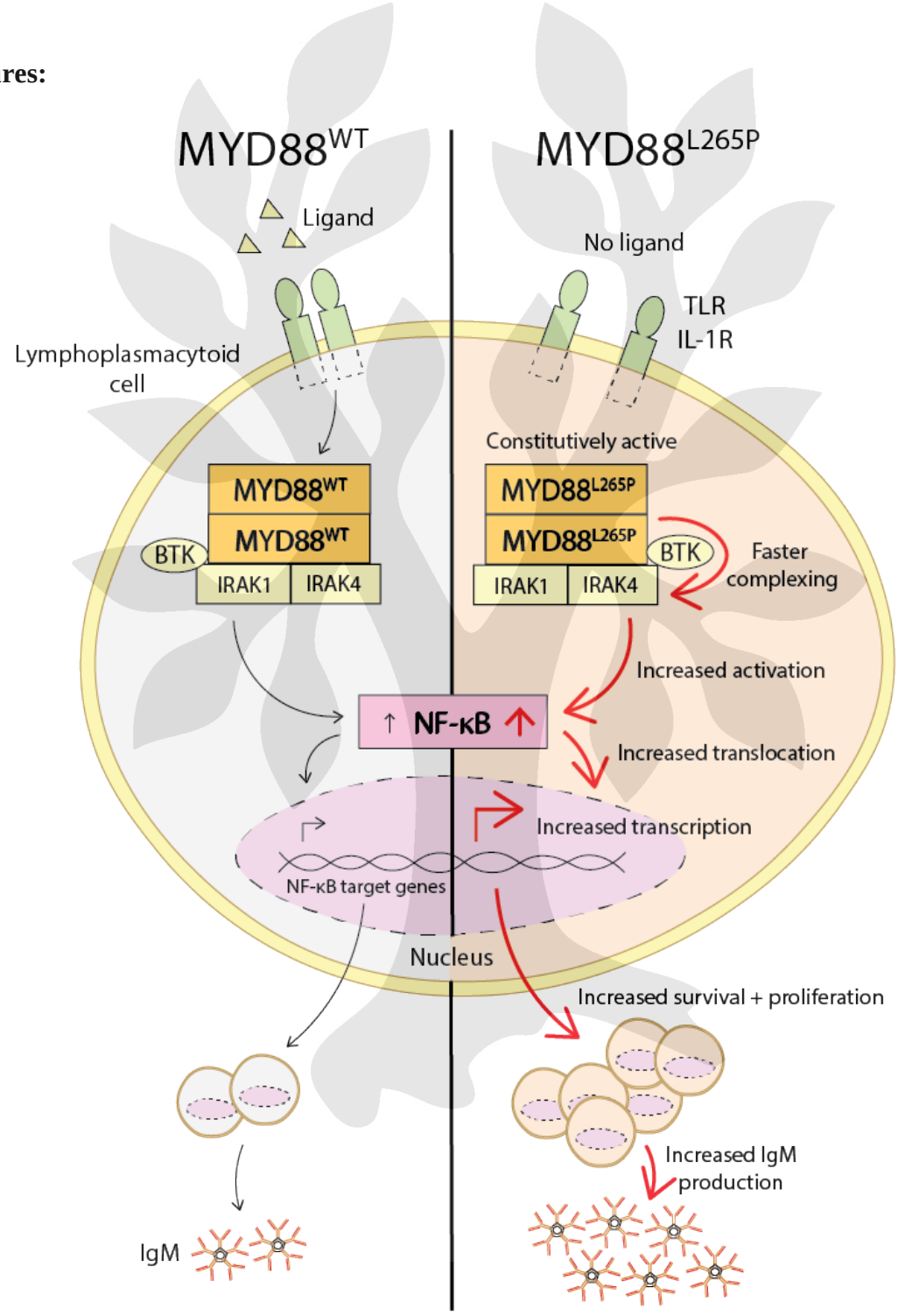

Figure 1: Pathways of development of lymphoplasmacytoid B cells in health and in WM. In WM patients, mutations and loss of DNA methylation occur to the lymphoplasmacytoid cells, with enrichment in B memory cells at an earlier stage of differentiation. The most common mutation, MYD88^{L265P}, results in increased BTK phosphorylation and faster MYD88 complexing with IRAK1/4 in response to low or absent TLR or IL-1R stimulus, which results in increased NF-κB translocation to the nucleus, increased target gene transcription, uncontrolled proliferation of WM lymphoplasmacytoid cells and overproduction of IgM. WM: Waldenström Macroglobulinaemia

MYD88: Myeloid differentiation primary response 88, BTK: Bruton's tyrosine kinase, IRAK: IL-1 receptor-associated kinase, TLR: Toll-like receptor, IL-1R: Interleukin-1 receptor, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, IgM: Immunoglobulin M.

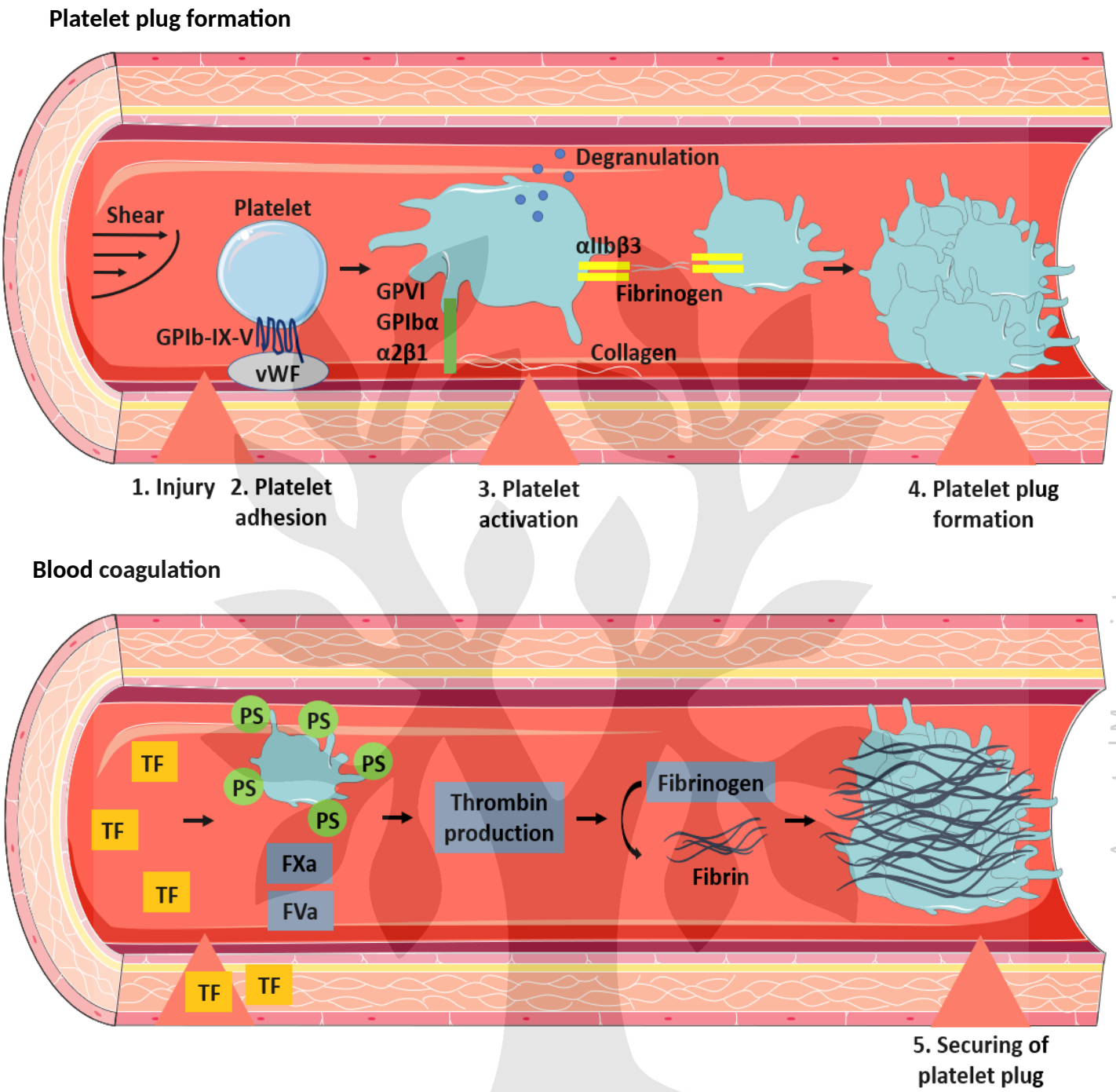

Figure 2: Simplified haemostasis. A) Platelet plug formation. Following an injury, GPIb-IX-V and GPVI bind exposed extracellular matrix ligands such as von Willebrand factor (vWF) and collagen respectively to enable platelet adhesion to the endothelium. Engagement of these receptors triggers platelet signalling, and platelets undergo changes to the cytoskeleton and release granules. The platelet-specific integrin, αIIbβ3 becomes active and binds fibrinogen, bridging adjacent platelets. Platelets aggregate forming a platelet plug at the site of the injury. B) Blood coagulation and the securing of the platelet plug. Tissue factor (TF) is exposed at the site of the injury triggering the extrinsic coagulation cascade, resulting in thrombin production. Thrombin converts fibrinogen into insoluble fibrin which secures the platelet plug in place. Blood hyperviscosity and high levels of IgM are likely to interfere with these haemostatic pathways, and potentially underpin bleeding events.

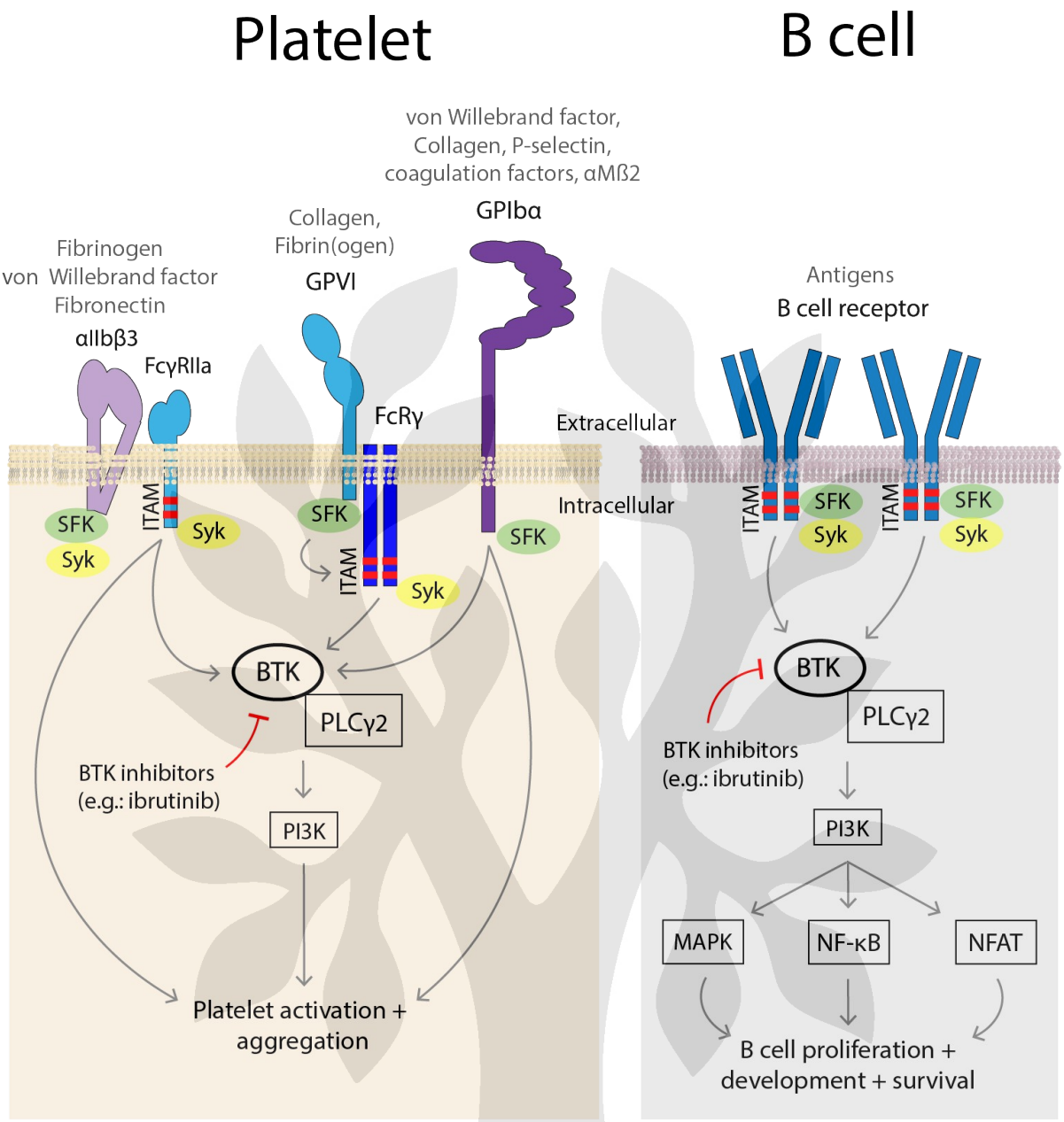

Figure 3: Platelets and B lymphocytes utilise common signalling pathways. BTK is differentially involved in the downstream signalling pathways triggered by ligand engagement of the major platelet adhesion/signalling receptors (αIIbβ3, GPVI and GPIbα of the GPIb-IX-V complex) and the BCR. In receptors with ITAMs, following ligand binding and receptor clustering, phosphorylation of the cytoplasmic ITAMs and recruitment of SFKs ensues, resulting in phosphorylation of Syk and activation of PI3K. PI3K mediates the conversion of phosphatidylinositol 4,5 bisphosphate to phosphatidylinositol 3,4,5 triphosphate, which engages BTK, resulting in phosphorylation of PLCγ. In platelets, this leads to activation and aggregation. In B cells, activation of MAPKs, NF-κB and NFAT, leads to B cell proliferation, development and survival. BCR and GPVI ITAM signalling are more reliant on the BTK pathway compared to GPIbα and αIIbβ3, thus signalling downstream of these receptors is more sensitive to BTK inhibition. BCR: B cell receptor BTK: Bruton's tyrosine kinase, GP: glycoprotein, ITAM: immunoreceptor tyrosine-based activation motif, SFK: Src-family kinases, PI3K: phosphoinositide 3-kinase, PLCγ: phospholipase C γ, NF-κB: nuclear factor κB, NFAT: nuclear factor of activated T cells, MAPK: mitogen-activated protein kinase.

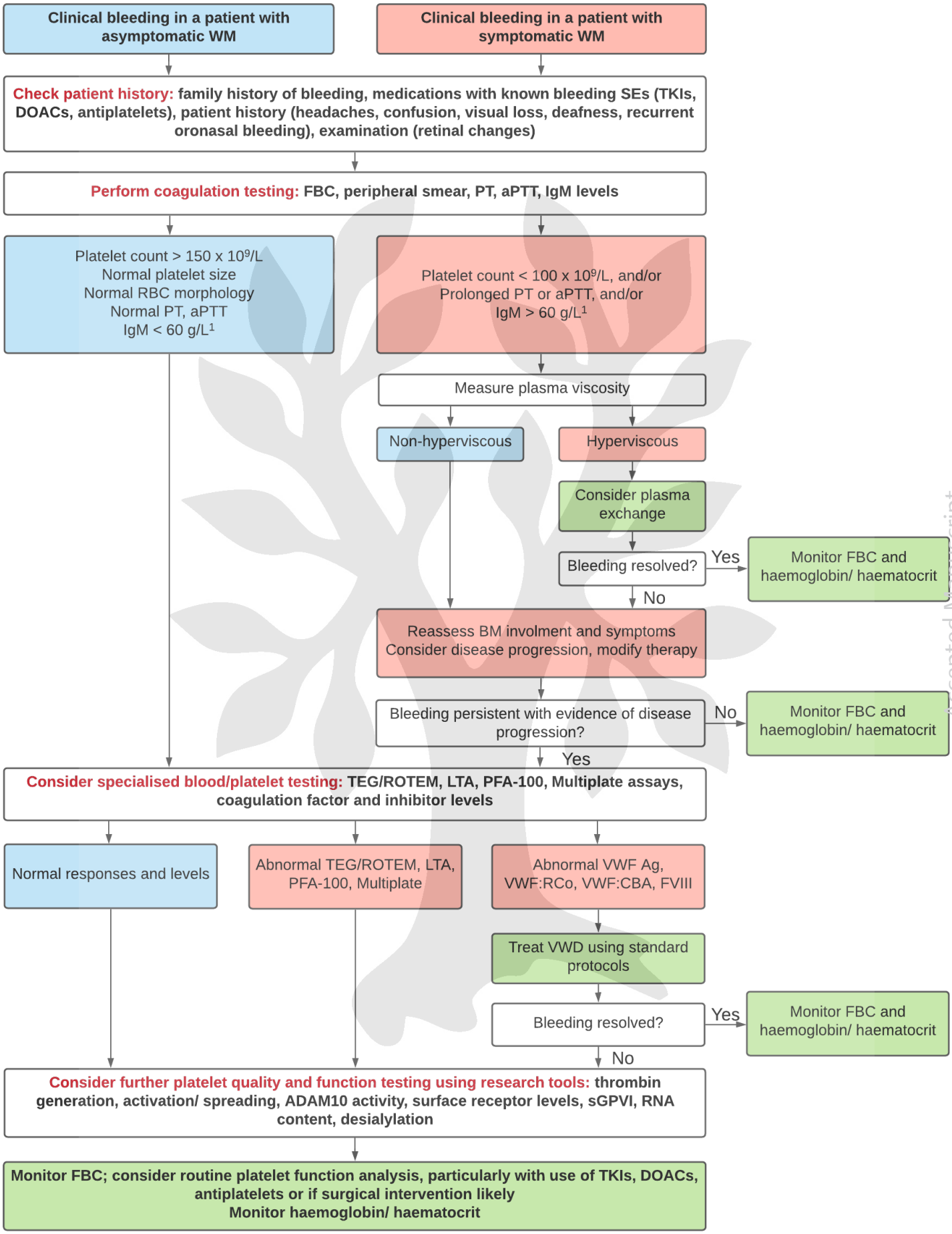

Figure 4: Recommended pathway to evaluate bleeding phenotypes in Waldenström macroglobulinaemia, with treatment options. Blue) asymptomatic WM, Red) symptomatic WM, Green) therapy recommendations. [1]: Castillo et. al. 2019. SE: side-effect, TKI: tyrosine kinase inhibitor, DOAC: direct oral anticoagulant, FBC: full blood count, PT: prothrombin time, aPTT: activated partial thromboplastin time, IgM: immunoglobulin M, RBC: red blood cell, BM: bone marrow, TEG: thromboelastography, ROTEM: rotational thromboelastomatry, LTA: light transmission aggregometry, PFA: platelet function analyser, VWF: von Willebrand Factor, Ag: antigen, R:Co: ristocetin cofactor assay, CBA: collagen binding assay, FVIII: Factor VIII, VWD: von Willebrand disease, ADAM10: a disintegrin and metalloproteinase 10, sGPVI: soluble glycoprotein VI.