Pathophysiological considerations to thrombophilia in the treatment of multiple myeloma with thalidomide and derivates

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Summary
Lenalidomide, a derivate of thalidomide, has recently been approved in Europe for the treatment of patients with multiple myeloma. Although the substance has a better effect/side-effect profile, especially with regard to teratogenicity and neurotoxicity, the rate of therapy-induced thrombosis seems comparable to thalidomide. The observed thromboembolic events were accompanied with a high rate of deleterious pulmonary embolism. Interestingly, the substances alone are not thrombogenic but combination with anthracyclines, dexamethasone or erythropoiesis-stimulating factors increases the risk considerably. As up to one third of patients treated with such combinations are affected, antithrombotic co-medication is highly recommended. This review elucidates the complex interactions between an activated coagulation-system in myeloma patients and the molecular effects of these drugs. This perception is important to choose the proper prophylactic co-medication without increasing the risk of bleeding, especially in first-line treatment, patients with high paraprotein-levels, or thrombopenia, either therapy-induced or due to bone-marrow infiltration.

Keywords
Thalidomide, lenalidomide, thrombosis, prophylaxis, multiple myeloma

Modern drugs used in the treatment of multiple myeloma

The development and introduction of thalidomide and bortezomib resulted in fundamental changes in the treatment of multiple myeloma (MM). Today, the combination of thalidomide plus dexamethasone (Thal/Dex), often in combination with melphalan, is one of the most commonly used regimens for patients with newly diagnosed MM. Thalidomide has proven anti-angiogenic activity as it can inhibit angiogenesis induced by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (1), even though there was no significant reduction in microvascular density or in the plasma levels of VEGF or bFGF following treatment with thalidomide (2–5). During the past few years, a number of thalidomide analogues with distinct biological activities have been developed. These analogues seem to have a better side-effect profile, especially with regard to neurotoxicity, than the parent drug. In addition, they demonstrate enhanced in-vitro activities that might predict superior clinical antitumour efficacy. The two major classes of analogues are known as "selected cytokine inhibitory drugs" or SelCIDs and "immunomodulatory drugs" or IMiDs. SelCIDs are phosphodiesterase type 4 inhibitors and reduce tumour necrosis factor alpha (TNFα) production, whereas IMiDs do not inhibit phosphodiesterase type 4 but stimulate T-cell proliferation as well as the production of interleukin 2 (IL-2) and interferon-gamma (IFNγ) (6, 7). The exact molecular mechanisms of action are still uncertain, although it is known that thalidomide analogues impair VEGF-induced mitogen-activated protein kinase (MAPK) signalling pathways (8). Lenalidomide displays significantly increased potency in inhibiting TNFα production, following lipopolysaccharide stimulation of peripheral blood mononuclear cells (PBMC). Lenalidomide is 50 to 2,000 times more active than thalidomide at stimulating T-cell proliferation and 50 to 100 times more active in augmenting the production of IL-2 and
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Thrombophilic side effects of combined drug therapies

The appearance of thrombotic side effects during treatment with thalidomide or its derivatives exhibits several strong differences in comparison to other pathophysiological causes of thrombosis. While thalidomide-derivatives have better side-effect profiles than the mother compound, particularly with regard to the induction of birth deformities or neurological side effects, the initiation of thrombotic events seems not to be altered. Although no study with direct comparison has been performed, in various clinical studies the proportion of patients with thrombembolic events receiving IMiDs, especially lenalidomide, is in the same range as with thalidomide (11–14). Neither thalidomide nor lenalidomide induce thrombosis by itself; the incidence of deep vein thrombosis (DVT) in single drug-treated patients is between <2% and 4% with a faint dose relation (12, 15–19). These facts, and the observation that the incidence of DVT after combination-treatment increases over months (20, 21), raise the question of a pathophysiological explanation and, eventually, the development of an appropriate prevention-strategy.

The fraction of patients with clinically relevant and potentially fatal DVTs rises considerably when these drugs are combined with dexamethasone, doxorubicin or erythropoietin. In first-line studies using the combination of thalidomide with dexamethasone up to 26% of patients experienced DVT when these drugs were administered without prophylaxis (22–26). Interestingly, the DVT rate has been reported to be only 2 – 7% in patients with relapsed or refractory myeloma receiving the same drug combination (15, 17–19). A similar phenomenon, although not as evident as with dexamethasone, has been observed combinations in anthracyclines: whereas the proportion of patients with DVT was 26 – 34% in newly diagnosed patients, and a 16% rate has been described in relapsed or refractory patients receiving thalidomide and doxorubicin-containing schedules (DT-PACE) (21). From these data it can be suggested that factors related to the tumor biology are decisive for the development of DVT. On the other hand, a study performed with patients suffering from myelodysplastic syndrome, a disease with distinctive biological features, receiving thalidomide in combination with darbepoietin-alpha had to be stopped due to the occurrence of DVT in three patients with one patient dying after pulmonary embolism (PE) (27).

Clinical features of thrombophilia in the treatment with thalidomide and derivates

The majority of patients with thrombotic complications experience proximal DVT with or without PE. Interestingly, a central venous line is not an additional risk factor, as the number of patients with thrombotic events is in the same range when the drugs are applied orally (e.g. MP-Thal) (28). Another remarkable aspect of DVT induced by thalidomide and derivates in combination with anthracyclines and dexamethasone is that the number of afflicted patients is constantly increasing over a time period of up to 12 months with 80% of incidences within six months after initiation of therapy (21, 29). A recently performed regression analysis of effects of different variables on venous thromboembolism (VTE) revealed that, besides a time from diagnosis of less than six months, an increased C-reactive protein (CRP) as well as an acquired protein C resistance are statistically relevant risk factors (30). CRP level is a highly significant prognostic factor for patients with MM, essentially reflecting the interleukin (IL)-6 activity (31). IL-6 is a pleiotropic cytokine acting on several cell lineages including myeloma cells, and, at the hepatocyte level, stimulates the synthesis of acute phase proteins, such as CRP (31, 32). IL-6 was demonstrated to be a strong autocrine or paracrine plasmacytoma cell growth factor, and IL-6 serum level correlates with tumour cell mass in patients (33).

An additional relevant risk factor for the development of thrombosis during therapy with thalidomide is acquired resistance to activated protein C (aAPCR). In multiple myeloma aAPCR, not associated with factor V Leiden, there is a transitory abnormality that can be found in approximately 10% of MM patients (29, 30, 34). The risk of DVT in myeloma patients receiving thalidomide rises from 12% to 66% when patients develop aAPCR (29). The production of auto-antibodies against protein C in these patients could be the explanation for the transient APC resistance phenotype (29, 35, 36).

These details show that thalidomide and derivates, when given in combination with anthracyclines, dexamethasone or

Table 1: Clinical and laboratory risk factors for the development of deep vein thrombosis (DVT) in the treatment of multiple myeloma (MM) patients with thalidomide and derivates.

<table>
<thead>
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<th>Patients’ and treatment risk factors:</th>
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<tr>
<td>First-line treatment</td>
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<td>Higher dose of thalidomide</td>
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<td>High dose dexamethasone</td>
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<td>Additional anthracyclines</td>
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<td>Additional erythropoiesis-stimulating factors</td>
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<tr>
<td>General risk factors not associated with multiple myeloma (history of thrombosis, contraceptives, factor V Leiden, immobility, etc.)</td>
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</tbody>
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<th>Myeloma-specific risk factors:</th>
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<tr>
<td>Acquired protein C resistance (prolonged aPTT)</td>
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<td>Elevated CRP (reflecting high IL-6 levels)</td>
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<tr>
<td>Light-chain disease</td>
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<td>Higher tumor stage (reflecting tumor cell mass)</td>
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erythropoiesis-stimulating factors, induce a long-lasting systemic pro-thrombotic effect. For the decision which patients should receive antithrombotic prophylaxis, a predictive laboratory marker would be helpful. Unfortunately, no identifiable laboratory abnormality has been found to be predictive of VTE. A recent retrospective analysis of 1,178 patients has shown that none of the classical coagulation parameters are independently associated with increased risk of VTE in univariate and multivariate analysis. In particular, neither the levels of protein C, protein S and antithrombin (AT) III, nor the incidence of anti-phospholipid antibodies, factor V Leiden and prothrombin gene promoter mutation have been found to correlate with the increased risk of VTE in patients receiving thalidomide. The factors that have been found to be positively associated were: light chain disease, recent diagnosis <12 months, and high CRP levels (30) (see Table 1).

Pathophysiological considerations

Regulation of thrombin activity

It has been shown that 63 – 71% of patients with newly diagnosed and previously treated MM have an activated coagulation and, subsequently, fibrinolysis system as demonstrated e.g. by elevated D-dimers (28, 29). The central event in coagulation and eventually the formation of a thrombus is the cleavage of thrombin (aFII) from prothrombin by the serine-protease aFX. Subsequently, thrombin converts fibrinogen to fibrin (“initiation”), and activates platelets and co-factors, such as factor (F)V and FVII (“amplification, propagation”). The regulation of thrombin-activity is an essential necessity of coagulation-homeostasis. Basically two different ways of thrombin-inhibition, one humoral (antithrombin) and one cellular (thrombomodulin), are important (Fig. 1).

AT is a circulating plasma protease-inhibitor that neutralizes most of the enzymes in the clotting cascade. Thrombin, FXa, and FIXa, as well as FXIIa and FXIa, are inhibited by forming equimolar, irreversible complexes (37, 38). AT has two active functional sites, the reactive center, Arg393-Ser394, and the heparin-binding site located at the amino terminus of the molecule. The binding of endogenous or exogenous heparins to the heparin-binding site on AT produces a conformational change in AT, which accelerates the inactivating process 1,000– to 4,000-fold (37, 38). Although AT is an important negative regulator of thrombin, no reduction of AT levels could be observed in patients treated with thalidomide and dexamethasone in comparison to untreated patients (39).

Thrombomodulin (TM) is a glycoprotein with endothelial growth factor-like domains presented on the luminal vascular endothelial cell surface. It plays a functional role as endothelial cell anticoagulant converting thrombin from a procoagulant protease to an anticoagulant and inducing its interaction with protein C (40, 41). Binding of thrombin to TM induces a conformational change in thrombin, which changes its substrate specificity such that it acquires the ability to activate protein C and no longer promotes platelet activation or the cleavage of fibrinogen (42, 43). Eventually, the TM/thrombin-complex at the cell mem-

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**Figure 1:** Molecular mechanisms resulting in hypercoagulation in myeloma patients. A) Physiological regulation. B) Pathological alterations during treatment (red, decrease; green, increase). Abbreviations: APC, activated protein C; F1,2, prothrombin fragment; GpIb, thrombocyte glycoprotein Ib; PAR1, proteinase activated receptor type I; PC, protein C; T, thrombin; TM, thrombomodulin; vWF, von Willebrand factor.
brane is internalized and degraded. TM has also been demonstrated to be present in a free form in circulating blood. Although the real origin of plasma TM has not yet been identified, it is currently thought that the smaller forms of TM that are found in plasma are the result of the proteolysis of endothelial cell molecules after endothelial injury (44). Patients treated with thalidomide and dexamethasone had significantly decreased TM plasma levels. TM levels dropped to less than half within the first month of treatment and did not fully recover after three months (39). The decrease of TM results in an increase of thrombin, which is a rational explanation for the enduring activation of the coagulation system in treated myeloma patients.

Cellular effects of thrombin
Besides its central role in the coagulation process, thrombin is able to induce cellular effects, mediated by a family of G-protein coupled protease-activated receptors (PARs) (45). Platelets as well as a number of other cells including endothelial cells have a dual receptor system for thrombin, with two distinct receptors (PAR-1, and PAR-4) (46, 47). Thrombin cleaves the amino-terminal exodomain of PAR, exposing a new amino-terminus, which then serves as a tethered ligand that binds to the body of the receptor to initiate transmembrane signaling (40, 48, 49). Activation of tumor cells by thrombin through the PAR1/GPR pathway seems to be an important survival and resistance factor for tumor cells during progression and metastasis (40, 50–52). The increased incidence of venous thromboembolism in patients treated with chemotherapy followed by thalidomide may be explained by altering the expression of PAR-1 on injured endothelium (53). Doxorubicin given as single drug reduces the expression of thrombin receptor PAR-1 on endothelial cells; in combination with thalidomide, PAR-1 positive endothelial cells are increased from 60% to 80% as compared to controls, resulting in endothelial dysfunction (54).

Von Willebrand factor and platelet activation
A factor associated with platelet activation is von Willebrand factor (VWF). High levels of VWF antigen (VWF-Ag, 374%) were found in patients receiving thalidomide. In patients with VTE, VWF-Ag levels were significantly higher as compared with patients without VTE (55). VWF is stored and released from platelet alpha-granules and from Weibel-Palade bodies of endothelial cells. The release of VWF from platelet alpha-granules and Weibel-Palade bodies of endothelial cell can be induced by thrombin through PAR-1 activation (56–58), thus being rather the consequence than the cause of the activated coagulation system in myeloma patients. VWF enables the platelet, via its surface glycoprotein receptors, to adhere to exposed subendothelium (59, 60). In a recent study with patients with unstable angina pectoris enoxaparin was able to effectively block the release of VWF (61).

These observations, the reduction of soluble TM, an increase of PAR-1 expression on endothelial cells, the development of acquired protein C resistance, and the high levels of von Willebrand factor antigen, adequately explain the distinct phenomena in thalidomide-induced thromboembolism, namely the deleterious effect of combination with endothelium-damaging agents such as anthracyclines, and the long-lasting effect of the treatment upon thrombophilia up to several months.

The risk of bleeding in myeloma
If prophylactic treatment of thrombosis in the treatment with thalidomide and derivates is discussed, the risk of bleeding complications of myeloma patients has to be taken into consideration. Although no exact data are available, clinical experience shows that myeloma patients per se are at risk of experiencing bleeding complications. A number of molecular mechanisms are discussed. The majority of bleeding complications are due to acquired von Willebrand disease or the consequences of hyperviscosity syndrome, especially in Ig-A myeloma with the triad vision changes, neurologic abnormalities, and bleeding (62–66). The most frequent laboratory abnormality related to coagulation is prolongation of the thrombin time, a reflection of the inhibition of fibrin polymerization by the paraprotein (67).

Patients with MM or Waldenstrom’s macroglobulinemia may have severe platelet dysfunction. Alterations in platelet function, probably due to an interaction between the paraprotein and platelet surface membrane glycoproteins, can result in prolongation of the bleeding time, impaired clot retraction, defective platelet aggregation in vivo, and decreased platelet adhesion in vitro. The abnormal paraproteins found in these conditions can affect all stages of platelet function including adherence, activation, aggregation, and procoagulant activity (68–70). Also, specificity of the paraprotein for GPIIb3a has been described in a patient with MM with a fatal bleeding disorder (71). In addition, the post-chemotherapy thrombocytopenic period raises considerable concern of bleeding, and aspirin treatment should definitely be avoided in this phase. Also, it has been suggested not to use aspirin or other cyclooxygenase (COX) inhibitors without monitoring TNFz in inflammatory states and specifically in myeloma as the increase of TNFz could exacerbate disease (72). Nevertheless, a number of studies have shown the effectiveness of aspirin prophylaxis in the treatment of myeloma patients with thalidomide or derivates in combination with dexamethasone or anthracyclines (13, 73–77).

Thrombosis prophylaxis in the treatment with thalidomide or derivates
Warfarin as well as aspirin and LMWH have been used in various studies to prevent thrombosis during therapy with thalidomide and lenalidomide (23). In comparison to LMWH or aspirin, warfarin had less pronounced effects on preventing thrombosis. In the used dosages (1–2 mg/day) a reduction of thromboembolic events of no more than 50% has been described (21, 39, 78). The use of warfarin, at least in the mentioned dosages, is not appropriate to prevent thrombosis. Aspirin and LMWH on the other hand, where both able to significantly reduce the rate of thrombosis during therapy. Aspirin has been used in dosages from 81–325 mg/day with no dose correlation; obviously lower dosages of aspirin are sufficient; reduction of thrombosis rate was 70–80% (13, 14, 73–75, 77, 79, 80). The effect of LMWH was superior to aspirin with a reduction of thrombosis rates to the rate
observed in the control arms in most studies; mostly enoxaparin with 40 mg/kg has been used (13, 20, 73, 81, 82). A comprehensive overview of actual study data can be found in (81). In December 2007, the American Society of Clinical Oncology has published guidelines with recommendations for VTE prophylaxis and treatment in patients with cancer has been published (83). The recommendations in summary are that routine prophylaxis of ambulatory cancer patients is recommended for patients receiving thalidomide or lenalidomide. The authors propose LMWH or adjusted-dose warfarin (international normalized ratio [INR] ca. 1.5) for myeloma patients receiving thalidomide plus chemotherapy or dexamethasone, but admit that these recommendations are based on extrapolation from studies of postoperative prophylaxis in orthopedic surgery and a trial of adjusted-dose warfarin in breast cancer (83).

As elucidated above, the major molecular events resulting in a prothrombotic condition in the treatment of myeloma patients are the reduction of soluble thrombomodulin and the development of acquired protein C resistance. Both increase the thrombin concentration, which is a central activator of numerous coagulatory processes. One of them is the activation of thrombocytes, which may explain the high levels of von Willebrand factor antigen found in patients receiving thalidomide. PAR-1, one of the known thrombin receptors expressed by various cells, is upregulated upon endothelial cells; both effects increase the risk of thromboembolic events considerably. Aspirin can block the activation of thrombocytes and alpha-granule secretion by irreversible inhibition of platelet COX, as well as their release of VWF (56). There are no published data available regarding PAR-1 expression and the cellular effect of thrombin on myeloma cells. The failure of aspirin to inhibit the other prothrombotic effects of thrombin might explain its limited prophylactic effect in the treatment of myeloma patients. Also, aspirin should not be used in patients with thrombocytopenia, either due to a high degree of bone marrow infiltration or as a consequence of treatment with cytotoxic drugs. In summary, aspirin prophylaxis cannot be recommended at the beginning of combination therapies with thalidomide or derivatives where the myeloma cell mass is high and the hematological side effects of cytotoxic treatments are difficult to anticipate.

The direct inhibition of thrombin or its activator FXa is another approach with consequences upon various downstream effects of thrombin. As direct thrombin or FXa inhibitors are in development but not yet available, LMWH are a useful alternative. Although they are expensive and inconvenient to use, LMWH have shown to be effective in various studies. In addition to their potency to stimulate the "tissue factor pathway inhibitor" and block the activation of FX at an early stage, they inhibit aFX and, although to a minor degree, thrombin. The decline of circulating thrombin reduces PAR-1 signaling in endothelial cells and tumor cells. This mechanism has also been discussed as an explanation for the survival prolongation of patients receiving LMWH (40, 82, 84). These characteristics make LMWH suitable for thrombosis prophylaxis in patients with high myeloma cell mass and at the beginning of therapy. Mostly enoxaparin at a dosage of 40 mg/kg has been used. As LMWH are mainly cleared through the kidneys, their dosage must be adjusted in patients with reduced kidney function.

In summary, both aspirin and LMWH have shown to be sufficiently efficient in the prevention of thrombosis with a slight advantage of LMWH that can be explained by the fact that they interfere at an earlier stage with the deleterious molecular mechanisms responsible for clot formation. With respect to the importance of the TF/aFVII complex, LMWH with a high potency of tissue factor pathway inhibitor induction, such as tinzaparin, reviparin, certoparin or enoxaparin should be preferred. Aspirin should be used with care in patients with the risk of thrombocyte dysfunction, e.g. with high paraprotein levels at the beginning of therapy or therapy-induced thrombocytopenia. For future drug developments, it would be interesting to examine the potency of direct thrombin or direct aFX inhibitors.

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**Recommended based on pathophysiological considerations**

Patients treated first-line with thalidomide or derivatives in combination with dexamethasone, anthracyclines or erythropoiesis stimulating factors are at high risk of developing DVT and potentially deleterious PE. Prophylactic treatment with either aspirin or LMWH is highly recommended and supported by various clinical studies. Prophylaxis with warfarin did not sufficiently reduce the thrombosis rate. Prophylaxis must be continued for the time of treatment, at least for 12 months. Aspirin should be used with care in patients with the risk of thrombocyte dysfunction or the risk of therapy-induced thrombocytopenia, e.g. with high paraprotein levels and at the beginning of therapy.

Based on the results of various clinical studies and considering to pathophysiological mechanisms, we recommend performing prophylaxis during the first six months of treatment with LMWH in prophylactic doses. Mostly enoxaparin at a dosage of 40 mg/kg has been used with success. During this time, 80% of thrombotic events have been observed. In patients with reduced renal function the dosage must be adjusted. After six months, prophylaxis should be continued and may be performed with aspirin at a dosage of 81 – 100 mg/day, which costs less and is more convenient.

In the rare cases in which patients receive thalidomide or lenalidomide monotherapy, the risk of therapy-induced thrombosis is not increased, and no prophylaxis is necessary.

**Abbreviations**

SelCID, selected cytokine inhibitory drug; IMiD immunomodulatory drug; DVT, deep vein thrombosis; PE, pulmonary embolism; Thal, thalidomide; Dex, dexamethasone; VTE, venous thromboembolism; CRP, C-reactive protein; aAPCR, acquired resistance to activated protein-C; AT, anti-thrombin; TM, thrombomodulin; MM, multiple myeloma; LMWH, low-molecular-weight heparin.
References


