Chapter 8

Summary and Discussion

8

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THROMBOCYTOPENIA, BLEEDING AND SURVIVAL – THE END OR START OF A CHAPTER?

Introduction

The use of platelet concentrates (PC) is generally recommended for the prophylaxis and treatment of haemorrhagic complications in patients with thrombocytopenia due to myelosuppression.[1–4] Based on the observations of Gaydos et al as well as two post mortem studies, the concept of prophylactic platelet transfusions became standard practice since the late sixties and early seventies of the past century.[5–7] Indeed, after the introduction of platelet transfusions, the incidence of lethal haemorrhages steadily declined from more than 60% in the sixties to less than 5% in the last two decades.[6 – 8] Although it has to be noted that other supportive care measures as well as leukaemia treatment without doubt contributed major to this decline, a Cochrane analysis, which included these older studies, showed a small but significant effect of prophylactic platelet transfusions in the reduction of severe haemorrhages as compared to therapeutic transfusions.[9] Over the past three decades several endpoints have been engaged in successive trials starting with more patient centred endpoints such as bleeding prevention efficacy and the incidence of adverse transfusion reactions (e.g. alloimmunisation, febrile non-haemolytic transfusion reactions), as well as transfusion efficacy, a more transfusion product oriented endpoint. This thesis is based on the results of two product-based randomised trials, looking at transfusion efficacy in terms of increments, with adverse transfusion reactions and haemostatic efficacy as secondary outcome measures.[10, 11] This discussion reviews the most relevant outcomes of these trials and debate the clinical relevance of these (surrogate) endpoints, focussed on refractoriness and bleeding incidence.

The trials

The first randomised controlled trial (RCT) we performed aimed to investigate the clinical efficacy of a platelet additive solution as compared to conventionally plasma stored platelets in non-selected, thrombocytopenic patients in two large hospitals. Despite platelets stored in platelet additive solution (PAS2, Trombosol) had a significantly lower 1- and 24-hour corrected count increment, the wide chosen 30% margin of non-inferiority, as well as the fact that a comparable transfusion interval and no difference of hemorrhagic consequences were observed has not led to abandoning of platelets stored in PAS2 for up to five days in clinical practice (Chapter 3).[10] The transfusion efficacy results are in line with the findings of three other studies using this additive solution.[12-14] Adverse transfusion reactions occurred less frequently after transfusion with platelets in additive solution, consistent with the concept of plasma as important factor in FNHTRs. Platelet transfusion refractoriness defined as a 1-hour CCI < 7.5 and/or a 24-hour CCI < 4.5 occurred more frequent after transfusions with PAS2-PCs, however a multivariate analysis showed that patient factors like enlarged spleen, fever and infection and not product factors like storage time and storage solution determined the incidence of non-immunological refractoriness.[10]

As a consequence of the growing awareness of transfusion-transmitted infections along with a public debate, the second randomised trial investigated the transfusion efficacy of pathogen-reduced platelet products (PR-PAS3PC, Intercept) as compared to two non-pathogen-reduced platelet products (PAS3 PC, Intersol; plasma PC) in a comparable

non-selected hospitalised patient population as the first trial. With the chosen 20% margin of non-inferiority, inferiority was shown for pathogen-reduced platelets as compared to both control arms. Moreover, patients treated with pathogen-reduced platelets had significantly more bleeding complications and was reason for the Data Safety Monitoring Committee to stop the study arm PR-PAS3PC (Chapter 4).[11] Although the overall bleeding incidence, scored by clinicians in this trial, was considerably less as compared to other trials in which bleeding was scored according to rigorous prescriptive protocols; the reported outcome is consistent with these trials.[15-17] Whether the increase in haemorrhagic complications was due to a difference in platelet dose or impaired platelet function could no be determined. Platelet dose, which was significantly lower in the pathogen reduction arm, has been suggested as possible explanation for the increase in haemorrhagic complications.[18] A recently published large trial, however, testing 3 different platelet doses showed no difference with regard to the bleeding incidence.[19] In vitro studies reported that platelets treated with pathogen reduction, especially after prolonged storage, show reduced aggregation capacity, reduced glycoprotein expression, increased expression of annexin V as well the activation marker P-selectin, suggesting functional alterations that may play a role in an increase in bleeding complications.[20, 21] Apart from this unresolved issue, the outcome of this second trial has lead to both a debate with regard to bleeding as an endpoint as well as enormous (legal) consequences of reporting an adverse outcome and consequently stopping a study arm. The outcomes of the second trial as well as the intention to include bleeding as a primary outcome measure in current trials have led to a number of questions. To what extend is an increase in haemorrhagic complications acceptable using manipulated platelets to reduce the already very low incidence of transfusion-transmitted infections? And, if so what margin of non-inferiority is acceptable? What are the clinical consequences of bleeding complications?

THE ENDPOINTS: REFRACTORINESS AND BLEEDING

Refractoriness

Although still incompletely understood, the association of refractoriness with several clinical factors has been noted and investigated by several studies (table 1). Bishop et al showed that the recovery of platelets is affected by several clinical factors, including diffuse intravascular coagulation (DIC), administration of amphotericin B, splenomegaly and HLA antibodies. Antibiotic therapy, bleeding and temperature were less important factors.[22-24] Norol et al studied the impact of platelet storage time in the context of clinically "stable versus unstable" patients (i.e. patients with bacterial infection, GVHD, veno-occlusive disease as well as patients with splenomegaly) showing that only stored platelets performed worse as compared to fresh platelets in "unstable" patients.[25]
Bock et al demonstrated that fever as well as the use of certain antibiotics, either causal or as confounder (amphotericin B, ciprofloxacin, vancomycin), significantly influenced platelet increment.[26] The relation of fever with refractoriness has been explained by circulating cytokines, among others IL-1 and TNF, which induce activation of endothelial cells leading to the expression of adhesion molecules and induction of procoagulant activity.[27, 28]
In line with this,, the association between a higher dose of total body irradiation with refractoriness also suggests that endothelial damage could play an important causal role.[27]
Heim et al showed in a large group of haemato-oncology patients that patient age, female sex and the administration of antithymocyte globulin (ATG) were associated with better post-transfusion platelet recovery, whereas increasing storage time, ABO-mismatched platelets, additive solution (T-Sol), allogeneic haematopoietic stem cell transplant (HSCT),

transfusion sequence > 40 and fever before transfusion had a significant adverse affect.[29] Similar results were found in an analysis using the data of the TRAP trial. In this analysis refractoriness was defined as 2 sequential 1-hour post transfusion increments (recovery) of less than 11 x 10^9/L (equalling an average CCI of 5.0). The strongest negative impact on 1- as well as 24-hour increment had male sex and females with previous pregnancies. Other negative factors were enlarged spleen, amphotericin B, bleeding, fever, infection, weight, height and transfusion sequence number. Platelet product factors negatively influencing 1- and 24-hour increments were ABO-incompatibility and storage time. By estimation, patient factors determined over 80% of the variation of both the 1- and 24-hour increment. The authors suggested that decreasing transfusion efficacy with increasing platelet transfusions might be related to progressive endothelial damage with increased platelet adherence and thus a more rapid loss from circulation.[30, 31] In our first RCT platelet transfusion failure occurred at least once in 49.6% of the patients. Platelet transfusion failure was, independent from thrombocytopenia, positively associated with bleeding complications, non-transplant related chemotherapy, severe mucosal damage and age.[10, 32] To summarize, non-immunological refractoriness, a frequent observed complication of platelet transfusions, is largely determined by several patient factors including bleeding complications, infectious complications and chemo- and radiotherapy induced tissue damage, whereas product factors such as storage time and storage solution play a minor role. In this sense platelet refractoriness may be a consequence as well as an indicator of increasing endothelial activation and damage.

Table 1: *Factors associated with refractoriness*[22, 24, 25, 26, 29, 31, 32]

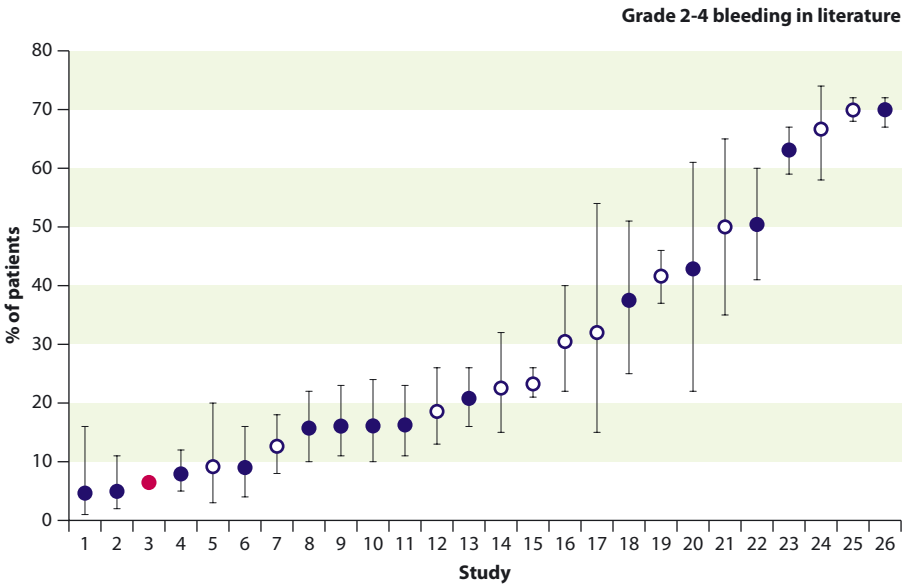
	Bishop	Norol	Bock	Heim	Slichter	Kerkhoffs
Gender				●	●	●
Age						●
BSA					●	
Enlarged spleen	●	●			●	●
Bleeding	●				●	●
Infection		●			●	●
Fever	●		●	●		●
VOD		●				
GVHD		●				
DIC	●					
Mucosal damage						●
TBI		●				
ATG						●
Stem cell Tx				●		
Chemotherapy						●
Antibiotic therapy	●		●		●	
Transfusion sequence				●	●	
Storage time		●			●	
ABO mismatch				●	●	
Additive solution				●		

BSA =Body Surface Area; VOD = Veno Occlusive Disease; GVHD =Graft versus Host Disease; DIC = Diffuse intravascular Coagulation; TBI =Total Body Irradiation; ATG =Anti Thymocyte Globulin; Tx = Transplantation

Bleeding

The reported incidences of grade 2 – 4 bleeding varies from 5 – 70% (figure 1). Clinical platelet transfusion studies evaluating platelet products report major bleeding > grade 1 in 48% of the patients, whereas the incidence of major bleeding in AML RCTs evaluating patient outcome is only 7%, comparable with the percentage found in our second RCT. These figures reflect the subjectivity of the grading of bleeding. Active versus passive reporting of bleeding complications together with the frequency and timing of bleeding observation are the main factors explaining for the enormous variation in reported bleeding incidences in literature.[33] Apparently the difference between platelet product driven studies and clinical outcome studies in AML trials is explained by the perception of "clinically relevant", i.e. the perception of "what is meaningful".[34] Adapting the active and rigorous bleeding observation of the PLADO trial, which encompasses an active bleeding observation of 8 WHO defined sites by trained personnel and an independent adjudication, the Bleeding Observation Pilot (BOP) study showed bleeding grade 2 – 4 in 54% of the 68 participating patients on 18% of the observed days, which is in agreement with reports using similar bleeding score systems.[19, 35] Comparing the data of the BOP trial with our second RCT shows that the large difference in bleeding incidence is mainly due to the underreporting of skin- and mucosal bleeding events, underlining the impact of clinical perception (Chapter 6) .

Figure 1: *Figure 1 shows reported percentages of patients with grade 2 - 4 bleeding complication. The error bars reflect the 95% confidence interval. The filled markers represent randomised controlled trials, whereas the open markers represent observational studies. 1 = Blumberg et al[71]; 2 = Sensebe et al[72]; 3 = AML Trials 83 = Kerkhoffs et al[11]; 4 = Oka et al[16]; 5 = Tinmouth et al[17]; 6 = Gil-Fernandez et al[18]; 7 = Zumberg et al[19]; 8 = Kerkhoffs et al[10]; 9 = Mirasol[76]; 10 = Diedrich et al[77]; 11 = Wandt et al[78]; 12 = Rebulla et al[52]; 13 = Gmur[79]; 14 = Nevo et al[55]; 15 = Wandt et al[80]; 16 = Sagmeister et al[81]; 17 = Murphy et al[82]; 18 Pihush et al[53]; 19 = Higby et al[83]; 20 = Navarro et al[84]; 21 = Heddle et al[35]; 22 = McCullough et al[16]; 23 = Lawrence et al[85]; 24 = Friedmann et al[46]; 25 = Slichter et al[19].*



The use of bleeding as an endpoint assumes the level of thrombocytopenia to be an important etiological factor as well the potency of transfused platelets to correct this level to decrease bleeding complications. Despite a preventive platelet transfusion policy major bleeding is occurring in half of the patients. We still lack the tools in understanding which patients are going to have bleeding complications and to what extend platelet transfusions are aiding in their prevention or even may be harmful in certain clinical situations.[36, 37]

Experimental studies in thrombocytopenia

In rabbits with severe thrombocytopenia flattening and increased fenestration of the endothelium has been shown using electron microscopy.[38] Also an increased leaking of red cells associated with the level of thrombocytopenia was shown in thrombocytopenic rabbits.[38, 39] The relation between thrombocytopenia was explored using radio labelled red cells in stable thrombocytopenic patients. Substantial faecal loss of radio labelled red cells only occurred at platelet counts less than 5 x 10^9/L.[40] In a landmark study using radio labelled platelets an endothelium supportive role for platelets was suggested with an average consumption of 7 x 10^3/μL/day.[41] Although these studies show that platelets play an important role in the maintenance of endothelial integrity, it is likely that increased fenestration and flattening of the endothelium causes petechiae and mucosal bleeding, but it is unknown whether this explains for the major bleeding complications.
Using mice experiments, Ho-Tin-Noé et al showed mice deficient of β-integrines, which have decreased neutrophil infiltration capabilities, were protected from thrombocytopenia-induced tumor hemorrhage. The same group showed that platelet adhesion in it self is not required to maintain vascular integrity and that in the absence of platelets hemorrhage only occurred in an inflamed microcirculation.[42, 43]

Bleeding susceptibility

Gaydos et al first described the relationship between thrombocytopenia and haemorrhage, although no threshold could be recognized and most of the lethal cerebral haemorrhages described in this study occurred in patients with cerebral leukaemia involvement. Moreover aspirin was frequently used as an antipyretic agent.[5] Estey et al studied the causes and risk factors of remission induction failure in 378 previously untreated AML patients in the period 1973 – 1979. Only 22% of these patients were primary chemotherapy resistant, the majority of patients failed coming into remission due to infectious complications and in 33% of the patients failure occurred due to fatal haemorrhages despite prophylactic platelet transfusion support. The main risk factor for fatal hemorrhage were an initial white blood count of ≥ 25 x 10^9/L (OR 2.7; 95%CI 1.3 – 5.3) and the incidence of death from haemorrhage was highest during the initial 2 weeks of treatment. In the discussion an etiologic role for leukaemia infiltration of vessel walls is considered as also reported by Freireich.[44, 45] Friedmann et al studied clinical and laboratory features predicting for severe hemorrhage in 2942 patients. 368 patients (12.5%) suffered severe bleeding complications. Uraemia, hypoalbuminemia, recent BMT, platelet transfusion, the administration of aminocaproic acid and recent bleeding were associated with increased bleeding. Platelet count was not significantly associated with bleeding in untransfused patients. The main limitation of this retrospective study was the lack of information regarding the temporal relationship between platelet count, platelet transfusion and bleeding.[46] Studying the relationship of thrombocytopenia with bleeding post stem cell transplant, Nevo et al compared 321 bleeding BMT patients with 287 non-bleeding matched controls. There was a small but significant increased risk for bleeding in patients

with more days of platelet counts ≤ 10. However profound thrombocytopenia was present in only 8.6% of bleeding patients. Pulmonary hemorrhage was significantly associated with thrombocytopenia in contrast to bleeding from other sites.[47] This might be associated with GVHD and endothelial damage, as has been shown by a post-mortem study.[48]
In line with the report of Gil-Fernandez et al, who identified high platelet consumption factors (VOD, fever, treatment with amphotericine B and mucosal damage) in most cases of bleeding in two transfusion trigger groups (10 vs. 20 x 10^9/L), Nevo et al showed that profound thrombocytopenia was not the primary cause of bleeding in both groups.[49, 50]
In contrast, in the re-analysis of the Rebulla trigger trial, including 255 patients with acute myeloid leukaemia, six variables were multivariate associated with grade I-II bleeding: administration of antifungal medication, steroid administration, a higher platelet count and platelet transfusion decreased the risk, whereas the presence of infection and fever increased the risk. Grade II – IV bleeding was associated with fever as well as platelet count. Grade III – IV bleeding was associated with the administration of antifungal therapy (increased risk!). The presence of a grade I bleeding was associated with at 2.6 times increased risk of grade II – IV bleeding (95%CI 1.18 – 5.49) and grade I – II bleeding was associated with a 3.1 times higher risk of grade III – IV bleeding (95%CI 1.17 – 7.95).[51, 52] Pihusch et al studied hemorrhagic and thrombotic events in 447 transplant patients (autologous n = 83; allogeneic n = 364). Haemostatic (thrombotic as well as haemorrhagic) events occurred in 83.2% of the patients. Severe haemorrhage occurred in 41.5% of the patients and 3.6% suffered lethal bleeding. Intracranial haemorrhage was observed in 21 patients (4.7%) and associated in a majority of patients with infection. Allogeneic BMT patients had a higher bleeding incidence as compared to autologous BMT patients. Patients with GVHD > grade I had a significantly higher incidence of bleeding. A strong correlation was found between the duration of thrombocytopenia and bleeding events. GVHD and duration of thrombocytopenia were the only "predictors" for the occurrence of bleeding. Also a thrombotic event such as microangiopathic haemolytic anemia (MAHA) was more frequent in the allogeneic group. Interestingly in 23.5% of the MAHA cases a shortened aPTT was found indicating endothelial perturbation and activation. In the discussion authors suggest a role for TNF-α, essential in the pathogenesis of GVHD, known to modulate endothelial haemostatic function and enhance the production of Plasminogen activator inhibitor-1 and Tissue Factor as well as down regulating Tissue factor pathway inhibitor.[53, 54]
To summarize (see also table 2), bleeding is a frequent but variably reported complication in patients with thrombocytopenia due to myelosuppression. Apart from the level of thrombocytopenia, also inflammation and vascular damage are associated with an increased risk of bleeding. It could be postulated that both the dynamics of thrombocytopenia as well as hemorrhage are determined by endothelial activation and damage, associated with inflammation.

Table 2: *Factors associated with increased bleeding (severity)*[5, 44, 46, 47, 51, 53, 32]

	Gaydos	Estey	Friedman	Nevo	Gil	Rebulla	Pihush	Kerkhoffs
Age								
WBC	●	●						
Uraemia			●					
Hypalbuminemia			●					
Bleeding			●			●		
Fever					●	●		
Infection						●	●	
VOD					●			
GVHD							●	
Mucosal damage					●			●
Platelet count	●			●		●	●	
Stem cell Tx			●				●	
Chemotherapy								●
Antifungal Rx					●	●		

WBC = White Blood cell Count; VOD = Venoocclusive Disease; GVHD = Graft versus Host Disease; Tx = Transplantation; Rx = Therapy

Refractoriness and bleeding in relation to survival

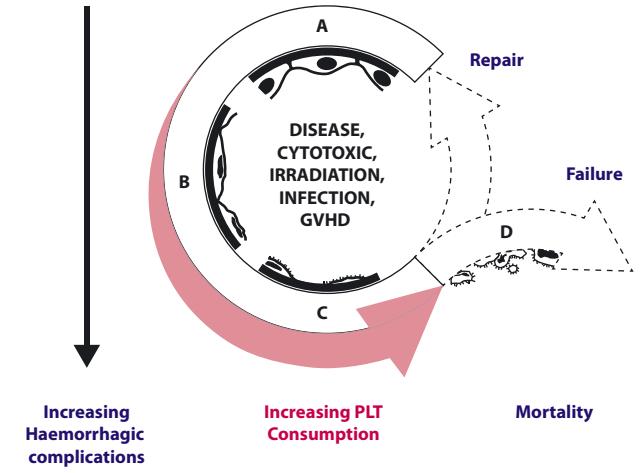
In a study in 1,402 patients, the first 100 days post transplant, Nevo et al showed that bleeding was both for allogeneic as well as well autologous patients associated with reduced survival.[55] Bleeding severity correlated with GVHD severity in 463 allogeneic transplant patients studied by Nevo et al. A significant association was found with gastrointestinal bleeding, hemorrhagic cystitis and pulmonary bleeding. Acute GVHD occurred early in the course post transplant and in 88% of the patients with bleeding and GVHD, bleeding episodes started after GVHD initiation. Both bleeding as well as GVHD were significantly associated with a reduced survival. Also, survival in non-bleeding patients was significantly reduced in patients with more pronounced thrombocytopenia perhaps suggesting more extensively injured endothelium.[47, 56, 58] The association between hemorrhagic complications, reduced survival and GVHD was also shown in another study of 807 allogeneic HSCT patients.[57] In the study by Pihush et al, haemostatic events (both thrombotic as well as hemorrhagic) were associated with an increased mortality risk (RR 1.7; 96%CI 1.0 – 3.2).[53] Intrigued by these findings, we studied the association of platelet refractoriness with patient survival. Surprisingly, patients experiencing one or more 24-hour platelet transfusion failures had, as compared to patients always showing a sufficient 24-hour increment, a significantly reduced survival, independent of therapy, diagnosis and age (Chapter 6).[32] As it is unlikely, that bleeding and refractoriness are directly causally related to the reduced survival, it is hypothesised that these are both confounders for vascular damage and /or microthrombosis.

Damaged endothelium: a common pathway?

Haemorrhagic complications, platelet refractoriness and deep, prolonged thrombocytopenia are repeatedly observed to define a category of patients with reduced survival. Without a logical direct causality, this leads to the hypothesis that these patients have more pronounced damage of the vascular endothelium (figure 2). As has been mentioned above, although thrombocytopenia in itself leads to endothelial flattening and fenestration, in the absence of an inflammatory process thrombocytopenia does not lead to haemorrhage in animal models unless deep thrombocytopenia which is associated with leakage of erytrocytes. Endothelial damage is regarded as a pathologic hallmark of vascular complications after HSCT, such as veno-occlusive disease of the liver, thrombotic microangiopathy, and capillary leak syndrome. In GVHD, the vasculature is sequentially affected. Endothelial damage is caused by the conditioning regimen, followed by neovascularisation and recruitment of inflammatory cells with in the third phase alloreactive T-cells targeting the endothelium.[59] The intensity of the conditioning regimen positively correlates with endothelial damage as measured by plasma levels of vWF, sVCAM-1 and sTNF receptor I.[60] Cyclic GMP, also a marker for endothelial damage, was a negative predictive factor for survival after HSCT.[61] Pericapillary hemorrhage was shown in areas EC lesions in severe intestinal GVHD and associated with severe hemorrhagic enterocolitis.[62] Circulating endothelial cells (ECs) are increased with endothelial damage and in patients after myeloablative conditioning an increasing number of ECs was found.[63, 64] In patients with GVHD significantly more EC microparticles were found as compared to patients without GVDH as are vWF and thrombomodulin.[65, 66] Moreover, factors like interleukin-1 and TNF-α have been shown to induce ultra structural changes in the blood-retina barrier.[67] Apart from GVHD, which might be a model to study bleeding susceptibility also for other conditions, several cytotoxic agents have been shown to cause endothelial damage and interestingly treatment with vascular endothelial growth factor inhibitors in oncology patients resulted in an increased incidence of both thrombotic as well as haemorrhagic complications.[68-70]

In conclusion deep aplastic thrombocytopenia causes endothelial fenestration and capillary leakage of erythrocyts. Its association with skin and mucous membrane bleeding is obvious, but an association with major bleeding is not proven. In contrast high blast counts, chemotherapy, irradiation, infection and GVHD associated endothelial damage are recognized to be associated with bleeding at varying degrees, as well as with thrombocytopenia. Enhanced endothelial damage due to these causes leads to increased consumption of both autologous as well as transfused platelets, haemorrhagic complications as well as ultimately a decreased patient survival. It is unclear to what extend transfused platelets are preventing these complications, although it seems unlikely that we will be able to prevent these complications just by transfusing platelets. Future studies are needed to test novel grading systems for the bleeding complications, preferentially distinguishing between just lack of endothelial repair and endothelial damage associated with activation. Endothelial maintenance benefits from platelet substitution and a relatively low number of platelets is sufficient. Endothelial damage may need another approach and it is questionable whether increase of the transfusion threshold and increasing the transfusion dose is the answer. It is challenging to validate the corrected count increment as a surrogate outcome parameter for survival as well as trying to make an IPSS-like scoring system to predict hemorrhagic complications to improve the platelet transfusion strategy on a patient level.

Figure 2: *Figure 2 summarizes the hypothesis of endothelial damage in relation to increasing platelet (PLT) consumption, haemorrhagic complications and survival. The figure represents segments of vascular endothelium showing normal endothelium (segment A), flattened and fenestrated endothelium occurring in thrombocytopenia (segment B), activated endothelium due to disease, cytotoxic agents, irradiation, GVHD and/or infectious agents (segment C) and damaged, apoptotic endothelium (segment D).*



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