Journal of Hematology & Thromboembolic Diseases

Wautier and Wautier, J Hematol Thrombo Dis 2018, 6:2
DOI: 10.4172/2329-8790.1000289

Review Article Open Access

Molecular Links between Erythrocyte Adhesion and Vascular Dysfunction in Diabetes Mellitus, Polycythemia Vera, Retinal Vascular Occlusion

Wautier JL* and Wautier MP

Faculty of Medicine, University Denis Diderot Paris 7, France

***Corresponding author:** Wautier JL, Faculty of Medicine, University Denis Diderot 7, 8 Avenue Leopold II, 75016 Paris, France, Tel: 33142060561; E-mail: jlwautier@hotmail.fr

Received date: March 20, 2018; **Accepted date:** May 08, 2018; **Published date:** May 14, 2018

Abstract

Despite clinical and epidemiological studies quantitative and qualitative abnormalities red blood cell (RBC) role in thrombosis has been ignored. Abnormal interaction of RBCs with vascular endothelium has been demonstrated in diabetes mellitus, Polycythemia vera (PV) and retinal vascular occlusion (RVO) after the first description in sickle cell anemia. The molecular basis has been identified. In diabetes mellitus, glycation of RBC band 3 caused the ligation to the receptor for advanced glycation end products (RAGE) present on endothelium. An abnormal Lu/BCAM (*CD 239*) expression in PV is responsible for the binding to endothelium annexin V. In RVO, RBC phosphatidylserine overexpression mediated the attachment to vascular laminin alpha-5 chain. Red blood cell binding altered the non-thrombotic properties of endothelium leading to thrombosis.

Keywords: Polycythemia vera; Retinal vascular occlusion; Diabetes mellitus; Thrombosis; Red blood cell adhesion; CD239; RAGE; Annexin V; Laminin alpha-5 chain; NADPH oxidase

Introduction

Vascular dysfunction and thrombosis are predominant causes of death and disability. Arterial thrombosis has been considered to be due to platelet accumulation at vessel site, thrombus formation at damaged endothelium. Red blood cell (RBC) qualitative or quantitative abnormalities have been observed, in ocular and renal complications of diabetes mellitus, in venous thrombosis in Polycythemia Vera (PV) and in Retinal Vein Occlusion (RVO).

Diabetes mellitus

Since diabetes mellitus was defined by Claude Bernard in the nineteen century we know that this is the first risk factor for vascular thrombosis. Increase in blood glucose is often associated with lipid disorders and platelet hyper reactivity. In 1981 we have been able to demonstrate, using the same system as that described by Hebbel [1] that RBCs of diabetic patients have an increased adhesion to endothelium correlated with the severity of vascular injury [2]. RBC adhesion was measured in static conditions using chromium51 labelled erythrocytes and cultured human umbilical vein endothelial cells. Results were expressed as an adhesion ratio (AR) calculated by measuring the radioactivity remaining after washes corresponding to adherent RBCs from patients with diabetes mellitus versus normal subjects. The mean AR percentage of adhering blood cells from diabetic patients versus percentage of adhering control RBCs was 2.33 (range 0.8 to 5.2, $p<0.001$). The extent of adhesion expressed as an AR was correlated with severity of vascular complications. We have, in a group of diabetic patients followed for 1 year, observed that in the same patient adherence rates evolved in parallel with glycated hemoglobin HbA1c [3]. It took 13 years for us to discover what was the molecule responsible for endothelial adhesion [4]. With antibodies and recombinant molecules we demonstrated that the Receptor for Advanced Glycation End products (RAGE) is the endothelial receptor for RBCs of diabetic patients [5]. In Diabetes Mellitus RBC band 3 protein is glycated and binds to the receptor for Advanced Glycation End products [6]. In diabetes mellitus, one major mechanism of endothelial disturbance is the activation of a cascade reaction leading to NADPH oxidase stimulation, NFkB activation and consequently gene expression of factors implicated in inflammatory reactions [7]. RBC adhesion to endothelium alters several functions leading to an inflammatory reaction: Induction of adhesion molecules Vascular Cell Adhesion Molecule-1 (*VCAM-1*) Inter Cellular Adhesion Molecule-1 (*ICAM-1*) cytokine secretion Interleukin-6 (*IL6*), Vascular Endothelial cell Growth Factor (VEGF), Macrophage-Chemoattractant Protein-1 (*MCP-1*), tissue factor (TF) expression, augmentation of vascular permeability, alteration of Nitric Oxide (NO) metabolism (Figure 1).

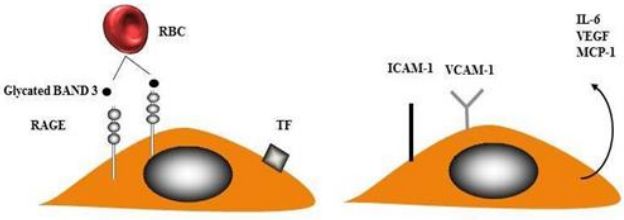

Figure 1: Erythrocyte adhesion in Diabetes mellitus.

RBC band 3 protein is glycated in patients with diabetes mellitus and binds to the receptor for Advanced Glycation End Products (RAGE). Engagement of endothelial RAGE induced Tissue Factor (TF) production, Intercellular Cell Adhesion Molecule-1 (*ICAM-1*) and Vascular Cell Adhesion Molecule-1 (*VCAM-1*) expression, and Interleukin-6 *(IL-6),* Vascular Endothelial Growth Factor (VEGF), Macrophage Chemoattractant Protein-1 *(MCP-1)* release.

Forty percent of NO released by endothelium cannot penetrate into RBC. Free hemoglobin binds NO and blocks its anti-adhesion properties and vasodilatation Protein glycation is associated with a high risk of vascular complications, especially in the microcirculation responsible for retinopathy and diabetic nephropathy [8]. Inhibition of AGE formation in animal models prevents or reduces the occurrence of vascular complications [9]. In the Han Chinese population a single nucleotide polymorphism in the myeloperoxidase (MPO) gene was associated with type 2 diabetes mellitus susceptibility. In the same population glutathione peroxidase-1 *(GPX1)* rs1050450 and MPO rs2107545 were significantly associated with increased risk of carotid plaques in type 2 diabetes mellitus patients [10]. Current literature has identified several biomarkers that are known to play a key role in cardiovascular disease. Many of these of biomarkers are influenced by an increased oxidative stress. The biomarkers stem from seven major pathways; NFkB, Nrf2 antioxidant response element signaling pathway *(Keap1-Nrf2)*, protein kinase-C, macrophage activation, arachidonic acid mobilization, endothelial cell dysfunction and AGE. It appears that RAGE is probably involved in different mechanisms leading to cardiovascular complications [11]. In human the most effective strategies are currently a good balance of diabetes, treatment with anti-free radicals. New strategies are tested including *SGLT1* and *SGLT2* inhibitors (glifozins), Dipeptidyl-peptidase-4 inhibitor (gliptins), *GLP1* analogs (incretins) or polytherapy which associated metformin, glifozins, and gliptins [12,13]. Dipeptidyl peptidase-4 *(DPP4)*-inhibitors, in addition to their effect through improvement of glycemic control, may act directly on the AGE-RAGE axis. Furthermore, while most studies aiming at blocking AGE-RAGE axis have been disappointing, trials investigating the effects on diabetic nephropathy of a AGE inhibitor, pyridoxamine dihydrochloride (Pyridorin©) are presently going on. Blockade of RAGE by antibodies anti-RAGE, RAGE analogs TTP488 (azeliragon) or recombinant rRAGE prevents or limits the deleterious effect of AGE (Figure 2) [14].

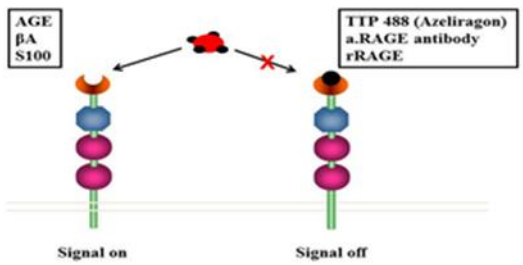

Figure 2: Schematic representation of receptor for AGE (RAGE) and ligands.

Interaction with ligands Advanced glycation end products (AGE) β-amyloid peptide (βA) S100 calgranulin (S100) and inhibition of this interaction by TTP488 (azeliragon), anti-RAGE antibodies, recombinant RAGE (rRAGE).

Polycythemia vera

Polycythemia Vera (PV) is a chronic disorder in which the clonal proliferation of multipotent hematopoietic cells results in an increase in the red cell mass. This expansion is associated with circulatory disturbances mostly related to hyperviscosity. PV is the most common myeloproliferative syndrome, characterized by erythropoietin-independent erythroid colony formation invitro, and was recently shown to be associated in most cases to a somatic point mutation of the *JAK2* tyrosine kinase *(JAK2 V617F)* [15,16], a substitution from valine to phenylalanine at position 617, which renders JAK2 constitutively active, leading to uncontrolled cell proliferation in the erythroid lineage and resulting in increased red cell mass. In addition another JAK2 exon 12 mutation *(K539L)* was described in patients with PV and idiopathic erythrocytosis [17]. Since the first description of PV in 1892 in a patient with thrombosis, thromboembolism remains a major cause of mortality and morbidity in PV. However the optimal management of patients with PV remains controversial. Phlebotomy and aspirin are still the first line therapy but despite red cell mass reduction, the risk of thrombosis remains higher than in a population of sex and age matched subjects. In addition the use of anti-platelet agents can increase the risk of hemorrhage. The most common vascular events were acute coronary syndrome and transitory ischemic attack [18]. Because adhesion of RBCs to endothelium was shown to be correlated to vascular risk in sickle cell anemia and diabetes mellitus we investigated whether RBCs from patients with PV had abnormal interactions with endothelium by measuring RBC adhesion to Human Umbilical Vein Endothelial Cells (HUVEC) under static conditions (Figure 3) [19].

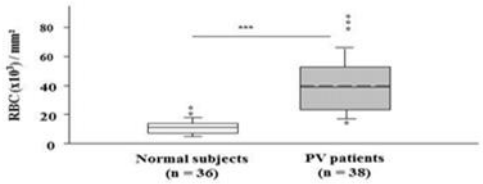

Figure 3: RBCs from patients with PV adhere in greater numbers to endothelial cells than RBCs from healthy volunteers under static conditions.

RBC adhesion was measured using 51Cr-labeled RBCs. The nonadherent RBCs were removed by successive washes and the radioactivity was measured in each wash and in the remaining RBCs attached to endothelial cells. The unbroken line within the box represents the median; the dotted line represents the mean. The vertical lines extending beyond the boxes indicate the 25% and 75% percentiles, while the horizontal bars outside the boxes represent the 10% and 90% percentiles. The open circles indicate the values outside this range.

We also evaluated adhesion molecule expression on RBC using specific antibodies and flow cytometry. Blocking experiments indicated that PV RBC adhesion was mediated by CD239, (Lutheran blood group/basal cell adhesion molecule Lu/BCAM), on the RBC side and laminin alpha-5 chain on the endothelial side. It has been shown that the adhesion molecule Lu/BCAM, the unique erythroid receptor for laminin alpha-5 chain, is phosphorylated when sickle RBCs are

stimulated by epinephrine and that this leads to increased adhesion to laminin alpha-5 chain [20]. We showed in our previous work that PV RBC adhesion to laminin alpha-5 chain was associated with Lu/BCAM phosphorylation (Figure 4).

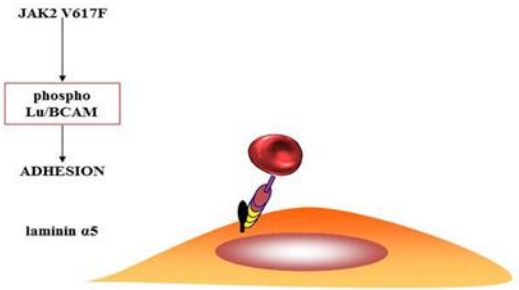

Figure 4: Adhesion of RBCs from patients with Polycythemia vera.

Erythrocyte Lu/BCAM *(CD 239)* from patients with polycythemia vera (PV) is phosphorylated and mediated adhesion to endothelial cell laminin alpha-5 chain (laminin α5).

Lu/BCAM phosphorylation was also strongly increased in a K562 cell line co-expressing Lu and *JAK2V617F*, suggesting that erythroid activation of Lu/BCAM could result from the presence of *JAK2V617F* in PV. Growing evidence supports the hypothesis that endothelial dysfunction might contribute to thrombotic events by orchestrating the recruitment of blood cell elements to sites of injury. In PV patients with Budd-Chiari syndrome and portal vein thrombosis, Sozer et al showed that cells with endothelial characteristics lining liver sinusoids and venules were *JAK2V617F* positive [21]. Using an immunodeficient mouse transplant assay system, the same group showed that JAK2V617F positive CD34+ cells were able to generate endothelial like cells *invivo* expressing either human wild type *JAK2* or *JAK2V617F*. Endothelial cells express a wide range of adhesion proteins including integrins and CAMs (ie Cell Adhesion Molecules). We showed that *JAK2V617F* is able to activate Lu/BCAM through Rap1 and Akt that are both ubiquitously expressed proteins regulating cell adhesion and cell-cell interactions. Several type I ATP-competitive JACK inhibitors were assessed in clinical trial and exhibited minimal hematologic toxicity. Ruxolitinib despite a weak effect on the cause of the disease improve the clinical state of patients and increases survival in myelofibrosis [22]. The lack of complete response in most patients treated with JAK2 inhibitor ruxolitinib indicates the need for identifying novel therapeutic strategies. Metformin is a biguanide that exerts anti-neoplastic activity in hematological malignancies. Metformin plus ruxolitinib demonstrated more intense reduction of cell viability and induction of apoptosis compared to monotherapy. Metformin produced a downregulation of JACK2/STAT signaling [23].

Retinal vascular complications

Retinal vein occlusion (RVO) is a common cause of permanent visual loss and is the fifth cause of unilateral blindness. The most frequent and less severe type, Branch Retinal Vein Occlusion (BRVO) is possibly driven by a mechanical factor because it generally occurs at an arteriovenous crossing. Retinal Artery Occlusion (RAO) may also occur. The most rare and sight-threatening form, Central Retinal Vein Occlusion (CRVO) remains of unknown pathophysiology Aging, arterial hypertension, and glaucoma are the only well-established risk factors for RVO. Despite a number of studies, thrombophilic risk factors have not been strongly associated with RVO, which suggests a very limited role of coagulation or anticoagulation factors in the pathophysiology of the disease. In contrast, substantial data exist suggesting that blood hyperviscosity is an important risk factor. Several blood viscosity parameters have been shown to be increased in RVO patients compared with normal subjects, including a higher hematocrit, higher whole blood viscosity, reduced red cell deformability, and enhanced index of erythrocyte aggregation. The laser induced RVO mouse model is characterized by a predominant inflammatory and tissue damage response. Hypoxia was observed in all RVO eyes for up to five days but no significant RVO-dependent changes in gene expression were detected for angiogenesis or hypoxia related genes [24]. Adhesion molecules *(CD36, CD47, CD49d, CD239)* when tested on RBCs by flow cytometry were similarly expressed on CRVO or RAO RBCs. CRVO patients had a higher number of RBCs expressing membrane phosphatidylserine (PS) compared with RBCs from normal subjects or RAO patients [25]. Under static conditions adhesion of RBCs from patients with CRVO to endothelial cells was significantly increased compared with adhesion of normal RBCs and of patients with RAO (Figure 5).

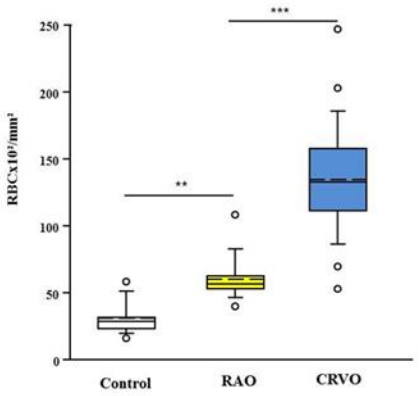

Figure 5: Adhesion of RBCs from patients with retinal vascular occlusion (**p<0.01, ***p<0.001).

RBCs from patients with Central Retinal Vein Occlusion (CRVO) adhere in greater numbers on human microvascular endothelial cells than do RBCs from patients with retinal artery occlusion (RAO) or RBCs from healthy volunteers under static conditions. The unbroken line within the box represents the median; the dotted line represents the mean. The vertical lines extending beyond the boxes indicate the 25% and 75% percentiles, while the horizontal bars outside the boxes represent the 10% and 90% percentiles. The open circles indicate the values outside this range.

In a flow based adhesion assay RBCs from patients with CRVO adhered in a greater number and were more resistant to washout than control RBCs ($p<0.001$). The number of RBCs remaining adherent after washout at the highest shear stress (0.3Pa) was 20 ± 8/mm^2 for

the patients and <1/mm^2 for the controls. The percentage of PS-positive RBCs was significantly higher in CRVO patients and was correlated with the extent of RBC adhesion. The anti-PS receptor antibodies inhibited 55% of RBC adhesion in static conditions. Annexin V, which binds to PS, was the most efficient RBC adhesion blocker (Figure 6) [25].

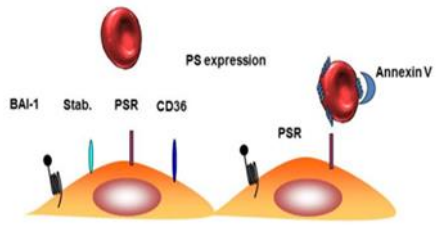

Figure 6: Schematic representation of adhesion of RBCs from patients with Central Retinal Vein Occlusion.

Blockade of Phosphatidylserine (PS) RBCs and endothelial PS receptor by annexin V or anti-PS receptor indicated that the couple PS-PS receptor is responsible for increased adhesion of RBCs from patients with CRVO. PS Receptor (PSR), Brain-specific angiogenesis inhibitor-1 (BAI-1), Stabilin-2 Stab (Stab) and CD36, potential ligands for PS are present on endothelial cells.

These results reinforce the hypothesis that PS exposure on CRVO RBCs is an important parameter for the increased RBC adhesion. The possible use of recombinant annexin V or derived peptides as a treatment for CRVO and similar disorders was patented in USA (US patent number 9463217, October 2016).

Conclusion

In a recent review article Byrnes and Wolberg [26] reactivate the concept that red blood cell could be a trigger for vascular thrombosis. The authors quoted numerous articles dealing with different routes by which RBCs may be involved in thrombosis, via platelet activation, rheological mechanisms and interaction with endothelium. Since the description of a link between increased erythrocyte adhesion and vascular complications in diabetes, several publications have described the consequences of RBC interaction with endothelium, mostly in sickle cell anemia. Different RBC molecules and different endothelial cell receptors have been identified to be responsible for the abnormal RBC-Endothelium interactions. In Diabetes Mellitus RBC band 3 protein is glycated and binds to RAGE. In PV the mutation of the kinase *JAK2* results in phosphorylation of Lu/BCAM (*CD239*) which is a ligand for endothelial laminin alpha-5 chain. In CRVO phosphatidylserine overexpression mediated the attachment to vascular laminin alpha 5-chain. These different examples indicated that RBC adhesion to endothelium may participate in the development of vascular complications in several diseases through different adhesion mechanisms. The role of RBCs in vascular complications suggests that new therapies might target the involvement of RBCs in vascular thrombosis. Indeed, previous experimental studies or ongoing clinical trials demonstrated that infusion of antibodies against adhesion molecules or peptides derived from adhesion molecules, may change the dramatic consequences of vascular occlusion and prevent in diabetic rats the occurrence of blindness [27].

References

1. Hebbel RP, Boogaerts MA, Eaton JW, Steinberg MH (1980) Erythrocyte adherence to endothelium in sickle-cell anemia. A possible determinant of disease severity. N Engl J Med 302: 992-995.
2. Wautier JL, Paton RC, Wautier MP, Pintigny D, Abadie E, et al. (1981) Increased adhesion of erythrocytes to endothelial cells in diabetes mellitus and its relation to vascular complications. N Engl J Med 305: 237-242.
3. Wautier JL, LeBlanc H, Wautier MP, Abadie E, Passa P, et al. (1986) Erythrocyte adhesion to cultured endothelium and glycaemic control in type 1 (insulin-dependent) diabetic patients. Diabetologia 29: 151-155.
4. Wautier JL, Wautier MP, Schmidt AM, Anderson GM, Hori O, et al. (1994) Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: A link between surface-associated AGEs and diabetic complications. Proc Natl Acad Sci USA 91: 7742-7746.
5. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, et al. (1996) Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. J Clin Invest 97: 238-243.
6. Grossin N, Wautier MP, Picot J, Stern DM, Wautier JL (2009) Differential effect of plasma or erythrocyte AGE-ligands of RAGE on expression of transcripts for receptor isoforms. Diabetes Metab 35: 410-417.
7. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, et al. (2001) Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 280: E685-694.
8. Wautier MP, Boulanger E, Guillausseau PJ, Massin P, Wautier JL (2004). AGEs, macrophage colony stimulating factor and vascular adhesion molecule blood levels are increased in patients with diabetic microangiopathy. Thromb Haemost 91: 879-885.
9. Tamarat R, Silvestre JS, Huijberts M, Benessiano J, Ebrahimian TG, et al. (2003) Blockade of advanced glycation end-product formation restores ischemia-induced angiogenesis in diabetic mice. Proc Natl Acad Sci USA 100: 8555-8560.
10. Liu D, Liu L, Hu Z, Song Z, Wang Y, et al. (2018) Evaluation of the oxidative stress-related genes ALOX5, ALOX5AP, GPX1, GPX3 and MPO for contribution to the risk of type 2 diabetes mellitus in the Han Chinese population. Diab Vasc Dis Res.
11. Robson R, Kundur AR, Singh I (2017) Oxidative stress biomarkers in type 2 diabetes mellitus for assessment of cardiovascular disease risk. Diabetes Metab Syndr.
12. Rosenstock J, Hansen L, Zee P, Li Y, Cook W, et al. (2015) Dual add-on therapy in type 2 diabetes poorly controlled with metformin monotherapy: A randomized double-blind trial of saxagliptin plus dapagliflozin addition versus single addition of saxagliptin or dapagliflozin to metformin. Diabetes Care 38: 376-383.
13. DeFronzo RA, Lewin A, Patel S, Liu D, Kaste R, et al. (2015) Combination of empagliflozin and linagliptin as second-line therapy in subjects with type 2 diabetes inadequately controlled on metformin. Diabetes Care 38: 384-393.
14. Wautier MP, Guillausseau PJ, Wautier JL (2017) Activation of the receptor for advanced glycation end products and consequences on health. Diabetes Metab Syndr 11: 305-309.
15. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, et al. (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365: 1054-1061.

16. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, et al. (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434: 1144-1148.
17. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, et al. (2007) JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 356: 459-468.
18. Hintermair S, Zwickl-Traxler E, Pecherstorfer M, Singer J (2018) Evaluation of vascular events in patients with myeloproliferative syndromes and mutations of either the januskinase-2 or calreticulin gene at the university hospital Krems from 2008 to 2015. Oncotarget 9: 8450-8462.
19. Wautier MP, El Nemer W, Gane P, Rain JD, Cartron JP, et al. (2007) Increased adhesion to endothelial cells of erythrocytes from patients with polycythemia vera is mediated by laminin alpha5 chain and Lu/BCAM. Blood 110: 894-901.
20. Gauthier E, Rahuel C, Wautier MP, El Nemer W, Gane P, et al. (2005) Protein kinase A-dependent phosphorylation of Lutheran/basal cell adhesion molecule glycoprotein regulates cell adhesion to laminin alpha5. J Biol Chem 280: 30055-30062.
21. Sozer S, Fiel MI, Schiano T, Xu M, Mascarenhas J, et al. (2009) The presence of JAK2V617F mutation in the liver endothelial cells of patients with Budd-Chiari syndrome. Blood 113: 5246-5249.
22. Vainchenker W, Leroy E, Gilles L, Marty C, Plo I, et al. (2018) JAK inhibitors for the treatment of myeloproliferative neoplasms and other disorders. F1000Res 7: 82.
23. Machado-Neto JA, Fenerich BA, Scopim-Ribeiro R, Eide CA, Coelho-Silva JL, et al. (2018) Metformin exerts multitarget antileukemia activity in JAK2 (V617F)-positive myeloproliferative neoplasms. Cell Death Dis 9: 311.
24. Martin G, Conrad D, Cakir B, Schlunck G, Agostini HT (2018) Gene expression profiling in a mouse model of retinal vein occlusion induced by laser treatment reveals a predominant inflammatory and tissue damage response. PLoS One 13: e0191338.
25. Wautier MP, Heron E, Picot J, Colin Y, Hermine O, et al. (2011) Red blood cell phosphatidylserine exposure is responsible for increased erythrocyte adhesion to endothelium in central retinal vein occlusion. J Thromb Haemost 9: 1049-1055.
26. Byrnes JR, Wolberg AS (2017) Red blood cells in thrombosis. Blood 130: 1795-1799.
27. Wautier JL, Wautier MP (nov.9 2017) Red blood cell a culprit for thrombosis. Blood 130: 1795-1799.