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Reflections on the unexpected laboratory finding of hemorheological alterations observed in some haematological disorders

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ABSTRACT

Hyperviscosity syndrome is a clinical condition characterized by the slowing of blood flow through the vessels and it may be associated with several diseases. The nosographic classification of primary hyperviscosity conditions (Wells classification 1970) divided the primary hyperviscosity syndromes in polycythaemic, sclerocytemic and sieric.

Recent and personal laboratory observations have highlighted an unexpected behaviour of the erythrocyte deformability observed in some haematological disorders such as polycythemia vera, multiple myeloma and monoclonal gammopathy of undetermined significance. The interest of this observation depends on the fact that up to now, according to the Wells classification, the hemorheological alteration present in PV was related to the increase of RBC mass while that present in MM and MGUS was attributable to the abnormality of plasma or serum viscosity only.

Through an extensive research among the literature, using MEDLINE/PubMed to identify all published reports on the hyperviscosity syndromes, issues that until now have been dealt with separately will therefore be analyzed in a unique paper, allowing a global view.

The aim of this paper is to provide some suggestions for reflection and emphasizing the need of a nosographic framework of hyperviscosity that, probably, deserves to be reviewed.

1. Introduction

In the past years, the data collected in our hemorheological laboratory on the major determinants of whole blood viscosity, has given us the chance to reflect on the encoded classifications. These are in compliance with Copley (1987) and the literature on modern clinical hemorheology. It is normal practice to follow the Well classification (Wells, 1970) when it comes to hemorheological laboratory findings. However, hemorheological data should not only consider the vascular system and the hemodynamic profile but include also the complex mechanism of the endothelial function.

At first, in fact, the hemorheological alterations in clinical and experimental research were related in particular to the vascular resistance and to the cardiac output (Dormandy, 1970; Dormandy, 1971), while later on the hemorheological variations were examined also in the light of the endothelium integrity and endothelium function (Forconi et al., 2011; Forconi and Gori, 2013). For instance, if on the one hand the increase in plasma viscosity affects the blood flow resistance, on the other this same increase in plasma viscosity reduces or mitigates the vascular resistance (Cabrales and Tsai, 2006; Salazar Vazquez et al., 2009), increasing the endothelial NO synthesis by activating the endothelial nitric oxide synthetases. As it is known, the activation of this enzyme results especially associated to the variations of the wall shear stress, this link justifies the disagreement found in the evaluation of whole blood viscosity in “vivo” and “vivo”.

With reference to the variations concerning the plasma viscosity on the endothelial function, it would be interesting to demonstrate the opposite condition, or rather in the event of plasma hypoviscosity in what way would it occur in some clinical disorders such as acquired or congenital hypofibrinogenemia and afibrinogenemia.

Another interesting focus is the behaviour of whole blood viscosity, without mentioning the different aspects observed in macro and in microcirculation (Gaehtgens et al., 1987; Lipowsky, 2007). As it is known, whole blood is an example of a non-Newtonian fluid, because of

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its nature it is a suspension of viscoelastic cellular components in plasma, therefore the whole blood viscosity varies with its shear rate. In the last decades, clinical and experimental data have demonstrated that erythrocyte deformability and plasma viscosity are important at high shear rates, while erythrocyte aggregation occurs at low shear rates. In this regard, it must be highlighted that in microcirculation, high shear flow is predominant and from this assertion, the pivotal role exerted by erythrocyte deformability and plasma viscosity in this vascular district may be properly explained; in the same district, together with these hemorheological determinants, also the platelets exert a significant role.

Going back to the Wells classification (Wells, 1970), published in 1970, that divides rigorously the primary hyperviscosity syndromes in polycythaemic, sclerocytemic and sieric, differently from the secondary hyperviscosity syndromes; some of our hemorheological results in clinical disorders have shown an unexpected hemorheological profile and these findings have underlined the complex task of classifying hemorheological alterations, also bearing in mind the new and advanced techniques employed to measure whole blood viscosity determinants.

This overview was necessary to introduce the findings collected by our research team in haematological disorders generally found in polycythaemia and sieric syndromes.

In all the haematological disease evaluated, we have examined the erythrocyte deformability as below: we mixed 30 μl of anticoagulated blood with 2 ml of dextran solution at a viscosity of 24 mPa. The measurement was obtained using the diffractometer Rheodyn SSD of Myrenne (Ruef et al., 1996; Schmid-Schönbein et al., 1996; Hardeman et al., 2007); which measures the diffraction pattern of a laser beam passing through erythrocytes suspended in a viscous medium and deformed by a force with defined shear stress. The shear stresses employed were 6, 12, 30 and 60 Pa. The erythrocyte deformation was expressed as elongation index (EI) = (L – W / (L + W) × 100, were L = length and W = width of the erythrocytes.

2. Polycythaemia vera

In the PV, a clonal myeloproliferative disease which is a part of the hyperviscosity polycythaemic syndrome, we have examined the erythrocyte deformability by using the laser diffractometer (Rheodyn SSD-Myrenne).

Specifically, we have selected 26 PV patients (16 men and 10 women; mean age 62.3 ± 9.3 yrs.; range 41–75 yrs.) of whom 21(81%) were JAK2 positive. The patients previously had been treated with phlebotomy or with cytoreductive therapy (hydroxyurea, pipobroman) or both. The same evaluation has been done for a group of 29 subjects (16 men and 13 women; age range 35–52 yrs.) free of any medical conditions as confirmed by their medical history, physical examination and routine haematological and urinalysis.

For PV patients, in comparison with normal controls (C), the erythrocyte deformability, expressed as elongation index (IE), was significantly decreased at the shear stress of 6 Pa (C = 28.81 ± 6.93, PV = 21.57 ± 5.14 $p < 0.001$), 12 Pa (C = 36.40 ± 6.73, PV = 29.86 ± 5.31 $p < 0.001$), 30 Pa (C = 43.72 ± 6.10, PV = 38.91 ± 5.36 $p < 0.01$) and 60 Pa (C = 46.94 ± 5.36, PV = 43.17 ± 5.44 $p < 0.05$).

We believe that this unexpected finding is related to the metabolic abnormalities described in red blood cells of PV patients. In fact, for the erythrocytes of PV patients, it has been observed respectively: a) a high activity of glycolytic enzymes (Streichman et al., 1986) b) a reduction of ATP and 2.3 DPG associated with an increased synthesis of lactate in vitro (Streichman et al., 1986; Arnaud et al., 1991); c) an increase in erythrocyte membrane lipids associated with increased phosphatidylserine exposure (Streichman et al., 1986; Fujita et al., 2010; Wautier et al., 2011); d) an increment in fetal haemoglobin (Hoffman et al., 1979); e) a higher electrophoretic mobility (Streichman et al., 1981); f) an increase in sialic acid concentration (Streichman et al., 1981) or a reduced variety of membrane sialic acids (Bratosin et al., 2007).

Other authors (Dabrowski et al., 2011) have found a significant decrease of erythrocyte deformability in patients with PV, which has been associated to the increased concentration of glutathione and malonyl dialdehyde.

Our previously published data (Lo et al., 2012), in agreement with other authors (Dabrowski et al., 2011), suggest that hemorheological determinants may affect the pathophysiology of the hyperviscosity syndrome which occurs with polycythaemia vera.

3. Multiple myeloma

Multiple myeloma is a neoplasm of the plasma cells ranging from asymptomatic to aggressive symptoms due to the production of abnormal immunoglobulin or part of them.

In this haematological disorder, that is part of primary sieric hyperviscosity syndromes, we have examined (Caimi et al., 2018a) the erythrocyte deformability by using the laser diffractometer (Rheodyn SSD- Myrenne). In such regard, we have enrolled 29 patients (18 men and 11 women; mean age 67.9 ± 10.6 yrs.) with multiple myeloma. Sixteen patients had been recently diagnosed or they were at the initial stage of therapy, 8 were under treatment for relapse of disease, whereas 5 patients had achieved a complete remission. The same hemorheological evaluation has been performed in a control group of 31 subjects (13 men and 18 women; age range 23–65 yrs.) free of medical conditions, as confirmed by their medical history, physical examination, routine haematological and urinalysis.

In MM patients, in comparison with normal controls (C), the erythrocyte deformability, expressed as elongation index (IE), was significantly reduced at the shear stress of 6 Pa (C = 24. ± 94 2.32, MM = 16.25 ± 4.87 $p < 0.001$), 12 Pa (C = 33.29 ± 2.18, MM = 26.54 ± 5.31 $p < 0.001$), 30 Pa (C = 41.67 ± 2.20, MM = 36.43 ± 5.07 $p < 0.001$) and 60 Pa (C = 45.19 ± 2.52, MM = 42.34 ± 4.65 $p < 0.01$).

The reduction of this hemorheological parameter observed in MM patients may be explained with the alteration of membrane lipid composition described in this haematological disorder. In MM patients an alteration of the erythrocyte membrane fatty acid composition has been found: an increase in saturated fatty acids and in total polyunsaturated fatty acids (n-6), and a decrease in monounsaturated fatty acids, in total polyunsaturated fatty acids (n-3), in total trans fatty acids, and in the ratio n-3/n-6 (Jurczyszyn et al., 2014). In MM patients also the plasma fatty acid pattern shows: an increase in saturated fatty acids, in monounsaturated fatty acids and in total polyunsaturated fatty acids (n-6) and a reduction in polyunsaturated fatty acids (n-3), in total trans fatty acids, and in the ratio n-3/n-6 (Jurczyszyn et al., 2015).

The observed alterations in erythrocyte membrane seem related to the functional alteration of the desaturase and elongase, enzymes that are pivotal in the maintenance of the lipid network in biological membranes (Nakamura and Nara, 2004; Rzehak et al., 2009; Wakil, 1989; Wakil et al., 1983).

Other authors (Zhang et al., 2012), by using the AFM, have demonstrated that the surface topographic image, the eight profile and the surface ultrastructure of the red blood cells distinguish the erythrocyte of healthy subjects from those of MM patients. Instead, other authors (Liu and Li, 2014), have found in MM erythrocytes also a marked irregularity of the outline of the histograms of the particle size extracted from the surface ultrastructure.

The altered membrane lipid profile, as well as the significant anomalies observed by using the AFM, may explain the behaviour of the erythrocyte deformability observed in MM patients.

From a hemorheological perspective, MM is then a haematological neoplasm that impairs significantly the microcirculation by an increase in the plasma viscosity and by a decrease in erythrocyte deformability at the same time; both these hemorheological determinants act upon the microcirculatory district, affecting the levels of oxygen reaching the tissues.

4. Monoclonal gammopathy of undetermined significance

MGUS is defined for the presence of a monoclonal protein at a serum concentration <30 g/l, clonal plasma cells in bone marrow <10%, no *endo*-organ damage and no evidence of B-cell lymphoma or other diseases is known to produce an M-protein. MGUS is not considered a neoplastic disorder since it does not always progress to overt malignancy. In this clinical disorder, observed in approximately 3% of subjects aged over 50 and in more than 5% of individuals older than 70 years, more common in men than women, the presence of a paraprotein may alter the whole blood viscosity and in particular plasma viscosity.

Also in MGUS, that theoretically might be part of primary sieric hyperviscosity, we have evaluated (Caimi et al., 2018b) the erythrocyte deformability by using laser diffractometer (Rheodyn SSD-Myrenne). In that regard, we selected 21 MGUS subjects (11 men and 10 women, mean age 66.4 ± 11.6 yrs.). The M-protein isotype was IgG in 18 subjects and IgM in 2 subjects; in 1 subject, instead, a monoclonal IgG and IgA were found together. The same evaluation was undertaken for a control group of 21 subjects (13 men and 8 women; age range 23–63 yrs.) whose good health was ascertained by their clinical history, physical examination, electrocardiography, routine blood and urine analyses.

In MGUS subjects, in comparison with normal control (C), the erythrocyte deformability, expressed as elongation index (IE) was significantly reduced at the shear stress of 6 Pa (C = 25.05 ± 2.39, MGUS = 14.64 ± 3.19 $p < 0.001$), 12 Pa (C = 33.37 ± 2.35, MGUS = 23.47 ± 3.27 $p < 0.001$), 30 Pa (C = 41.84 ± 2.42, MGUS = 34.34 ± 3.11 $p < 0.001$) and 60 Pa (C = 45.13 ± 2.67, MGUS = 40.24 ± 2.93 $p < 0.001$).

It is not easy to explain this hemorheological alteration in MGUS, that is a benign disorder and in which the only expected hemorheological alteration should regard plasma viscosity.

In MGUS subjects no abnormalities in the erythrocyte membrane lipid profile, plasma lipid composition or in fatty acid metabolism are known, whereas such alteration has been observed in patients with MM.

To date, the only abnormality of the red cell membrane observed by some authors (Fukumoto and Gotlib, 2006; Melitis et al., 2002) in MGUS subjects seems to refer to membrane proteins consisting of paroxysmal nocturnal haemoglobinuria-like (PNH-like) defect. This is a disease characterized by an altered synthesis of glycosylphosphatidylinositol that results essential for the binding of some surface proteins, such as CD55 and CD59, that protect the red blood cells from lysis complement-related-.

In order to verify if the reduced erythrocyte deformability observed in MGUS subjects was linked to the presence of a possible PNH clone, we performed a specific cytofluorimetric analysis (unpublished data) and the results show that 15 MGUS subjects examined have not this anomaly.

Although MGUS is an asymptomatic premalignant disorder, it seems to be associated with an alteration of the hemorheological profile including not only the increase in plasma viscosity but also an unexpected reduction in erythrocyte deformability.

5. Conclusions

Hyperviscosity syndrome is a clinical condition that can be observed in various diseases and, therefore, requires careful evaluation.

Over the years, the need for a complete knowledge of hyperviscosity syndrome has led to a specific nosographic framework, based on pathophysiology and which, according to Well's classification of 1970, divided the primary hyperviscosity syndromes into polycythaemic, sclerocytemic and sieric.

Up to now, this overview has represented the main tool for evaluating hyperviscosity syndromes, with particular attention to the possible mechanism responsible for the slowing of blood flow through the vessels.

However, from our data produced by the analysis of patients with different haematological diseases, it emerges that in some clinical conditions the viscosity may not only be related to the cell mass or to serum and/or plasmatic determinants. In PV, for example, a reduced erythrocyte deformability was observed, an element which, together with the increase in the mass of red blood cells, could contribute to the increase of hyperviscosity state.

The data from patients with MM and MGUS are interesting. In the specific case of MM patients, the serum protein share alone could explain the hyperviscosity, albeit with a mitigating effect induced by the low hematocrit values, but also in these patients the deformability of the erythrocyte is reduced, with a possible effect on the development of hyperviscosity. Even more intriguing is the case of subjects with MGUS in which the whole blood viscosity is higher than in normal controls, even in the absence of not very high concentrations of serum proteins, and in which an alteration of the erythrocyte deformability has also been observed.

All these information underline the hemorheological complexity of each haematological disease and in particular the difficulty to interpret the hemorheological data without any clinical contextualization.

Furthermore, the need for new evaluation and analysis tools becomes increasingly important, and, probably, it will be also necessary a new nosographic classification of hyperviscosity syndromes, in which other parameters should be evaluated.

Indeed, the Well classification (Wells, 1970) not covers all the hemorheological aspects associated to haematological and non-haematological alterations, and we believe that no other more recent classifications seem to achieve this aim. Specifically, by examining the classification suggested by Baskurt (2007), Dumas et al. (2015) and Sloop (2017) neither of them takes into account mixed forms of hemorheological alterations which may be found in clinical practice.

In conclusion, despite the clinical and scientific value of the above classifications, we believe could be useful a new overview including a specific clinic contextualization of hemorheological data.

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Declaration of competing interest

the authors report no conflict of interest.

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References

Arnaud, J., Pris, J., Brun, H., Constans, J., 1991. Consequences of moderate hypoxia on red cell glycoltic metabolism in polycythemia rubra vera. Ann. Biol. Clin. 49, 9–13.
Baskurt, O.K., 2007. Mechanisms of blood rheology alterations. In: Baskurt, O.K., Hardeman, M.R., Rampling, M.W., Meiselman, H.J. (Eds.), Handbook of Hemorheology and Hemodynamics. IOS Press, Amsterdam, pp. 170–190.
Bratosin, D., et al., 2007. Reduced diversity of the human erythrocyte membrane sialic acids in polycythemia vera. Absence of N-glycolyneuraminic acid and characterisation of N-acetylneuraminic acid 1,7 lactone. Biochimie 89, 355–359.
Cabrales, P., Tsai, A.G., 2006. Plasma viscosity regulates systemic and microvascular perfusion during acute extreme anemic conditions. Am. J. Phys. 291, 2445–2452.
Caimi, G., et al., 2018a. Red blood cell deformability in multiple myeloma. Clin. Hemorheol. Microcirc. 69, 233–238.
Caimi, G., et al., 2018b. Hemorheological parameters in monoclonal gammopathy of undetermined significance (MGUS). Clin. Hemorheol. Microcirc. 28, 51–59.
Copley, A.L., 1987. Introduction: on the way to modern clinical hemorheology. In: Chien, S., Dormandy, J., Ernst, E., Matrai, A. (Eds.), Clinical Hemorheology. Martinus Nijhoff Publishers, Dordrecht, pp. 1–8.
Dabrowski, Z., et al., 2011. Elongation index of erythrocytes, study of activity of chosen erythrocyte enzymes, and the levels of glutathione,malonyldialdehyde in polycythemia vera. Clin. Hemorheol. Microcirc. 47, 169–176.

Dormandy, J.A., 1970. Clinical significance of blood viscosity. Ann. R. Coll. Surg. Engl. 47, 211–228.
Dormandy, J.A., 1971. Influence of blod viscosity on blood flow and the effect of low lolecular weight dextran. Brit Med J 4, 716–719.
Dumas, G., et al., 2015. Syndrome d'hyperviscositè plasmatique. La Revue de Mèdecine Interne 36, 588–595.
Forconi, S., Gori, T., 2013. Endothelium and hemorheology. Clin. Hemorheol. Microcirc. 53, 3–10.
Forconi, S., et al., 2011. Endothelium and hyperviscosity. Clin. Hemorheol. Microcirc. 49, 487–491.
Fujita, H., et al., 2010. Increased phosphatidylserine exposure on the erythrocyte membrane in patients with polycythemia vera. British Jornal of Haematology 152, 238–240.
Fukumoto, J.S., Gotlib, J., 2006. A patient with paroxysmal nocturnal hemoglobinuria, T cell large granular lymphocyte clonal expansion, and monoclonal gammopathy of undetermined significance. Am J Hematoly 39, 245–249.
Gaehtgens, P., Pries, A.R., Ley, K., 1987. Structural, hemodynamic and rheological characteristics of blood flow in the circulation. In: Chien, S., Dormandy, J., Ernst, E., Matrai, A. (Eds.), Clinical Hemorheology. Martinus Nijhoff Publishers, Dordrect, pp. 97–124.
Hardeman, M.R., Goedhart, P.T., Shin, S., 2007. Methods in hemorheology. In: Baskurt, O.K., et al. (Eds.), Handbook of Hemorheology and Hemodynamics. IOS Press.
Hoffman, R., et al., 1979. Fetal hemoglobin in polycythemia vera: cellular distribution in 50 unselected patients. Blood 53, 1148–1155.
Jurczyszyn, A., et al., 2014. Erythrocyte membrane fatty acids in multiple myeloma patients. Leuk. Res. 38, 1260–1265.
Jurczyszyn, A., et al., 2015. Plasma fatty acid profile in multiple myeloma patients. Leuk. Res. 39, 400–405.
Lipowsky, H.H., 2007. Blood rheology aspects of the microcirculation. In: Baskurt, O.K., Hardman, M.R., Rampling, M.W., Meiselman, H.J. (Eds.), Handbook of Hemorheology and Hemodynamics. Ios Pres, Amsterdam, pp. 307–321.
Liu, J., Li, J., 2014. Detection of erythrocyte in patients with Waldenstrom macroglobulinemia using atomic force microscpy. Acta Biochim Biophys Sin (Shangai) 46, 420–425.
Lo, Presti R., et al., 2012. Erythrocyte deformability evaluated by laser diffractometer in polycythemia vera. Clin. Hemorheol. Microcirc. 50, 189–192.
Melitis, J., et al., 2002. Detection of CD55-and/or CD59-deficient red cell populations in patients with plasma cell dyscrasias. Int J Hemat 75 (1), 40–44.
Nakamura, M.T., Nara, T.Y., 2004. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Ann Rev Nutr 24, 345–376.
Ruef, P., Pöschl, J.M.B., Linderkamp, O., Schmid-Schönbein, H., 1996. The shear stress diffractometer Rheodyn SSD for determination of erythrocyte deformability. II. Sensitivity to detect abnormal erythrocyte deformability. Clinical Hemorheology 16, 749–752.
Rzehak, P., et al., 2009. Evidence for an association between genetic variants of the fatty acid desaturase 1, fatty acid desaturase 2(FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membrane. Br. J. Nutr. 101, 20–26.
Salazar Vazquez, B.Y., et al., 2009. Microvascular benefits of increasing plasma viscosity and maintaining blood viscosity: counterintuitive experimental findings. Biorheology 46, 167–179.
Schmid-Schönbein, H., Ruef, P., Linderkamp, O., 1996. The shear stress diffractometer Rheodyn SSD for determination of erythrocyte deformability. I. Principles of operation and reproducibility. Clinical Hemorheology 16, 745–758.
Sloop, G.D., 2017. A classification of disorsers of blood viscosity by pathologic mechanism. In: Sloop, G.D. (Ed.), In Blood Viscosity.Its Role in Cardovascular Pathophysiology and Hematology. Nova Science Publishers, Inc., New York, pp. 83–106.
Streichman, S., et al., 1981. Moving boundary electrophoresis and salic content of normal and polycythemic red blood cells. Br. J. Haematol. 48, 273–279.
Streichman, S., et al., 1986. Some characteristics of circulating erythrocytes in polycythemia vera: common features wth normal young and foetal red blood cells. Scandinavian Journal of Hematology 36, 33–38.
Wakil, S.J., 1989. Fatty acid synthase, a proficient multifunctional enzyme. Biochem 28, 4523–4530.
Wakil, S.J., Stoops, J.K., Joshi, V.C., 1983. Fatty acid synthesis and its regulation. Annu. Rev. Biochem. 52, 537–579.
Wautier, M.P., et al., 2011. Red blood cell phosphatidylserine exposure is responsible for increased erythrocyte adhesion to endothelium in central retinal vein occlusion. Jornal of Thrombosis and Haemostasis 9, 1049–1055.
Wells, R., 1970. Syndrome of hyperviscosity. N Engl J Med 283, 183–186.
Zhang, Y., et al., 2012. Detection of erythrocytes in patients with multiple myeloma using atomic force microscopy. Scanning 34, 295–301.