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Whole blood viscosity assessment issues III: Association with international normalized ratio and thrombocytopenia

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Abstract

Background: Anticoagulant and antiplatelet therapies are being used interchangeably or in combination. While international normalized ratio is assessed to determine anticoagulant's contraindication/need, whole blood viscosity is not assessed to determine the need for antiplatelet. **Aims**: The objective of this study is to investigate whether whole blood viscosity value is associated with levels of international normalized ratio and platelet count. **Materials and Methods**: De-identified archived clinical pathology data for the year 2008 were audited. All cases of international normalized ratio, which were concomitantly tested for haematocrit and total proteins, were extracted (n=7,387). Whole blood viscosity levels were extrapolated. Whether differences are associated with normal vs. high international normalized ratio and thrombocytopenia vs. thrombocytosis were evaluated. **Results**: Multivariate analysis show that whole blood viscosity levels statistically significantly differs between international normalized ratio and platelet counts ($p<0.001$). Platelet count is statistically significantly lower in low blood viscosity when compared with hyperviscosity and normoviscosity ($p<0.001$). Conversely, international normalized ratio is statistically significantly higher in low blood viscosity relative to hyperviscosity ($p<0.001$) and normoviscosity ($p<0.002$). No difference was observed between hyperviscosity and normoviscosity in platelet count or international normalized ratio. **Conclusion**: The observation corroborates with previous reports to suggest putting into perspective the specificity of whole blood viscosity relative to stasis, against which antiplatelet is employed. It indicates that low whole blood viscosity is synonymous to high international normalized ratio whereby anticoagulant and antiplatelet therapies are contraindicated. International normalized ratio, platelet count and blood viscosity are laboratory indices to consider in constituting antiplatelet monitoring panel.

Keywords: Anticoagulant therapy, antiplatelet, clinical laboratory indices, hypoviscosity syndrome, drug monitoring, platelet count, whole blood viscosity.

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Introduction

Whole blood viscosity (WBV) is one of Virchow's triad, which is an established concept of three phenomena including stasis, endothelial dysfunction and atherothrombosis that ultimately lead to, and/or result from cardiovascular complications [1-4]. Each phenomenon represents a subclinical vascular process, which in turn is indicated by a clinical pathology index. Specifically, WBV is an intrinsic resistance of blood flow in the vascular system [4, 5], and index for stasis [5].

Increase in WBV is a potential risk factor for future cardiovascular disease [6]. In current clinical practice, WBV is assessed mainly in the management of polycythemia and retinal ocular disease. Considering the implication of stasis in metabolic diseases, its clinical management with antiplatelet and the controversies

regarding antiplatelet and its bleeding complications [7-9], such usage is under-utility.

The use of antiplatelet is often in combination or as alternative to anticoagulant [10-12]. International Normalized Ratio (INR) is used to determine the clotting tendency of blood and the need for anticoagulant (especially warfarin) therapy. Normal range is about 1.0 for a healthy person, and 2.0-3.5 for people who are on anticoagulant therapy. A higher INR level indicates a high tendency of bleeding complications, whereas lower INR level is indication of high risk of having a thrombotic event in patients who are on anticoagulant therapy. Thus, while anticoagulant and antiplatelet are used interchangeably, there is an established laboratory index (INR) being employed for routine monitoring to assess contraindication to and/or need for anticoagulant; but none for antiplatelet. This is an issue worth addressing.

The objective of this work is to establish whether difference in WBV level is associated with different levels of INR and platelet count. It would be expected, and thus hypothesized that a patient with low INR requiring anticoagulant therapy should present with higher WBV. The findings from this study would provide evidence to suggest WBV as a potential laboratory index to assess for contraindication to antiplatelet therapy synonymous to how INR is being used for anticoagulants.

Materials and Methods

This work is part of Translational Biomedical Science Research initiative. It is supported materially by the Albury South West Pathology – a unit of Western Pathology Cluster of NSW Health Australia. One year of de-identified archived clinical pathology data for the period of January to December 2008 was audited.

7387 cases (female-male ratio = 3528-3859) for International Normalizing Ratio, which were concomitantly tested from one phlebotomy collection point for full blood count (FBC) haematocrit and total proteins, were extracted. Haematocrit results from the FBC were used in conjunction with total protein to extrapolate WBV values based on previously published algorithm [13]. Whether whole blood viscosity differs between normal vs. high International Normalizing Ratio and thrombocytopenia vs. thrombocytosis were evaluated.

INR level was commonly determined arithmetically from the result of prothrombin time using the formula

$$INR = PT_{patient}/PT_{normal}$$

INR data for this study were determined with this formula included in a standard operational procedure (SOP). The SOP included measurement of prothrombin time (PT) by quantitative analysis using the Sysmex CA540® method and INR calibration curve, which is plotted for every new lot of PT reagent [14]. Results of INR were reported with the following interpretative consideration to risk of bleeding

- Normal patients – no therapy = 1.0
- Anitcoagulated patient = 2.0 – 3.5 including
  - Prophylaxis for prosthetic heart valves = 2.5 – 3.5
  - All other indications = 2.0 – 3.0
- Critical results for anticoagulated patients = <1.5 or >4.5

In this study, numerical levels of INR results have been used as reported. It was assumed on the premise of the hypothesis that a pathology that impacts on INR should also impact on platelet count and WBV. The data were sorted by WBV results and categorized into three groups of low, normal or high WBV levels based on the continuum of ≤15.00, 15.01-19.01 and ≥19.02 respectively. High, normal and low WBV were named groups I, 2 and 3 respectively.

In the statistical analysis, it was considered to avoid errors due to unequal sample size such as exaggerating the effects of inequality of variance [15, 16]. The high WBV (group 1) had the least number (n = 173) and was used as the base sample size for the sub-groups. The discretional criterion for selection of [n = 173] from the other two groups was to make the comparison be between the lowest-median-highest intervals in ranking (Fig. 1). Therefore, the lowest [n = 173] in the low WBV rank and the median [n = 173] in the WBV groups were selected. To determine association of high, normal or low WBV with INR and platelet count, multivariable analysis of variance (MANOVA) was performed using S-Plus version 6.1.

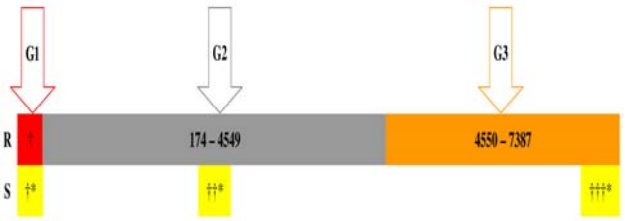

Fig. 1 Indication of selections from the ranks of WBV-groups. †1-173, ††2102-2274, †††7215-7387, *173, G1, G2 & G3: hyperviscosity, normoviscosity and hypoviscosity groups respectively, R:ranking, S:selection for statistical analysis.

The complete data set were further re-analyzed thrice to observe for consistency in result. First, data were sorted by, and selection of (n = 173) from groups 2 and 3 were repeated to match for age and gender. MANOVA was repeated. Second, data were sorted by INR. The top (n = 120) vs. the bottom (n = 120) were selected to represent high vs. low INR sub-groups respectively and WBV level was compared between the sub-groups. Third and similar to the second, data were sorted by platelet count. The top (n = 120) vs. the bottom (n = 120) were selected to represent thrombocytosis vs. thrombocytopenia sub-groups respectively and WBV level was compared between the sub-groups.

Results

The statistics of the groups for the complete data set are presented in Table 1. The result shows that platelet count increases with whole blood viscosity or vise versa, whereas low viscosity is associated with high INR (Table 1).MANOVA show that levels of international normalizing ratio and platelet counts statistically significantly differs between different ranges of whole blood viscosity levels ($p<0.001$).

Table 1 Group statistics

Group		**1**	**2**	**3**
WBV (208 Sec-1)		≥ 19.02	15.01 - 19.01	≤15.00
N	Female	47	2017	1464
	Male	126	2359	1374
	Total	173	4376	2838
Age (Year)	Max	92	100	100
	Min	14	<1	<1
	Mean	52	62	67
INR	Mean	1.29	1.28	1.44
	Median	1.1	1.1	1.2
	SD	0.65	0.65	69
	Max	6.3	9.4	10
	Min	0.9	0.8	0.8
PLT (x10^9/L)	Mean	263	252	236
	Median	257	239	209
	SD	109	101	151
	Max	1012	1728	1369
	Min	15	2	2

WBV: whole blood viscosity at high shear rate, N: number or sample size, INR: international normalized ratio, PLT: platelet count.

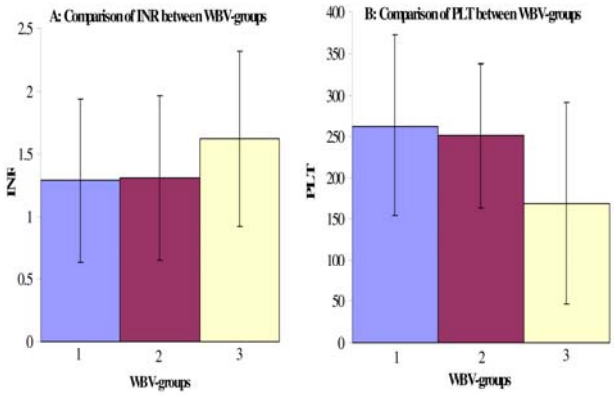

Fig. 2 Comparison of INR & PLT between WBV-groups. 1-3: hyperviscosity, normoviscosity and hypoviscosity groups respectively, INR: international normalized ratio, PLT: platelet count x10^9/L, WBV: whole blood viscosity.

Platelet count is statistically significantly lower in low whole blood viscosity compared to hyperviscosity and normoviscosity ($p<0.001$). Conversely, international normalized ratio is statistically significantly higher in low whole blood viscosity compared to hyperviscosity ($p<0.001$) and normoviscosity ($p<0.002$). No difference was observed between hyperviscosity and normoviscosity in platelet count or international normalized ratio (Fig. 2).

In the repeat analyses for observation of consistency, MANOVA on the age and gender matched data set also showed statistical significance ($P<0.005$). WBV was observed to statistically significantly differ between high vs. low INR ($P<0.001$), as well as between thrombocytopenia vs. thrombocytosis ($P<0.005$) (Fig. 3)

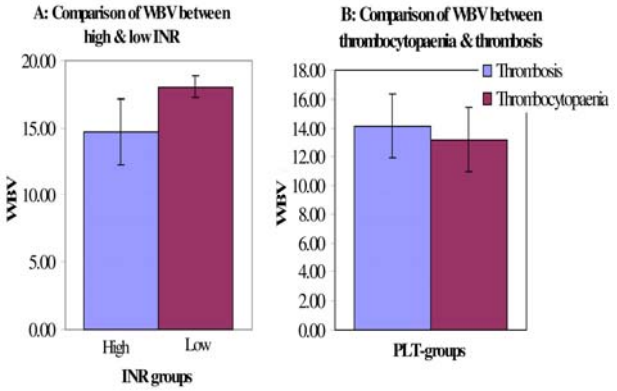

Fig. 3 Comparison of WBV levels between subpopulations of INR and platelet count. INR: international normalized ratio, PLT: Platelet count x10^9/L, WBV: whole blood viscosity.

Discussion

This study reports observation of significantly higher level of INR associated with lower level of WBV. This observation is in consonance with the study's hypothesis and has relevant applicability in clinical practice. On one hand, it affirms that the use of anticoagulant therapy is in tandem with relative high WBV level. More importantly on the other hand, low WBV being associated with relative high INR, whereby the latter indicates risk of bleeding, suggests that blood viscosity can be utilized as a laboratory index of contraindication/indication to anticoagulant and/or antiplatelet therapies.

The issue is that INR is mainly used to assess the status of extrinsic (clotting) pathway in order to determine indication/contraindication for anticoagulant therapy. It is not assessed on chronic disease patients who merely require antiplatelet prophylaxis. Neither is WBV assessed on the chronic patients, nor is the risk of bleeding complication of less concern. What this report contributes is that WBV is inversely associated with INR. Therefore, if the latter is assessed to determine possible risk of bleeding complication anticoagulant, chronic disease patients who are not qualified for INR assessment, but who are to be treated with antiplatelet would benefit from assessment of WBV. A recommended guideline is not to prescribe antiplatelet if contraindicated [17-20]. Yet, lack of laboratory assessment of compliance is an acknowledged major issue [19]. The report provides insight to assessment of hypoviscosity as part of antiplatelet drug monitoring and in compliance to guidelines and good evidence-based clinical practice.

Further, this study reports observation of significantly higher and lower level of platelet count associated with high and low WBV respectively. It is known that antiplatelet therapy is employed in the management of hyperviscosity/stasis through modulation of platelet hyperreactivity [21]. Therefore, it is not out of place that relative high platelet count is associated with high blood viscosity. This logic is not in line with the fact that several studies have reported an association between platelet hyperreactivity after antiplatelet therapy [22]. Nevertheless, platelet function can be measured by different methods and platelet counting has been in use, except that the usage to monitor antiplatelet drugs is not common [23]. Factors that increase WBV include haematocrit, total plasma protein, erythrocyte aggregation, erythrocyte deformability and oxidative stress [2, 13, 24]. In current clinical practice, a low platelet count is a factor that determines temporary stopping chemotherapy, but not antiplatelet therapy. This observation of thrombocytopenia associated with low blood viscosity provides evidence to suggest that low platelet count is also occasion to consider stopping any antiplatelet therapy.

A review has reported gender-specific differences in platelet function and response to antiplatelet therapy. The report further acknowledged role for laboratory monitoring of antiplatelet medications in predicting individual responsiveness [25]. The results from this study show that age and gender differences may not impact on the association between WBV and INR or platelet count. In corroboration with previous observation of infrequent prevalence of hyperviscosity in thromboembolic state ('whole blood viscosity issues II' on this series), it surmises that platelet count should necessarily be used as adjunct laboratory index to determine indication or contraindication for antiplatelet therapy. It is known that thrombocytopenia is associated with bleeding complications [26-28]. What this article contributes is that INR, platelet count and WBV are laboratory indices to consider in constituting antiplatelet monitoring panel.

The observations also corroborate with previous report to suggest putting into perspective the specificity of WBV relative to stasis. In addition, the result indicates that WBV identify individuals in whom anticoagulant and antiplatelet therapies may be contraindicated – that is, from the association and clinical utility of INR [8, 9, 29]. Perhaps, one question is: will hypoviscosity be associated with gastrointestinal bleeding to prove evidence of being a possible contraindication index?

Conclusion

This report presents that higher level of INR is associated with lower level of WBV and vise versa, whereas thrombocytopenia is associated with hypoviscosity. The results indicate that WBV identifies individuals in whom anticoagulant and antiplatelet therapies are indicated or contraindicated. The findings suggest that INR, platelet count and WBV are laboratory indices to consider in constituting antiplatelet monitoring panel.

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