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Whole blood viscosity issues VII: the correlation with leucocytosis and implication on leukapheresis

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Abstract

Background: Blood hyperviscosity has been acknowledged to be a complicating factor in polycythaemia and hyperproteinaemia. Hyperleucocytosis has also been implicated in hyperviscosity and may be a basis for therapeutic leukapheresis. **Aims**: This issue in the series seeks to determine the association and correlation between whole blood viscosity and white blood cell count (WBCC), with a view to advance the cause of a neglected clinical pathology index. **Materials & Methods**: Based on archived clinical pathology data, 10,857 cases that were concomitantly tested for full blood count and total proteins in the 2008 calendar year were audited for hyperleucocytosis. Whole blood viscosity level was determined and compared in the group with leucocytosis relative to the group with leucopenia and normal WBCC. The confounding effects of age, gender and red blood cell indices were evaluated. The correlation between whole blood viscosity and WBCC was also determined. **Results**: As a generalization, hypoviscosity is observed among individuals who presented hyperleucocytosis. There is no correlation (r = 0.20) between leucocytosis and blood viscosity. **Conclusion**: It is known that anaemia and thromboembolism, which can be associated with leucocytosis, predispose to hypoviscosity. The finding from this study provides evidence of association between hypoviscosity and leucocytosis. The absence of association and insignificant correlation between leucocytosis and hyperviscosity may be one explanation for ineffectiveness of therapeutic leukapheresis. Further, the non-correlation lends credence to specificity of blood viscosity, for which critical leucocytosis is not a substitute.

Keywords: Clinical laboratory, evidence-based practice, hyperleucocytosis, leukapheresis, whole blood viscosity.

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Introduction

Hyperviscosity syndrome has been acknowledged to be a vasculopathy especially arising from oxidative stress, polycythaemia and hyperproteinaemia [1-3]. Prospective studies have indicated that whole blood viscosity (WBV) is an independent risk factor to future cardiovascular disease [4]. However, the specificity and usefulness of WBV as a clinical laboratory tool has been doubt such that the test is not routinely requested. This series on WBV assessment issues has been geared towards investigating level of association or prevalence of hyperviscosity in conditions that are related.

In one review article, it is speculated that hyperviscosity is associated with hyperleucocytosis, retinoic acid therapy, and connective tissue diseases. The explanation was that critically high white blood cell count (WBCC) has the capacity to impact on the endothelium [5]. That is, a possibility of leucocytosis-induced endothelial dysfunction leading to hyperviscosity.

Leucocytosis generally implies abnormally high level of total WBCC. This can be any value greater than 11 $x10^9$/L. Hyperleucocytosis implies a very critically abnormal WBCC greater than 100 $x10^9$/L. Total WBCC constitute about one percent of the blood volume of an apparently healthy individual [6]. Leucocytosis often presents in appendicitis and leukaemia, and is associated with early mortality in the latter [7]. Leukapheresis is a therapeutic option in the management of hyperviscosity in leukemia

patients who are described as having leukostasis and hyperleucocytosis [8]. There are no evidence-based guidelines for use of leukapheresis, but it is a common practice [9]. Nevertheless, it is known that the therapeutic effect is short term and success against mortality is low [7].

Further, brain haemorrhage and thromboembolism are potential complications of hyperleucocytosis [10-12]. It is also known that both anaemia and leucocytosis do coexist in leukaemia patients [12]. Thus, on one hand anaemia with or without haemorrhage, which impacts negatively on more than 45% of the blood volume, potentially predisposes to hypoviscosity. On the other hand, leucocytosis involving only about 1% of the blood volume predisposes to leucostasis and hyperviscosity.

Since the association between hyperviscosity and hyperleucocytosis can be validated with archived clinical pathology data, investigation is worthwhile. If the results from this study validate the association, it would be a contribution of data towards formulation of evidence-based guidelines for monitoring leukapheresis. If the results from this study fail to provide any association or correlation between hyperleucocytosis and hyperviscosity, the findings will further lend credence to the specificity of WBV test.

Materials and methods

This work is part of Translational Biomedical Science Research initiative of Dr. Uba Nwose. It is supported materially by the Albury South West Pathology – a unit of Western Pathology Cluster of NSW Health Australia. Ten years of de-identified archived clinical pathology data for the period of January 1999 to December 2008 constitute the research database, of which year 2008 data was audited for this study.

10,857 cases (female : male = 5,035 : 5,822) that were concomitantly tested for full blood count, which included haematocrit; and total proteins in the 2008 calendar year, were audited for leucocytosis. Six subjects were less than one year old, which were approximated to 0.5yo in order to enable for analysis. WBV level was compared in the group with hyperleucocytosis relative to the group with normal WBCC.

Haematocrit (HCT) results from the full blood count (FBC) were used in conjunction with total protein to extrapolate WBV values based on previously published algorithm [13]. FBC data for this study were determined according to the standard operational procedure (SOP) of the South West Pathology Service,[14] which involved measurement using the Sysmex 1800® (Roche Diagnostics Australia Pty Ltd). Results were reported with the following interpretative consideration

- Critical leucocytosis: >11 $x10^9$/L for history of abdominal pain; >20 $x10^9$/L for others
- Leucocytosis: >11 $x10^9$/L
- Normal reference range for WBCC (normo-WBCC): 4.0 – 11.0 $x10^9$/L
- Leucopenia: <4 $x10^9$/L
- Critical leucopenia: <3 $x10^9$/L

In this study, numerical levels of WBCC results have been used as reported for the purpose of ranking and subsequent groupings. Only five (n=5) subjects presented with hyperleucocytosis indicated by WBCC >100 $x10^9$/L in the entire dataset. The prevalence of hyperviscosity associated with hyperleucocytosis was determined. In order to check for consistency vis-à-vis reproducibility of result, the data were thrice ranked/sorted and categorized into groups for analyses. First grouping was based on interpretative report of WBCC. The levels of WBV in leucopenia, normo-WBCC and leucocytosis subgroups were compared. Secondly, critical leucopenia [lowest n = 140] vs. critical leucocytosis [uppermost n = 140] subgroups were compared, as well as between 1st and 4th quartiles subgroups.

The third sub-grouping and analysis was a reverse of the first. Data were ranked by WBV and grouped into hypoviscosity, normoviscosity and hyperviscosity. The level of WBCC and red blood cell indices (incorporating MCH, MCHC, & MCV) was compared between hypoviscosity, normoviscosity and hyperviscosity groups in a multivariate analysis. Correlation between WBV and the WBCC was determined. Statistical analyses were performed included ANOVA, MANOVA and t-tests (Two sample standard t-test and Student's t-test) using S-Plus 6.1 professional.

Results

The summary statistics of comparative levels of WBV between WBCC subgroups is presented in table 1. It shows that the study population includes persons with normal as well as abnormal levels of WBCC. From the statistical analyses, it is observed that there is no unidirectional change in the WBV level with increasing WBCC. On the average population studied, hypoviscosity is observed in both leucopenia and leucocytosis subgroups, while the normo-WBCC subgroup has normoviscosity (Table 1).

Table 1 Summary statistics of WBV levels in WBCC-subgroups

	Leucopenia	Normo-WBCC	Leucocytosis
N	402	7,700	2,755
Mean Age	64 ± 18	67 ± 18	64 ± 19
Mean WBV (208 Sec-1)	14.67	15.77	14.80
Median WBV (208 Sec-1)	15.00	16.13	14.94
SD WBV	2.22	2.02	2.51

The [n = 5] cases of classical hyperleucocytosis presented average levels of 274 $x10^9$/L WBCC and 13.81 WBV (208 Sec-1). At the opposite extreme, [n = 5] very lowest

ranked leucopenia presented average levels of 016 $x10^9$/L WBCC and 12.35 WBV (208 Sec-1). Based on the number in the study population that presented WBCC level <4.0 $x10^9$/L leucocytosis vs. normo-WBCC vs. leucopenia comparison comprised topmost vs. median vs. lowest subgroups of [n = 402] each. The ANOVA shows a statistically significant difference between groups ($p < 0.001$). Evaluation of confounding effect of age and gender showed that neither of these factors was statistically significant.

Student's t-test also showed statistically significant difference between leucocytosis vs. leucopaenia subgroups ($p > 0.001$). However, the statistical significance was not observed when the critical leucopenia [lowest n = 140] vs. critical leucocytosis [uppermost n = 140] groups were compared. The normo-WBCC group is statistically significantly higher than leucopenia and leucocytosis subgroups ($p < 0.00001$). On the analysis of 1st vs. 4th quartile subgroups: The 4th quartile presented with average hypoviscosity level, which is statistically significantly lower than that of the 1st quartile subgroup (Two sample standard t-test p = 0; Student's t-test $p < 0.00001$). The comparative levels of observed averages of WBV in the various subgroups are presented in Fig. 1.

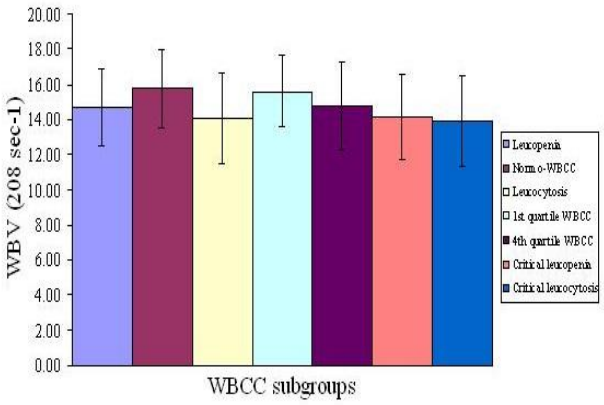

Fig. 1. Levels of mean WBV on different subgroups of WBCC. The figure shows that the level of blood viscosity is relatively lower in leucocytosis, 4th quartile WBCC and critical leucocytosis groups in comparison to leucopenia, 1st quartile and critical leucopenia groups respectively.

Table 2. Summary statistics of WBCC levels in WBV-subgroups

	Hypoviscosity	*Normoviscosity*	*Hyperviscosity*
Mean WBCC $x10^9$/L	12.4	8.7	10.0
Median WBCC $x10^9$/L	11.1	7.6	9.3
Standard deviation	7.0	4.4	4.7
Minimum WBCC $x10^9$/L	1.0	2.9	1.9
Maximum WBCC $x10^9$/L	34.1	36.3	43.0

Based on the number in the study population that presented WBV level >19.01, which is the subgroups with least size, hyperviscosity vs. normoviscosity vs. hypoviscosity comparison comprised topmost vs. median vs. lowest subgroups of [n = 251] each. The summary results of comparative levels WBCC in the WBV subgroups is presented (Table 2).

Multivariate comparison between hypoviscosity, normoviscosity and hyperviscosity groups show a statistically significant difference (MANOVA = 0). Further univariate analyses show statistically significant differences in the levels of MCHC ($p < 0.0001$) and WBCC ($p < 0.00001$). No statistical difference is observed in MCH and MCV levels. The evaluation of correlations for the ranked WBV data with levels of MCH, MCHC, MCV and WBCC presented values of 0.07, 0.15, 0.004 and 0.20 respectively.

Discussion

The observations from this study reveal that leucocytosis is associated with hypoviscosity. The results show that mean WBV in leucocytosis subgroup is in the range of hypoviscosity (Table 1), while mean and median WBCC levels are highest in the hypoviscosity subgroup (Table 2). In the second analysis where WBV was compared between lowest and highest of four categories of ranked WBCC, it is also observed that the 1st quartile (leucopaenia) subgroup with a statistically significantly higher mean age presented with a significantly higher level of WBV compared to the 4th quartile subgroup. This observation is contrary to expectations. The result from this study demonstrates no statistically significant association between leucocytosis and blood viscosity.

Further, correlation analysis shows non-significant relationship (r = 0.20) between WBCC and WBV. This result reaffirms that leucocytosis is not associated with hyperviscosity. This finding has important implication on leukapheresis therapy. First, it may be good practice to review the blood viscosity of a patient who is being considered for leukapheresis to rule out hypoviscosity, which is a contraindication against haemorrhage depending on why the leukapheresis is going to be carried out.

It is known that haemorrhage and thromboembolism are potential complications of hyperleucocytosis [10-12], whereby both complications predispose to hypoviscosity. This complication can be viewed to be synonymous with bleeding complications associated with haemorrhage and thromboembolism in sickle cell disease [15, 16]. A previous report has shown that low WBV level is more prevalent in positive D-dimer subpopulation compared to a subpopulation with negative D-dimer results. That report highlighted that hyperviscosity syndrome or stasis is not always associated with deep vein thrombosis [17]. A further contribution from this report is the observation of low WBV being associated with leucocytosis.

It is also known that therapeutic effect leukapheresis is short term and success against mortality is low. Indeed, it has been reported that four out of fourteen subjects died

within 2 weeks of presentation notwithstanding a prompt therapeutic leukapheresis that was deemed effective [7]. It is likely that bleeding complication could have confounded in the subjects. Thus, we suggest determination of WBV when considering therapeutic leukapheresis in hyperleucocytosis and hyperviscosity syndrome. That is, instead of just using WBCC as indication of leucostasis. Over and above the desire for laboratory parameters that have both high sensitivity and high specificity, the findings suggest putting into perspective the specificity of WBV for the clinical management of stasis.

Importantly, anaemia and leucocytosis do coexist in leukaemia patients [12]. Anaemia predispose to haemorrhage as well as hypoviscosity. The significant contribution by this report is that leucocytosis is associated with hypoviscosity. It would not be unlikely that this association may complicate therapeutic leukapheresis.

Limitation: The values of WBV in this study have been determined from extrapolation method, which is yet to be compared with other current diagnostic methods that have been validated. As participants in this study were de-identified and the outcome of this study provides for no direct or immediate personal clinical benefit to be offered, contact with patients was not made. This constitutes a limitation in that no clinical details of participants could be obtained. We further acknowledge that the size for hyperleucocytosis cases is too small to draw a conclusive statement for the subgroup. However, our observation of associated hypoviscosity is worthy of note.

Conclusion

There is a very weak or insignificant association or correlation between leucocytosis and hyperviscosity. This implies that hyperleucocytosis may not amount to hyperviscosity to justify therapeutic leukapheresis. It is calls for better appreciation of specificity of WBV test as a clinical laboratory tool.

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