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HEMATOLOGY AND ONCOLOGY RESEARCH
ISSN NO: 2372-6601

RESEARCH ARTICLE

DOI : 10.14302/issn.2372-6601.jhor-14-377

Acquired Abnormalities of Plasma Von Willebrand Factor Related Parameters and ADAMTS13 Autoantibodies in Aggressive Haematological

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Abstract:

Background

Abnormalities of plasma von Willebrand Factor (vWF) system has been described in solid tumors but more information is required to understand the pathophysiological process in haematological malignancies.

Objectives

This study was carried out to investigate the changes in vWF-related parameters including ADAMTS13 protein level in aggressive haematological malignancies and to identify the prevalence of anti-ADAMTS13 antibody as well as its correlations with vWF-related parameters.

Patients/Methods

Patient newly diagnosed or having relapse acute leukaemias or aggresive non-Hodgkin lymphomas were recruited into this study. Exclusion criterias include; pregnancy, patient already commenced chemotherapy, sepsis or has background congenital bleeding disorders. Blood specimen was subjected to; blood counts, ADAMTS13 protein, ADAMTS13 antibody detection, vWF:Ag, vWF activity, factor VIII level (FVIII) and vWF: CBA (collagen binding assays)

Results and Conclusion

A total of 60 subjects with median age at 42.5 (IQR: 23.25-57.5) were included. There were 34(56.7%) lymphomas and 26(43%) acute leukaemias. FVIII, vWF:Ag, wVF activity and vWF:CBA level were elevated whereas ADAMTS13 protein was reduced in majority of patients. Those with lymphomas showed significantly higher levels of FVIII, vWF:Ag, vWF:activity and vWF:CBA compared to the leukaemias. 38(63.3%) of patients showed presence of ADAMTS 13 autoantibody. There was however no correlation between ADAMTS13 protein and vWF-related parameters or with ADAMTS13 autoantibodies.

There was a high prevalence of ADAMTS 13 autoantibodies in this cohort despite the absence of thrombotic thrombocytopenic purpura (TTP). The more pronounced changes in vWF-related parameters among aggressive lymphomas compared to acute leukaemias are in tandem with the marginally higher rates of venous thromboembolism in the former.

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Citation: Dzarr AA, W Zaidah A, Azlan H (2015) Acquired Abnormalities of Plasma Von Willebrand Factor Related Parameters and ADAMTS13 Autoantibodies in Aggressive Haematological Malignancies.. Journal of Hematology and Oncology Research - 1(3):1-9. https://doi.org/10.14302/issn.2372-6601.jhor-14-377

Keywords: ADAMTS13 antibodies, ADAMTS13 protein, leukaemia, lymphoma, von Willebrand factor.

Short running title: von Willebrand factor parameters in aggressive haematological malignancies

Received : Dec 19, 2013 **Accepted** : Nov 27, 2014 **Published** : Jan 23, 2015

Introduction

Haemostatic abnormalities in malignancies are well recognized but the understanding on the pathogenesis and characterization of von Willebrand factor (vWF) system in malignant disorders are still growing.

Both lymphoproliferative and myeloproliferative disorders at a lesser degree has been associated with imbalance in the vWF system. The presence of anti vWF autoantibodies result in acquired von Willibrand disease (vWD) whose clinical manifestations are related to bleeding tendencies.[1] Despite the high prevalence of thrombocytopenia, coagulopathy and acquired vWD, haematological malignancies still ranked among the neoplasm most associated with venous thromboembolism (VTE). In a population-based case-control study, patients with haematologic malignancies in fact had the highest risk of VTE (odds ratio [OR] = 28.0; 95% CI, 4.0 to 199.7), followed by lung and gastrointestinal cancers. [2]

Studies on vWF system in non-haematological neoplasm showed elevated levels of vWF:Ag and vWF:Rcof as well as reduced plasma ADAMTS13 protein and activities in advanced stage tumors.[3-4] Severe plasma ADAMTS13 deficiency is associated with thrombotic microangiopathy an observation that first came to light in thrombotic thrombocytopenic purpura (TTP).[5] TTP results from deficiency of ADAMTS13, a metalloproteinase responsible in cleaving large vWF multimers which was intiated by inhibitory anti-ADAMTS13 antibody. It was subsequently known that these antibodies were not specific to TTP, but was also identified in low titer in non-TTP autoimmune disorders such as in systemic lupus erythematosus (SLE) and antiphospholipid syndromes (APS) without clinical TTP.[6] The prevalence of anti-ADAMTS13 antibody in malignant conditions remained to be known.

Hence this study was carried out to investigate the changes in vWF-related parameters in aggressive haematological malignancies, for example acute leukaemias and clinically aggressive non Hodgkin lymphomas. Abnormalities in the von Willebrand factor system may partially explain its pro-thrombotic state similar to those shown in studies on solid tumors. Another aim was to identify the prevalence of anti-ADAMTS13 antibody and its association with ADAMTS13 protein in haematological malignancies.

Methodology

A cross sectional study was conducted in Hospital Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, Malaysia from 2009 until 2012. This study was approved by the institution research and ethical committee.

Subject Recruitment

All patients attending the haemato-oncology service in HUSM diagnosed with haematological malignancies were screened. Informed consent was taken from the selected participants. Inclusion criteria included all male and female subjects above 12 years old newly diagnosed with acute leukaemias and clinically aggresive non Hodgkin lymphomas. Patient whose disease relapsed following a period of treatment

free remission was also eligible. Patients with the following attributes were excluded; Pregnant, patients who previously have been diagnosed with congenital haemorrhagic or thrombotic disorders, clinical or microbiological evidence of sepsis, and those already started on curative chemotherapy during blood specimen collection.

Materials and methods

Ten mls of blood was collected from consented subject in sodium citrate and EDTA anti coagulant containers (for blood counts only). Patients' biodata and clinical information were recorded. The following laboratory investigations were performed; full blood count and ABO blood grouping, prothrombine (PT) and activated partial thromboplastin time (aPTT), factor VIII assay, von Willebrand factor antigen (vWF:Ag), vWF activity, collagen binding assay (vWF:CBA), ADAMTS13 protein assay and ADAMTS13 auto-antibody detection assay.

All laboratory tests were conformed to the standard operating procedure for the haemostatic tests in the Coagulation section, Haematology Laboratory, USM. All the procedures were adhered to the Clinical and Laboratory Standards Institute (CLSI) guidelines for coagulation tests. Full blood counts were performed on Sysmex XE 2100 Hematology analyser whereas PT and aPTT were carried out on coagulation analyser, STA Compact using Diagnostica Stago reagents.

For Factor VIII assay, the test was done on STA Compact coagulation analyser using factor VIII deficient plasma and reagents (produced by Diagnostica Stago, France). Measurement of vWF:Ag assay was done by automated latex enhanced immunoassay for quantitative determination using the reagents from Diagnostica Stago. For the vWF activity assay, the test was performed using Hemosil TM reagents on ACL 9000 coagulation analyser (produced by Instrumentation Laboratory, USA and Italy). The test for CBA:vWF was based on Enzyme Linked Immunosorbent assay (ELISA) method using TECHNOZYM reagents (produced by Technoclone GmbH, Vienna Austria). All tests were carried out according to the manufacturer's instructions.

ADAMTS13 antigen assay and autoantibody detection were done according to manufacturer's instruction, using IMUBIND ADAMTS13 ELISA (product no, 813) and ADAMTS13 Autoantibody ELISA (product n. 814) respectively both from American diagnostic, West Avenue Stamford.

The normal ranges for the following tests were available from the established local laboratory reference ranges from healthy volunteers (Hb; Male: 12 – 16.5g/dl and female: 9.8 – 13.8 g/dl, WBC 4.3–10.8x10^9/L; Plt 150-400x10^9/L; PT 12.61-15.72s; aPTT 30-45.8s; FVIII assay 50-190%; vWF:Ag 50-160%; vWF activity 40-170%; vWF:CBA 60-130). For ADAMTS13 related tests, the manufacturer recommended ranges were used (ADAMTS13 protein 630-850ng/mL; ADAMTS13 autoantibody cutoff value at 9.6 AU/mL. The autoantibody is expressed in Arbitrary Units/mL which is equal to to 1 ug/mL of affinity purified human anti-ADAMTS 13 IgG). The manufacturer recommended ranges were verified using healthy volunteers (n=20).

The detection limit of the analytical method for ADAMTS13 related parameters was not given by manufacturer and not tested in this study.

Data were analyzed using IBM SPSS (Statistical Package for Social Sciences) version 20. Normality tests were performed to determine the choice of statistical tests. For non parametric tests, the results were expressed as median (Interquartile range) and differences between two groups were analysed using Mann Whitney test.

Results:

A total of 60 Malay subjects were included in this study. The median age of these subjects was 42.5 (IQR: 23.25-57.5). There were 34(56.7%) male subjects and the rest were female. The types of haematological malignancies and their proportions are shown in Fig 1 below. Diffuse large B cell lymphoma consisted the most common pathology, 38.3% of all cases. These cases were then grouped either into acute leukaemia; 34 (56.7%) or lymphomas 26 (43.3%).

vWF antigen and acitivity were found to be predominantly elevated in all pathologies except in T-ALL. vWF:CBA was also found to be elevated in 88% of studied samples and this trend is consistently seen across all pathologies. ADAMTS13 antibody was found in 63.3% and reduced plasma ADAMTS13 protein was seen in 76.7% of studied population. However there was no significant correlation between plasma ADAMTS13 protein and antibody levels as shown in the table 2 matrix. Plasma ADAMTS13 protein levels were re-tabulated into low and normal or elevated and compared to plasma ADAMTS13 antibody in a 2x2 table, yet no significant association was seen by Fisher Exact test (p=0.597). There were four subjects with

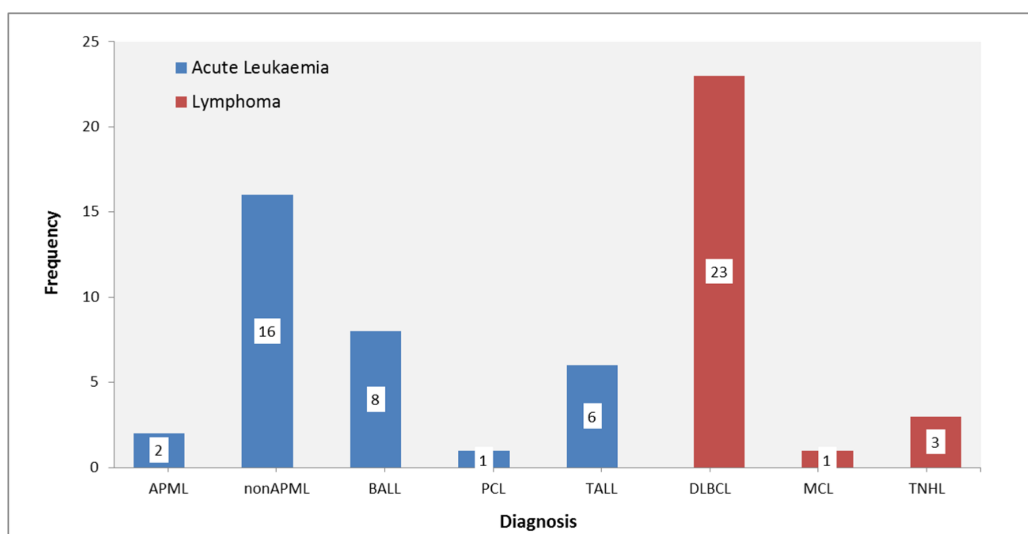

Figure 1: Primary haematological neoplasm in the studied subjects.

*Abbreviations: **APML** (acute promyelocytic leukaemia), **BALL** (B-lineage ALL), **DLBCL** (Diffuse Large B-cell Lymphoma), **MCL** (Mantle cell Lymphoma), **non APML** (non APML acute myeloid leukaemia), **PCL** (plasma cell leukaemia), **TALL** (T-lineage ALL), **TNHL** (T cell non Hodgkin lymphoma)

Table 1: Subject biological and laboratory data

Variables	Median(IQR)	**P value
Prothrombin time (sec)	14.75(13.8-15.85)	**0.027**
Leukaemia	15.3(14.05-16.28)	
Lymphoma	14.25(13.25-15.17)	
Activated Partial Thromboplastin Time (sec)	40.40(35.13-47.55)	0.899
Leukaemia	40.2(35.38-47.65)	
Lymphoma	40.40(34.37-47.48)	
Plasma Factor VIII level (%)	222.00(159.00-331.25)	**0.050**
Leukaemia	189.00(146.25-270.50)	
Lymphoma	275.00(166.75-433.25)	
Plasma vWF Antigen level (%)	221.00(156.75-347.25)	**0.016**
Leukaemia	194.50(151.25-266.50)	
Lymphoma	295.50(172.00-420.00)	
Plasma vWF Activity (%) *	226.48(144.73-316.51)	**0.026**
Leukaemia	187.38(144.20-274.93)	
Lymphoma	314.01(145.08-404.70)	
Plasma ADAMTS13 protein (ng/mL)	263.78(21.58-583.86)	0.881
Leukaemia	249.65(21.58-649.16)	
Lymphoma	263.78(10.50-525.84)	
Plasma ADAMTS13 autoantibody (AU/mL)	12.9(7.20-21.94)	0.090
Leukaemia	13.27(8.22-28.06)	
Lymphoma	11.47(5.24-18.45)	
Collagen Binding Assay (%)	178.19(151.01-234.28)	**0.021**
Leukaemia	165.18(136.92-202.06)	
Lymphoma	205.26(163.94-246.64)	

Total number of subjects = 60

* Only 54 total samples were evaluated (6 missing data)

** Asymptotic p value (2-tailed) is displayed and analysed using Mann Whitney.

undetectable ADAMTS13 protein; one each for AML with t(15,17) translocation, AML M5b, AML M5a, and relapse DLBCL, but only one patient with APML had the antibody.

There was also no significant correlations between either ADAMTS protein or antibody with vWF antigen, its acitivity or vWF:CBA.

Subsequently, each parameter was then compared between acute leukaemia and lymphoma grouping using

non-parametric, Mann-Whitney independent sample test. As expected, both the hemoglobin level and platelet count were significantly lower in the leukaemia group compared to the lymphoma group; 9g% (Intraquartile range; IQR: 8.18-10.08) versus 10.9g% (IQR: 9.48-11.63) (P=0.001) and 41.50 x 10^9/L (IQR: 18.75-83.25) versus 309.50 x 10^9/L (IQR: 189.75-444.75) (P<0.001) respectively. However the difference in total white cell count was not statistically significant; 14.50 x 10^9/L (3.32-42.05) for leukaemia versus 8.70 x 10^9/L (7.03-13.49) for lymphoma (p=0.596). The rest of the data are displayed in table 1.

Discussion

This study was done to investigate the abnormalities of vWF related parameters in aggressive haematological malignancies.

The elevated plasma vWF:Ag and activity as well as lowered plasma ADAMTS13 protein level seen in this study were in keeping to similar changes reported in earlier studies on solid tumors. (3-4) Oleksowicz showed that the level of plasma ADAMTS13 protease to be significantly lower in disseminated tumors compared to localized disease. Mean plasma FVIII:C, vWF:Ag and vWF:Rcof were all significantly elevated in patients with advanced stage solid tumors but not in localized disease when compared to normal patients.[3] In a separate rather similar study involving Persian population, patients with both disseminated and localized tumors had significantly higher plasma vWF:Ag and lower ADAMTS13 protein compared to normal controls. The ADAMTS13 protein was shown to inversely correlated with vWF:Ag level (r=-0.4).[4] It is possible that increased consumption of ADAMTS13 as demonstrated by high vWF antigen and activities in these cases have resulted in low ADAMTS13 protein.

We made use of manufacturer's reference normal values and grouped the subjects into two groups; acute leukaemias or lymphoma to gain reasonable numbers for comparative analysis. Plasma cell leukaemia is not an acute leukaemia but was arbitrarily grouped together due to it's dispersive and clinically aggressive nature. The leukaemias are naturally more disseminated compared to lymphomas and are expected based on previous results to have higher vWF:Ag, vWF activity, and vWF:CBA. On the contrary the result of this study was the exact reversed. The lymphomas showed significantly higher level of vWF activities compared to the leukaemias. Similar to the previous study we also demonstrated reduced ADAMTS13 protein level with 76.7% of all subjects having levels below the reference range. However the protease levels were not significantly different between the two groups. Unlike the previous study, there was no correlation between ADAMTS13 protein levels with vWF-related parameters.

The relatively higher vWF-related parameter levels among lymphomas compared to acute leukaemias provide a possible explanation to a marginal higher rate of venous thromboembolism seen among lymphomas compared to the latter (local data not included). It is difficult to compare the various rates derived from different population and different diagnostic criteria. Epidemiological data from the Californian Cancer Registry showed that the 2-years cumulative rates for

venous thromboembolism (deep vein thrombosis or pulmonary embolism) was 3.6, 3.7 and 4.7 in acute myeloid leukaemia, acute lymphoblastic leukaemia and aggressive lymphomas respectively.[7-8]

Measures were taken to remove patients with sepsis from this study sample yet the depicted vWF-related parameters have similar profile to those shown during severe sepsis (elevated plasma vWF antigen, activity, vWF:CBA and reduced plasma ADAMTS13 protein) suggesting possible sharing of pathophysiological mechanism between neoplasm and inflammation.[9] In addition to lowered ADAMTS13 synthesis in the liver, it was shown that sepsis lead to increase ADAMTS13 catabolic digestion by various cleaving protease among them, such as granulocyte elastase.[5]

In TTP, acquired anti-ADAMTS13 antibodies contribute to low ADAMTS13 protease. These antibodies has since also been identified in other diseases i.e sepsis, inflammatory diseases etc. We showed that there was a high prevalence with almost two third of patients with acute leukaemias and agressive lymphomas having detectable IgG anti-ADAMTS13 antibodies. However these detected IgG antibodies do not appear to contribute in lowering plasma ADAMTS13 protein level or affecting the vWF:CBA. None of the subjects were found to have clinical features of TTP in this study. There are several possibilities to explain these findings. We did not assess the sensitivity of the reagents and perform local validation studies. It is also possible that these are non-inhibitory antibodies targeting on less significant domains. Autoantibodies derived from plasma of patients with clinical TTP are most commonly directed against the cystein-rich spacer (cys-rich/spacer) domain of ADAMTS13. Activities against various other domains have also been registered at lesser frequencies.[10] In a study evaluating patients with immunopathology other than TTP, low titers of IgG anti-ADAMTS13 antibodies (measured using ELISA assay) were found in 3.6% ,13% and 5% of healthy donors, patients with systemic lupus erythromatosis and antiphospholipid syndrome respectively. The ELISA technique was found to have more sensitive detection compared to the ADAMTS13 neutralizing inhibitor technique. In that study too there was no correlation between antibody levels and plasma ADAMTS13 protease activity.[6]

Presence of anti-ADAMTS13 IgG antibodies was common among the aggressive types of hematological malignancies (76.7%). The presence of these antibodies among AML patients was an unexpected finding. Though the presence of autoantibodies is rather explainable in lymphoid malignancies, in AML the mechanism of its development requires further exploration. The persistence positivity of auto IgG is more important to exclude false positive results due to transient circulating antibodies of unknown clinical significance. The titre level may also indicate the clinical significance of the antibody thus requiring further confirmation.

In the recent guideline on diagnosing TTP cases[11], severely reduced ADAMTS13 activity < 5% ± the presence of an inhibitor or IgG antibodies, confirms the diagnosis. However none of the cases in this study had typical clinical manifestation of TTP.

It would have been beneficial if this study also looked into vWF multimeric composition analysis and ADAMTS13 activities rather just the protein. Increased proteolysis of ADAMTS13 was associated with reduced high molecular weight vWF multimers. vWF multimer analysis may further characterise the vWF changes in aggressive haematological malignancies. In future, anti -ADAMTS13 antibodies detection should be combined with neutralizing technique to confirm their functional effect, as this test is more specific.

Conclusion

In conclusion, the vWF:Ag, vWF activity,and vWF:CBA were elevated with corresponding reduction in ADAMTS13 protein level in haematological malignancies similar to other solid tumors. The changes in the vWF related parameters were significantly more pronounced in aggressive lymphomas compared to acute leukaemias. These findings may partly explain the prevalence of higher VTE in lymphomas as compared to acute leukaemias. Presence of non-inhibitory anti-ADAMTS13 antibodies was common among hematological malignancies but they did not appear to affect ADAMTS13 protein levels or the vWF parameters.

Conflict of interest: Nil

Acknowledgement: This study was supported under Malaysian Fundamental Research Grant Scheme, FRGS 203/PPSP/6171116 and Mr Mohd Anuar Nordin for his technical assistance.

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