

Acquired Von Willebrand Disease in Hematologic Malignancies at the National Cancer Institute (NCI) Egypt

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ABSTRACT

Acquired von Willebrand disease (AvWD) is an acquired bleeding disorder, which may suddenly become manifest in individuals, in the absence of a past personal or family history of bleeding and frequently in association with monoclonal gammopathies, lymphoproliferative, myeloproliferative and autoimmune disorders. In a minority of cases AvWD may develop in association with drugs or solid tumors. One hundred and fourteen patients attending to the Medical Oncology Department, NCI, presenting with a recent development of non-thrombocytopenic mucocutaneous bleeding were included in the study. Due to the limitations and variability of each assay and because of vWD heterogeneity, no single test procedure was sufficiently robust to permit detection of all subtypes. However, this is not the case in AvWD, since the most common pattern observed is deficiency of large vWF multimers 20/39. In this regard, the multimer analysis would depict most of the cases. Four separate assays for vWF were included: vWF; Multimer analysis, vWF; Antigen (vWF:Ag), factor eight coagulant activity (FVIIIc) and Ristocetin cofactor (vWF: RiCof). 39/114 cases were diagnosed as AvWD (17 CML, 11 NHL, 8 CLL & 3 MM). The 39 cases of AvWD were subclassified: 3 cases as type 1, 20 cases as type 2A, 8 cases as probable type 2N, 7 cases as probable type 2M (based on the low Ri: Cof/vWF: Ag ratio < 0.36) and 1 case as AvWD (unrecognized subtype). The increased incidence of AvWD in CML patients was statistically significant ($p = 0.04$). Using receiver operator characteristic (ROC) analysis, the best diagnostic single test was found to be RiCof, followed by FVIIIc, APTT and lastly vWF: Ag. Although patients with blood group O were most frequently and significantly ($p = 0.046$) affected when the multimer test was used, this turned insignificant when the Ri:Cof test was analyzed. This could be relevant to the normal distribution of blood groups among the population rather than to the inherent low level of vWF:Ag in blood group O individuals. This report emphasizes that AvWD is not an extremely rare disorder, particularly in the setting of hematologic malignancies in Egypt.

Key Words: AvWD - Ri:Cof - vWF:Ag - FVIIIc - Multimer - Blood group O.

INTRODUCTION

Acquired von Willebrand disease (AvWD) was first recognized in 1968 [26]. Subsequently, it was described in the presence of several clinical conditions [2,9,12,15]. AvWD is extremely rare, with more than 200 well-documented cases reported in the English-language literature. The most frequent clonal disorder associated with AvWD is monoclonal gammopathy of undetermined significance (MGUS) [6,27]. Monoclonal gammopathies associated with AvWD include multiple myeloma, Waldenstrom's macroglobulinemia and chronic lymphoproliferative disorders. Of interest is that most, if not all, reported cases of lymphomas described in association with AvWD have been low-grade non-Hodgkin's lymphoma [21,27] and some [29] but not all [21,27,29] have been associated with monoclonal gammopathy. AvWD was also reported in extranodal lymphoma [23]. Other hematologic malignancies described in association with AvWD include chronic myeloproliferative disorders [3,24,30] (polycythemia vera, essential thrombocythemia, agnogenic myeloid metaplasia with myelofibrosis and chronic myeloid leukemia). Similar to the diagnosis of congenital vWD, the diagnosis of AvWD requires careful correlation of clinical information with the results of clinical laboratory tests. This paper reports the prevalence of AvWD among various hematologic malignancies registered at the

NCI, the influence of ABO blood group and the diagnostic efficacy of BT, APTT, vWF: Ag, Ri:Cof, FVIIIc and vWF multimer analysis.

PATIENTS AND METHODS

Patient selection:

This was a prospective study and included a total of 114 patients attending the medical oncology department, NCI, Cairo University. Patients presented with recent and sudden occurrence of non-thrombocytopenic mucocutaneous bleeding with absence of personal and family history. They included 73 males and 41 females. The age of our population ranged from 15 years to 67 years.

Control group:

Ten normal healthy subjects were used as control, 4 males and 6 females. Their ages ranged from 35 to 50 years.

Laboratory work up included:

- Complete blood picture.
- Blood group.
- Coagulation assays:

Bleeding time (BT): Ivy's method [14].

APTT: (DADE Behring) [7].

vWF: Ag: (READS Medical Products) [1]. An enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of von Willebrand factor antigen (vWF: Ag) in citrated human plasma. Antigen levels of less than 50U/dl were considered deficient.

vWF: Ricof [18]. FVIIIc [13]. vWF: multimer analysis [8].

Statistical analysis:

Chi-square and/or Fisher exact tests were used for testing proportion independence. ROC curve (Receiver Operator. Characteristics) was plotted to get maximum joint sensitivity and specificity (Validity measures). It was done by plotting the true positive rate on y axis versus the 1-specificity (false positive rate) on x axis.

RESULTS

Out of the 114 cases presenting with recent occurrence of mucocutaneous bleeding, AvWD could be diagnosed in 39 cases. No sex difference was determined as 25/73 (34%) were males and 14/41 (34%) females.

The results are presented in the tables (1-8), and Figs. (1-3).

Table (1): AvWD among different study groups.

Different study groups (No. of cases)	Multimer and Ri:Cof				p value
	-ve		+ve		
	No.	%	No.	%	
	N=56		N=39		
CML (34)	17	26.2	17	43.6	0.04 (S)
CLL (26)	18	27.6	8	20.5	
MM (19)	16	24.7	3	7.6	
NHL (25)	14	21.5	11	28.3	

S: Significant.

Table (2): Relation of B.T and APTT to vWF multimer and Ri:Cof.

	Multimer and Ri:Cof		Total accuracy (%)	p value	Specificity	Sensitivity
	-ve No. (%) N=78	+ve No. (%) N=36				
B.T. (min)						
+ve (> 7)	36 (46.2%)	19 (52.8%)	53.5%	0.511 NS	54%	53%
-ve (3-7)	42 (53.8%)	17 (47.2%)				
APTT (sec)						
+ve (> 40)	13 (16.7%)	15 (41.7%)	70.2%	0.004 S	83%	42%
-ve (20-40)	65 (83.3%)	21 (58.3%)				

S: Significant; NS: Not significant.

Table (3): Correlation of F VIII:C to APTT.

APTT (Sec)	F VIII:C				Total accuracy (%)	p-value
	Normal		Decreased			
	No.	%	No.	%		
	N=98		N=10			
+ve (> 40)	19	19.4	6	60	78.7%	0.003 S
-ve (30-40)	79	80.6	4	40		

S: Significant.

Table (4): Correlation of Ri:Cof to BT, APTT, vWF:Ag and F VIII:C.

	Ri:Cof				Total accuracy (%)	p-value	Specificity	Sensitivity
	Normal		Decreased					
	No.	%	No.	%				
	N=101		N=13					
B.T. (min)								
+ve (> 7)	47	46.5%	8	61.5%	54.4%	0.308 NS	54%	62%
-ve (3-7)	54	53.5%	5	38.5%				
APTT (sec)								
+ve (> 40)	21	20.8%	5	38.5%	74.6%	0.152 NS	79%	39%
-ve (20-40)	80	79.2%	8	61.5%				
VWF:Ag IU								
+ve (< 50)	-	-	2	16.7%	88.3%	0.042 S	100%	17%
-ve (50-200)	101	100%	11	83.3%				
F VIII:C%								
+ve (< 50)	16	9.2%	3	18.2%	84.3%	0.281 NS	92%	18%
-ve (50-200)	85	91.8%	10	81.8%				

NS: Not significant.

Table (5): Ri:Cof test as compared to blood groups.

ABO blood group	Ri:Cof				p-value
	-ve (50-200) U/dl		+ve (< 50) U/dl		
	No.	%	No.	%	
	N=101		N=13		
O	41	38	6	46	0.652 NS
A	26	27	4	31	
B	31	29	2	15	
AB	3	5	1	8	

NS: Not significant.

Table (6): Multimer test as compared to blood groups.

	Multimer test				p-value
	-ve		+ve		
	No.	%	No.	%	
	N=20		N=22		
O	7	35	10	45	0.046 S
A	9	45	4	18	
B	2	10	8	36	
AB	2	10	-	-	

S: Significant.

Table (7): FVIII:C level in AvWD subtypes.

	Type 1	Type 2A	Type 2M (probable)
	50	46	55
	63	67	57
	-	69	
		71	
		113	
		83	
		85	
		77	
		93	
		43	
Mean	56.5	74.7	56

Table (8): Distribution of AvWD subtypes among different study groups.

Disease	Type 1	2A	2M	2N
CML	2	6	5	3
CLL	-	4	1	3
MM	-	2	-	1
NHL	1	8	1	1
Total	3	20	7	8

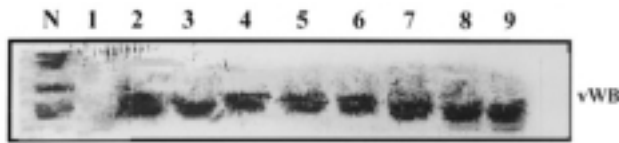


Fig. (1): vWB multimer analysis in acquired vWD absence of HMWM (1 through 9)

CML: 1,4,6,7,9
 NHL: 3,5,8
 CLL: 2

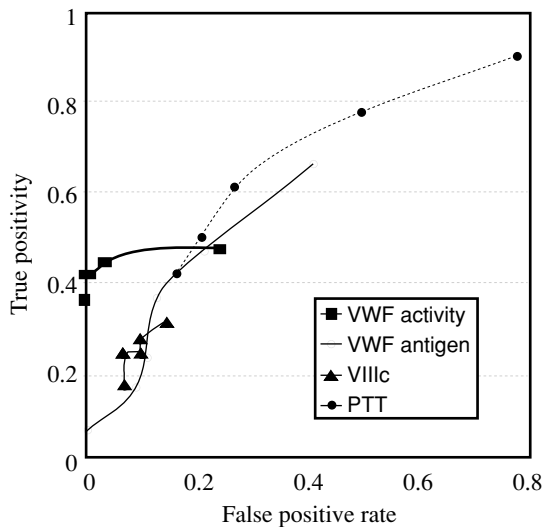


Fig. (2): Receiver operator characteristic (ROC) curve of diagnostic tests for vWD.

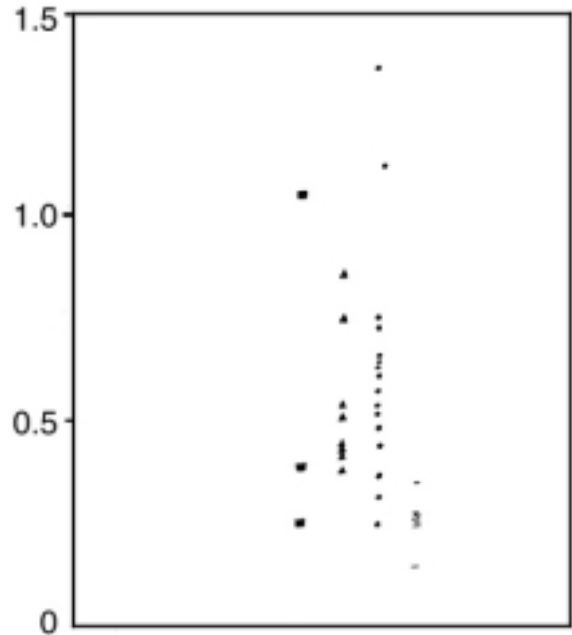


Fig. (3): Seven patients with probable type 2M AvWD can be identified by a markedly reduced ratio of Ri:Cof/vWF:Ag when compared to 16 type 2A, 8 type 2N and 3 type 1 vWD.

- : type 2 M
 • : type 2 A
 ▲ : type 2 N
 ■ : type 1

DISCUSSION

A revised classification of vWD has been introduced [25]. The Arabic numerals 1,2 and 3, respectively, define the primary categories of partial quantitative, qualitative and virtually complete deficiency of vWF. Type 2 is subdivided into four categories: 2A refers to qualitative variants with decreased platelet-dependent function with absence of HMWM, 2B refers to qualitative variants with increased affinity for GPIIb. 2M refers to qualitative variants with decreased platelet-dependent function with normal HMWM. 2N refers to qualitative variants with decreased affinity for FVIII [19]. A variety of laboratory assays [16,32] may be performed, not necessarily restricted to an assessment of vWF. Determination of vWF quantity, structure and function is required to appropriately diagnose vWD. Both the bleeding time (BT) and the APTT were poor tests for identifying vWD. In the present study the former was giving a sensitivity of 53% and a specificity of 45%, while the sensitivity of the latter test was 42% and the specificity was 83% (Table 2). However, there

was a significant ($p < 0.003$) correlation between VIIIc and APTT (Table 3). In our cases with AvWD, the most common abnormality observed was absence or reduction of high molecular weight multimers (HMWM-2A), leaving the rest of the molecule intact, thus probably preserving the site of FVIII binding. This notion is supported by the finding of a higher FVIIIc level in the 2A subtype as compared to the subtypes I and 2M. Although the difference was statistically insignificant, increasing the numbers might bring it to the significant level (Table 8). The vWF:Ag assay is a quantitative test and provides no information concerning the quality. Thus, the vWF:Ag on its own will not permit detection of many qualitative defects. Consequently, the use of this assay alone will lead to many type 2 vWD patients being missed by the laboratory [10]. In our laboratory, only 2/39 had vWF:Ag level below reference level (< 50 IU/dl). While the specificity of this test was 100%, its sensitivity was only 17%. On the other hand, Ri:Cof is a functional assay that measures the ability of test plasma to induce agglutination of a standardized platelet suspension in a fixed concentration of the obsolete antibiotic ristocetin. In the present study, Ri:Cof was reduced in 14/39 (36%) AvWD (10 CML, 1 CLL, 1MM & 2NHL). However, when correlating Ri:Cof to various other coagulation parameters (Table 4), none was significant except with vWF:Ag ($p < 0.042$), that is why two ratios (vWF:Ag/Ri:Cof and Ri:Cof/vWF:Ag) had been proposed by Caron et al. [4] and Montgomery & Goller [20], respectively. Using the first ratio and applying various cutoffs proved nonreproducible when applied to our AvWD cases. However, type 2N lies always within the uncertain group. Using the second ratio (Table 7, Fig. 3) allowed us to diagnose probable type 2M in 7 out of 8 AvWD unclassified (Ratio < 0.36). Also, the Ri:Cof assay was found to have a significantly higher diagnostic accuracy using the ROC analysis (Fig. 2). Ri:Cof assay, along with the F VIIIc concentration were considered probably the most critical test for guiding appropriate therapy [30]. Favaloro and Koutts [10] suggested that analysis of plasma vWF multimer distribution would be useful for further evaluation of cases with decreased vWF: Ag or vWF: RiCof, especially when the latter is discordantly lower than the former, suggesting a decreased abundance of higher-molecular-weight vWF multimers. Although this test does

not distinguish between subtypes 2A or 2B vWD, in our cases type 2B was excluded by primarily selecting non-thrombocytopenic patients.

Several pathogenetic mechanisms [28] have been reported for AvWD [24], but none was disease-specific. The mechanism reported most frequently involves inhibition of vWF either nonspecifically as part of a generalized autoimmune reaction or development of specific anti-vWF antibodies [26,31]. A similar but alternative mechanism involves formation of immune complexes between the vWF protein and monospecific antibodies, resulting in accelerated catabolism or elimination of high molecular weight multimers (HMWM) of von Willebrand factor (vWF). These vWF multimer-antibody complexes are subsequently cleared from the circulation either by the reticuloendothelial system or by adsorption onto tumor cells. Clearance of HMWM of vWF thus results in extremely low functional levels and variable antigenic levels. A similar mechanism is suggested in AvWD patients with high platelet counts [2]. Additional mechanisms of AvWD have included decreased synthesis associated with hypothyroidism, accelerated proteolysis of HMWM associated with the administration of HES, ciprofloxacin [5], Valproic acid (usually type I) [17] and selective adsorption of vWF by tumor cells. The latter mechanism was shown to be the aberrant tumor-cell expression of the platelet vWF receptor CD42 [glycoprotein Ib (GPIb)] but not CD41 (glycoprotein IIb/IIIa) or CD61 (glycoprotein IIIa) [27]. In our series, vWF multimer analysis reveals mostly a type 2 defect with decreased abundance of HMWM. 20/39 of AvWD cases were type 2A (Fig. 1). Recent data are in support of the hypothesis that conformational changes in the A3 domain of vWF might affect vWF function by regulating the ability of the AI domain to bind to platelet GPIb [22].

A further factor which is important in the assessment of the diagnosis of vWD is blood group. A number of studies have shown that plasma vWF:Ag levels differ significantly between blood groups, in the rank order $O < A < B < AB$ [11,21]. The significance of this is that vWD may be under- or over-diagnosed if vWF:Ag concentrations are interpreted without knowledge of the patient's blood type. It is noteworthy that, in one large study, the proportions

of blood groups O (77%), A (18%), B (4%) and AB (0%) were significantly ($p < 0.001$) different among type 1 congenital vWD patients compared with those observed in the normal population (45%, 45%, 7% and 3%, respectively), that was not the case with the other types [11]. In the present study, the rank of AvWD cases was 48.7%, 23%, 23%, 5.3%, respectively. Although it is similar to those found in the normal population, the preponderance of group O was borderline significant ($p < 0.046$) only when using the multimer test and it was insignificant when using the Ri:Cof test, thus reflecting the normal distribution of blood groups rather than a genuine increase in group O. Only 3 cases of AvWD were type 1, 20 were type 2A, 8 2N and 7 2M.

In the present study (Table 1), CML was significantly ($p < 0.04$) the most frequent malignant haematological disorder affected by AvWD (44%) followed by NHL (28%), CLL (21%) then MM (7%). This is not in agreement with the literature [24], where the most frequent disorder affected by AvWD was monoclonal gammopathy with undetermined significance (MGUS). This could be explained by the fact that MGUS was not included in the present study. The higher percentage of cases with AvWD at the NCI could be attributed to the selection criteria put forward.

In conclusion, at the Egyptian NCI, CML is the most frequent haematological disorder affected by AvWD followed by NHL, CLL and MM. Blood group O is the commonest blood group affected, reflecting the distribution among the normal population, unlike vWD type 1. The prevalent subtype is type 2A, thus multimer analysis would be a good additional screening test. Ri:Cof assay had a significantly higher diagnostic accuracy over all other coagulation tests (ROC analysis). The ratio Ri:Cof/vWF:Ag may be used to predict type 2M. Being a heterogeneous group, no single test is sufficiently enough to diagnose AvWD and the diagnostic approach of a bleeding tendency with normal platelet count should include assays for VIIIc, vWF:Ag and Ri:Cof before proceeding to platelet function tests.

REFERENCES

- 1- Bartlett A., Dormandy K.M., Hawkey C.M., Stableforth P. and Voller A.: Factor VIII related antigen: measurement by enzyme immunoassay. *Br. Med. J.*, 1: 994-997, 1976.
- 2- Budde U. and Van Genderen P.J.J.: AvWD in patients with high platelet counts. *Seminars in thrombosis and hemostasis*, 23: 425-431, 1997.
- 3- Budde U., Schaefer G. and Mueller N.: AvWD in the myeloproliferative syndrome. *Blood*, 64: 981-985, 1984.
- 4- Caron C., Rugen L., Reade V., Thoumoys A. and Goudemand J.: Von Willebrand Factor: Assays. PS-2573, p 63, 1997.
- 5- Castaman G., Lattuada A., Mannucci P.M. and Rodeghiero F.: Characterization of two cases of acquired transitory von Willebrand syndrome with ciprofloxacin: evidence for heightened proteolysis of von Willebrand factor. *Am. J. Hematol.*, 49: 83-86, 1995.
- 6- Castaman G., Rodeghiero F., Di Bona E. and Ruggeri M.: Clinical effectiveness of desmopressin in a case of acquired von Willebrand's syndrome associated with benign monoclonal gammopathy. *Blut.*, 58: 211-213, 1989.
- 7- Dacie J.V. and Lewis S.M.: Practical hematology. Eighth edition. Churchill Livingstone. p 308-309, 1995.
- 8- Enayat M.S. and Hill F.G.: Analysis of the complexity of the multimeric structure of factor VIII related antigen/von Willebrand protein using a modified electrophoretic technique. *J. Clin. Pathol.*, 36: 915-917, 1983.
- 9- Facon T., Caron C., Courtin P., Wurtz A., Dehaye M., Bauters F. and Goudemand J.: Acquired type II von Willebrand's disease associated with adrenal cortical carcinoma. *Br. J. Haematol.*, 80: 488-494, 1992.
- 10- Favaloro E.J. and Koutts J.: Laboratory assays for von Willebrand factor: relative contribution to the diagnosis of von Willebrand's disease. *Pathology*, 29 (4): 385-91, 1997.
- 11- Gill J.C., Endres-Brooks J., Bauer P.J., Marks W.J.J.R. and Montgomery R.R.: The effect of ABO blood group on the diagnosis of von Willebrand's disease. *Blood*, 69: 1691-1695, 1987.
- 12- Gouault-Heilmann M., Dumont M.D. and Inractor L.: AvWD with IgM inhibitor against von Willebrand's factor. *J. Clin. Pathol.*, 32: 1030-1035, 1979.
- 13- Hardisty R.M. and Macpherson J.C.: A one-stage FVIII (antihaemophilic globulin) assay and its use on venous and capillary plasma. *Thrombosis et Diathesis Haemorrhagica*, 7: 215-217, 1962.
- 14- Ivy A.C., Nelson D. and Bucher G.: The stan-

- ardization of certain factors in the cutaneous venostasis bleeding time technique. *J. of Laboratory and Clinical Medicine*, 26: 1812-1813, 1940.
- 15- Jakway J.L.: Acquired von Willebrand disease. *Hematol. Oncol. Clin. North Am.*, 6: 1409-1419, 1992.
 - 16- Konkle B.A.: Laboratory evaluation of von Willebrand disease. *Clin. Chem.*, 41: 489-490, 1995.
 - 17- Kreuz W., Linde R. and Funk M.: Induction of vWD type 1 by valproic acid. *Lancet*, 335: 1350-1351, 1990.
 - 18- MacFarlane D.E., Stibbe D.E., Kirby J., Zucker M.B., Grant R.A. and McPherson J.: A method for assaying Willebrand factor (ristocetin cofactor). *Thrombosis et Diathesis Haemorrhagica*, 34: 306-309, 1975.
 - 19- Mazurier C.: Von Willebrand disease masquerading as haemophilia A. *Thromb. Haemost.*, 67: 391-396, 1992.
 - 20- Montgomery R.R. and Collier B.S.: Von Willebrand disease. (eds): R. Colman, J. Hirsh, V. Marder and E. Salzman. In: *Hemostasis and Thrombosis, basic principles and clinical practice*, 1994.
 - 21- Nitu-Whalley I.C., Lee C.A., Griffioen A., Jenkins P.V. and Pasi K.J.: Type 1 von Willebrand disease-a clinical retrospective study of the diagnosis, the influence of ABO blood group and the role of the bleeding history. *Br. J. Haematol.*, 108: 259-264, 2000.
 - 22- Obert B., Houllier A., Meyer D. and Girma J.P.: Conformational changes in the A3 domain of von Willebrand factor modulate the interaction of the A1 domain with platelet glycoprotein Ib. *Blood*, 93: 1959-1968, 1999.
 - 23- Rao K.P., Kizer J. and Jones T.J.: AvWD associated with an extranodal pulmonary lymphoma. *Arch. Pathol. Lab. Med.*, 112: 47-50, 1988.
 - 24- Rinder M.R., Richard R.E. and Rinder H.M.: Acquired von Willebrand's disease: a concise review. *Am. J. Hematol.*, 54: 139-45, 1997.
 - 25- Sadler J.E.: A revised classification of von Willebrand disease. *Thromb Haemost.*, 71: 520-525, 1994.
 - 25- Sadler J.E.: A revised classification of von Willebrand disease. *Thromb Haemost.* 71: 520-525, 1994.
 - 26- Simone J.V., Comet J.A. and Abildgaard C.F.: Acquired von Willebrand's syndrome in systemic lupus erythematosus. *Blood*, 31: 806-812, 1968.
 - 27- Tefferi A., Hanson C.A., Kurtin P.J., Katzmann J.A., Dalton R.J. and Nichols W.L.: AvWD due to aberrant expression of platelet glycoprotein Ib by marginal zone lymphoma cells. *Br. J. Haematol.*, 96: 850-853, 1997.
 - 28- Tefferi A. and Nichols W.L.: AvWD: Concise review of occurrence, diagnosis, pathogenesis and treatment. *Am. J. Med.*, 103: 536-540, 1997.
 - 29- Tran-Thang C., Mannucci P.M. and Schneider P.: Profound alterations of the multimeric structure of von Willebrand factor in a patient with malignant lymphoma. *Br. J. Haematol.*, 61: 307-314, 1985.
 - 30- Van Genderen P.J., Leenknegt H., Michiels J.J. and Budde U.: Acquired von Willebrand factor in myeloproliferative disorders. *Leuk. Lymph.* 22 (Suppl. 1): 79-82, 1996.
 - 31- Van Genderen P.J., Vink T. and Muchiels J.J.: AvWD caused by an autoantibody selectively inhibiting the binding of von Willebrand factor to collagen. *Blood*, 84: 3378-3384, 1994.
 - 32- Werner E.J., Abshire T.C., Giroux D.S., Toker E.L. and Broxson E.H.: Relative value of diagnostic studies for von Willebrand disease. *J. Pediatr.*, 121: 34-8, 1992.