von Willebrand factor in Italian centenarians

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Background and Objectives. Subjects with blood type O have lower concentration of von Willebrand factor (VWF) than those with type A, B or AB. Since we recently observed that laboratory signs of marked hypercoagulability are compatible with health and longevity in Italian centenarians, we determined VWF and blood groups in healthy centenarians to see whether levels of this marker of endothelial perturbation were altered and whether its correlation with blood groups was similar to that among the general population.

Design and Methods. In 74 centenarians and in 110 controls (55<45 years old; 55>45 years old), we studied VWF antigen (VWF:Ag), ristocetin co-factor activity (VWF:Rco), multimeric pattern of VWF and cleaving protease (VWF:CP), and plasmin-antiplasmin complexes (PAP).

Results. The levels of VWF:Ag and VWF:Rco in centenarians were significantly higher than in controls without significant difference between blood group O or non-O. Fifty-one percent of centenarians have a reduction of the relative proportion of high molecular weight multimers (HMV); furthermore VWF:CP was lower and PAP significantly higher than in young controls.

Interpretation and Conclusions. The loss of large VWF multimers in 51% of centenarians could depend on degradation protease(s) in the circulation. VWF, a well-known independent predictor of atherothrombotic disease, was increased in centenarians, independently of the blood group, confirming the previous results of a state of hypercoagulability. The finding that the VWF:CP levels are low when VWF levels are high in centenarians could be a corollary of the previous described paradox of successful aging, adding another marker of increased risk of atherothrombosis to the scenario.

Key words: von Willebrand factor, centenarians, hypercoagulability, cleaving proteases, plasmin-antiplasmin complexes.

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Centenarians have a state of hypercoagulability with striking signs of high coagulation enzyme activity, enhanced formation of fibrin and secondary high hyperfibrinolysis.[1,2]

It is of interest that centenarians have a significantly higher frequency than young individuals of the high-risk 4G allele of the PAI-1 -675 (4G/5G) polymorphism,[3] mutant factor V (Arg506Gln)[4] and prothrombin gene G20210A mutation.[5] Nevertheless, they have aged successfully and escaped major thrombotic diseases. The elevation of activated factor VII (FVIIa) concentration in centenarians could be the result of enhanced expression and availability of tissue factor. While tissue factor is usually not expressed in intact vasculature, high levels of FVIIa could be an expression of endothelial cell perturbation. von Willebrand factor (VWF) is the major adhesive protein, circulating in the plasma as a series of heterogeneous multimers of molecular weight 400 to 2000 Kdaltons; only the highly polymeric forms are hemostatically active.[6] VWF is involved in the initiation of platelet adhesion to the damaged vascular wall[7] and is a well-known marker of endothelial perturbation.[8,9,10] In addition, VWF is an independent predictor, together with fibrinogen and tPA antigen, of myocardial infarction or sudden cardiac death.[11] Elevated circulating levels of VWF may be associated with an increased risk of death following stroke.[12] The ABO blood group type is associated with the plasma concentration of VWF antigen (VWF:Ag)[13,14] i.e., concentrations are higher in individuals of non-O blood groups than in those of blood group O. Higher risks of coronary heart disease, atherosclerosis, and venous thromboembolism have been shown in individuals with non-O blood groups.[15,16] Proteolytic cleavage of VWF takes place in the circulating blood of healthy subjects.[17] The hemostatically active large VWF multimers are degraded to smaller less active forms. There is little knowledge on the behavior of proteases in longevity, but is seems than the protease level is usually low when VWF is high: thus the contribution of the protease to the atherothrombotic risk of VWF could be explored.

We measured VWF:Ag levels and determined the blood groups in 74 healthy centenarians to see whether the levels of this marker of endothelial damage were higher and the correlation with blood groups was similar to that among the general population. In the same patients VWF:Rco was determined to see whether corresponding levels are altered in comparison with levels of VWF:Ag. We also performed VWF multimeric analysis of VWF by SDS-agarose discontinuous gel electrophoresis; the rel-

ative proportion of high molecular weight (HMV) multimers was calculated by scanning the autoradiography with a densitometer. VWF:CP levels were measured as previously described. Plasmin-antiplasmin complex (an index of plasmin formation and neutralization) was also measured.

Design and Methods

Subjects

A total of 184 individuals were studied; 74 were *cases* more than 100 years old and 110 were *controls* less than 100 years old. Approval for these studies was obtained from the Institutional Review Board of the University of Milan and informed consent was obtained according to the Declaration of Helsinki. Centenarians (age range 100 to 107 years; 18 men and 56 women) were ambulatory, self-sufficient and lived in their homes in metropolitan communities of Northern Italy (Milan, Modena). They were chosen on the basis of the strict criteria set by the Senieur Protocol.[18] Chronic disorders such as infection, inflammation, malignancy, dementia, diabetes, cardiovascular, renal and liver disease were causes for exclusion. An electrocardiogram and hemocytometry were performed and erythrocyte sedimentation rate and plasma concentrations of glucose, sodium, potassium, albumin, alkaline phosphatase, aminotransferases, urea and creatinine were measured. Centenarians with values outside the 2.5 and 97.5 percentiles of the reference values of these measurements were excluded. Controls (n=110, aged 21 to 86, 49 men and 61 women) were chosen to make up two groups of equal size: younger controls, aged < 45 years, who were blood donors or hospital staff members, and older controls, aged > 45 years. On the basis of history and physical examination , none of these control subjects had any of the conditions taken as causes for exclusion for the group of healthy centenarians.

Blood sampling and processing

Venous blood was obtained between 8:00 and 10:00 a.m. from the antecubital veins by the two-syringe technique, using 19-gauge needles. After the first 4 mL of blood had been discarded, samples were collected directly into vacutainers containing Na_2-EDTA. All blood specimens were centrifuged at 4°C for 20 min at 2000×g and platelet-poor plasma was stored at -80°C until assayed within 2 months. Blood groups were determined by the courtesy of Dr. Moroni at the S. Paolo Hospital, Blood Bank Center, Milan, Italy.

Assays

VWF:Ag was assayed by ELISA using monoclonal antibodies, as previously described.[19] Pooled normal plasma calibrated against the WHO standard for VWF (97/586) was used as a reference. The technicians were at all times unaware of whether samples came from patients or controls. VWF:Rco was performed with a modification of Macfarlane's method.[20] VWF multimeric analysis of VWF was performed by SDS-agarose discontinuous gel electrophoresis; the relative proportion of HMV multimers was calculated by scanning the autoradiography with a densitometer with a modification of Ruggeri's method.[6] VWF cleaving proteases assay was performed using a previously described enzyme immunoassay.[17] Plasmin-antiplasmin complex (PAP) was measured by an ELISA (Behringwerke, Marburg, Germany).

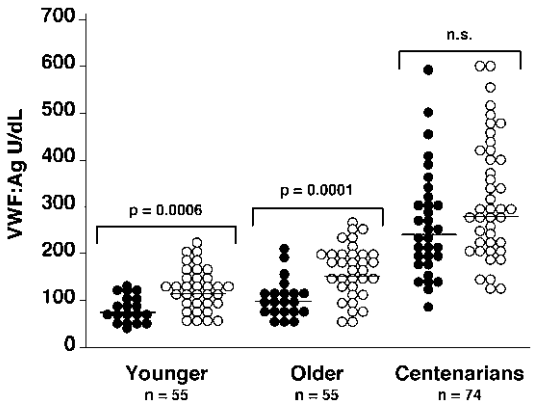

Figure 1. von Willebrand factor (U/dL) related to blood groups in younger, older, and centenarian individuals. Closed circles represent blood group type O, open circles type non-O.

Statistical analysis

The values obtained in controls and centenarians were analyzed after transformation into logarithms, but for descriptive purposes are given as geometric means and 95% confidence intervals. Comparisons between the groups were made by analysis of variance and between-group differences assessed with the Sheffé test.

Results

VWF:Ag and VWF:Rco levels are higher in centenarians than in the controls, both older and younger. The distribution of blood types among the controls mimics that in the general population (35% type O, 65% type non-O), whereas 43% of the centenarians had blood type O, and 57% had type non-O. Figure 1 shows that the correlation between VWF:Ag levels and blood types agrees with the well known fact that individuals with blood type O have lower mean concentrations of VWF:Ag than those with blood type non-O: (p<0.0006 and p<0.0001) (Figure 1).

Table 1. Geometric means (95% confidence intervals) of von Willebrand factor antigen and VWF:Rco levels (U/dL) in younger, older and centenarian individuals, related to blood group.

	VWF:Ag (U/dL) O	*vWF:Ag (U/dL) non-O*	*p*	*VWF:Rco (U/dL) O*	*VWF:Rco (U/dL) non-O*	*p*
Younger controls (n=55)	77 (67-91)	115 (100-132)	0.0006	80 (60-107)	112 (76-164)	0.0017
Older controls (n=55)	99 (84-117)	152 (133-174)	0.0001	98 (67-145)	151 (100-228)	0.0003
Centenarians (n=74)	245 (215-279)	285 (251-325)	NS	230 (159-332)	251 (174-360)	NS

Table 2. Relative proportion of high molecular weight VWF multimers in centenarians and controls.

		Cut-off%	
	Range %	*≤17*	*>17*
Younger	18-28	0	100
Older	15-20	25	75
Centenarians	10-20-	51	49

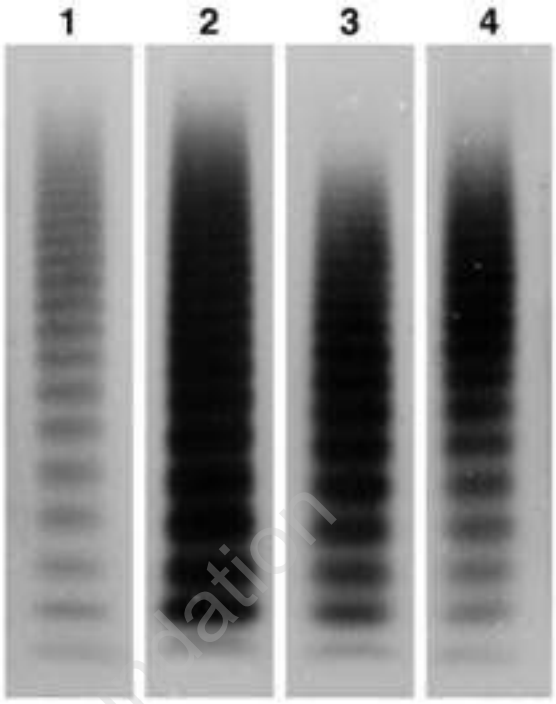

Figure 2. Multimeric structure of VWF protein: 1 normal plasma sample, 2 centenarians with normal pattern, 3 and 4 centenarians with a reduction in the relative proportion of high molecular weight multimers.

The levels of VWF:Rco corresponded to antigen levels (Table 1) and were well-correlated (r = 0.87, *p*=0.0001). In contrast, centenarians with blood group O and those with non-O had equally high levels of VWF:Ag. Using a cut-off of 17%, established in our laboratory database from younger and older subjects, we observed that 51% of the centenarians had a reduction of the relative proportion of HMW multimers as compared to that in younger controls, and in only 25% of *older* controls was a similar multimeric pattern present (Table 2, Figure 2). In 62% of centenarians the VWF:CP was lower than in young controls: 76% (50-97%) vs. 105% (52-175%) (*p*=0.01). There was a statistically significant inverse correlation between the proteases, VWF:Ag (r = -040) and VWF:Rco (r = -0.39). PAP were much higher in centenarians than in controls: 858 (768-928 ng/mL), compared to 330 (315-400 ng/mL) and 285 (253-321 ng/mL) in old and young controls, respectively.

Discussion

The level of VWF:Ag is extremely high in centenarians. The predominant site of VWF synthesis is the vascular endothelium,[21] which is the major source of plasma VWF *in vivo*.[22] In addition to this continuous release of VWF, rapid secretion of preformed VWF, localized in intracellular storage organelles called Weibel-Palade bodies[23,24] can be provoked *in vivo* and *in vitro* by a number of agents. Paleolog *et al*. demonstrated that cytokines have no direct effects on VWF release from cultured vascular endothelial cells but can significantly modulate its acute secretion in response to thrombin.[25] It has been shown that in centenarians there is a marked increase in the number of circulating activated T-lymphocytes (CD3 and HLA-DR positive) which may interact with endothelial cells in response to thrombin.[26] Furthermore, in centenarians increased thrombin generation was expressed as high levels of FVIIa correlated with factor X and factor IX activation peptides.[1]

The underlying mechanism of the effect of the blood group on VWF might be related to differences in the processing, release, or catabolism of VWF in people with different blood groups.[13] Evidence for these differences includes the finding of different oligosaccharides in the blood group A, B, and O structures of human VWF,[27,28] and the find-

ing that antiserum against blood groups A and B reacts only with VWF immunoprecipitate from individuals with the appropriate blood group. We found no significant differences in VWF:Ag levels between centenarians of blood group O and non-O. In order to investigate the possible mechanisms responsible for the decrease of VWF:Ag in people with blood group O, Gill *et al*.[29] studied VWF:Ag and VWF antigen II (VW AgII) in blood donors. Neither VWF:Ag nor VW AgII was different in secretors of H substance, as determined by the Lewis blood group, in either group O or non-O individuals. The cleavage of pro-VWF into mature VWF and its propolypeptide, VW AgII, is thought to occur in the storage granule and both are thought to be released into plasma simultaneously. Since previous studies of VW AgII in type II von Willebrand's disease suggest that VW AgII may reflect the rate of VWF synthesis, the findings of decreased VWF:Ag but not of VW AgII in normal group O individuals may be due to a more rapid clearance of VWF from plasma. We can surmise that centenarians, who had equally high levels of VWF:Ag whether they had group O blood or not, could have slower clearance of VWF from the circulation.

The loss of large VWF multimers in 51% of centenarians could depend on degradation protease(s) in the circulation, as suggested by very high PAP levels. The finding that the VWF:CP levels are low when VWF levels are high in centenarians could be a corollary of the previously described paradox of successful aging, adding another marker of increased risk of atherothrombosis to the scenario.

The concentration of plasmin-antiplasmin complex is much higher in centenarians than in controls (858 ng/mL compared to 330 ng/mL and 285ng/ml in older and younger controls, respectively, $p<0.01$). The presence of a modified form of VWF in centenarians could counteract the apparently unfavorable hemostasis profile due to the high levels of VWF:Ag. Nevertheless, we have also demonstrated this paradoxical marked hypercoagulability by genetic markers.[3,4,5] Furthermore, it has been shown that most centenarians have high serum levels of lipoprotein(a), low HDL cholesterol and relatively high trygliceride levels, which together are considered to be strong risk factors for coronary artery disease.[30] Thus , a long life is compatible with the possession of several high-risk alleles and high concentrations of well-known cardiovascular risk factors.

Parameters considered risk factors for the atherosclerotic vascular diseases in young people may lose their biological significance in advanced age and assume a different role.

Our data about the hemostasis profile of centenarians once again emphasize the need for a multidisciplinary approach if we wish to understand the mechanisms of successful aging.

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Contributions

RC, DM contributed to the conception and design, wrote the article and with the contribution of CF revised it. AL contributed to the analysis and interpretation of the data. All authors approved the final version to be published. Primary responsibility for the paper: RC; Tables 1, 2 and Figure 1: RC; Figure 2: AL.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received July18, 2002; accepted November 18, 2002. In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Although centenarians show a state of hypercoagulability, they have aged successfully and escaped major thrombotic diseases.

What this study adds

This study shows that the plasma levels of von Willebrand antigen are significantly higher in centenarians, thus confirming the above paradox of successful aging.