



Vilnius University
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Clinical Utility of von Willebrand Factor Multimers Assay

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INTRODUCTION

Von Willebrand disease is one of the most common blood clotting disorders, affecting up to 1% of the population. The disease is classified into three types, which are further divided into subtypes. Due to the varied symptoms of the disease, diagnosis and treatment present challenges. One of the diagnostic challenges is the analysis of von Willebrand multimers in gel electrophoresis. This test requires highly qualified laboratory personnel and very expensive equipment. For this reason, within the Baltic region, the test is performed only in Lithuania and Estonia, specifically in third-level hospital laboratories. The high cost of resources raises the question of the justification for this test when it is performed on all patients exhibiting symptoms of heavy bleeding.

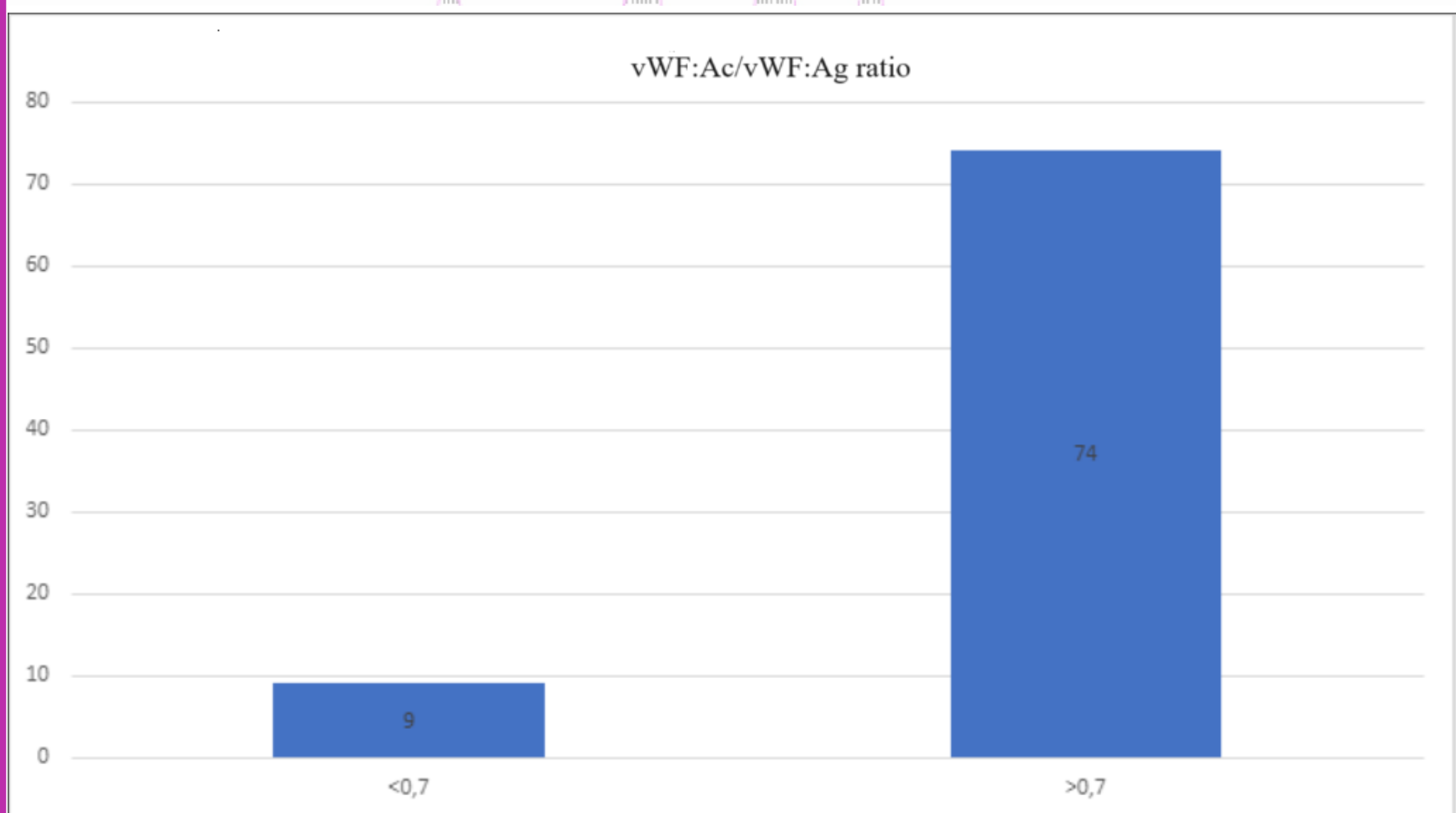
MATERIALS AND METHODS

Off the 143 subjects, 83 patients were selected who underwent von Willebrand factor multimer (vWF:MM) electrophoresis in agarose gel and had von Willebrand factor activity and antigen concentration determined. The activity/antigen (vWF:Ac/vWF:Ag) ratio was calculated for these patients.

RESULTS

vWF:Ac/vWF:Ag ratio

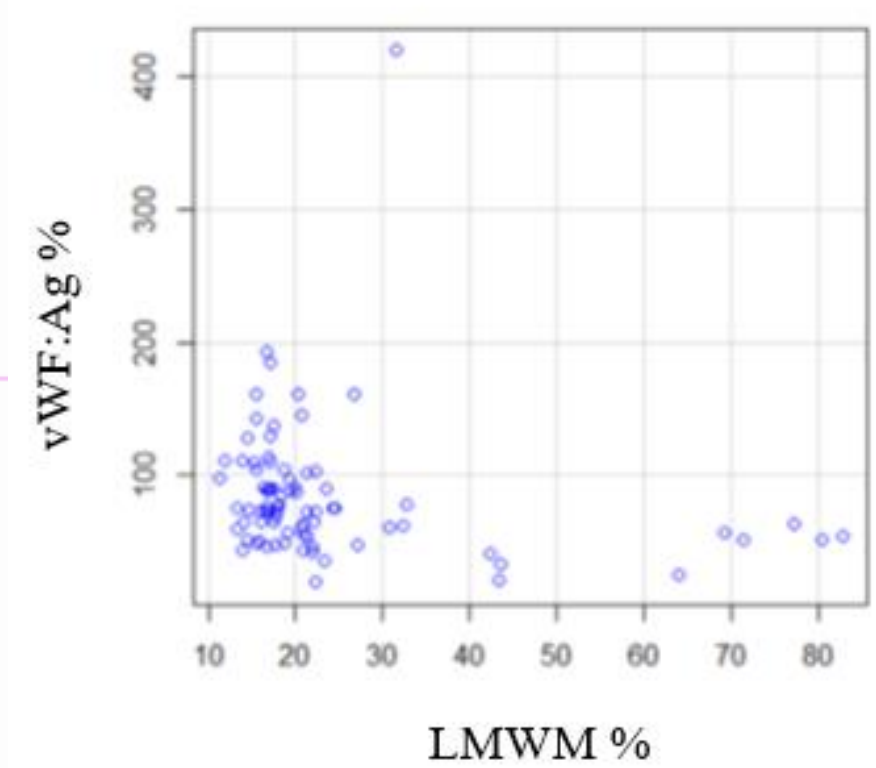
Von Willebrand multimer testing is often ordered without considering guidelines or coagulation test results. Among the selected subjects, 10,8 % of patients had a vWF:Ac/vWF:Ag ratio <0,7 (Graph 1). However, this is not an excessive use of resources, as out of 83 patients who underwent the test and had no altered coagulation test results, 7 patients (8,4 %) were found to have altered multimer fractions, suggesting a diagnosis of von Willebrand disease.



Graph 1. Ratio of von Willebrand factor activity (vWF:Ac) and antigen (vWF:Ag) calculated according to ISTH recommendations.

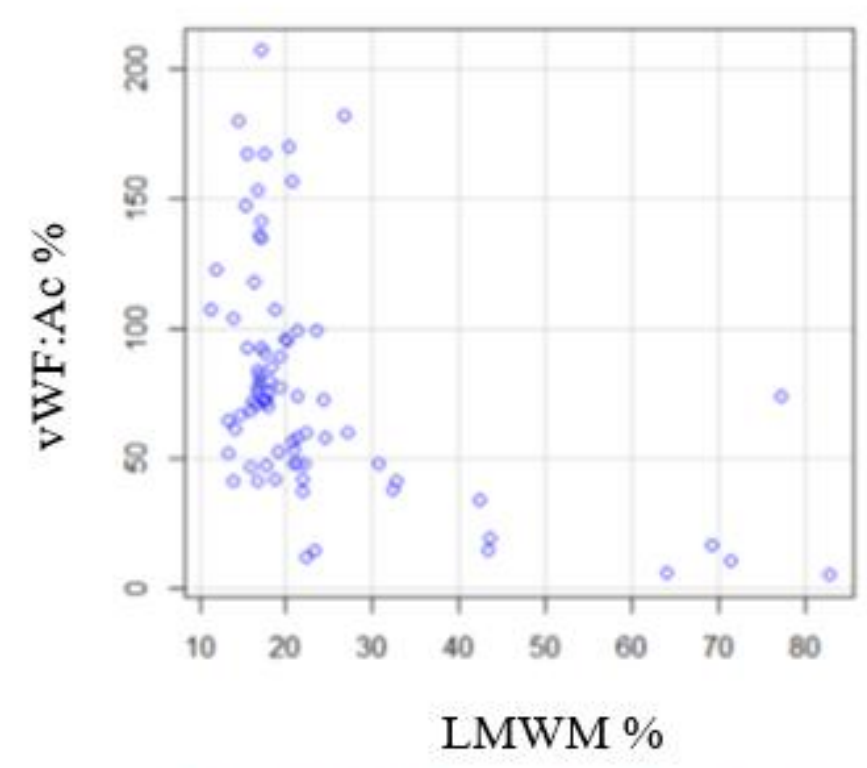
vWF:MM

To assess whether there is a relationship between the concentration of LMWM in the blood and the concentrations of vWF antigen and activity in the blood, a correlation analysis was conducted (Graph 2 and 3). Upon determining that the concentration of LMWM is dependent on both vWF activity and antigen concentration in the blood, a further correlation analysis was performed to determine whether changes in the vWF:Ac/vWF:Ag ratio have any correlation with the concentration of LMWM in the blood (Graph 4).



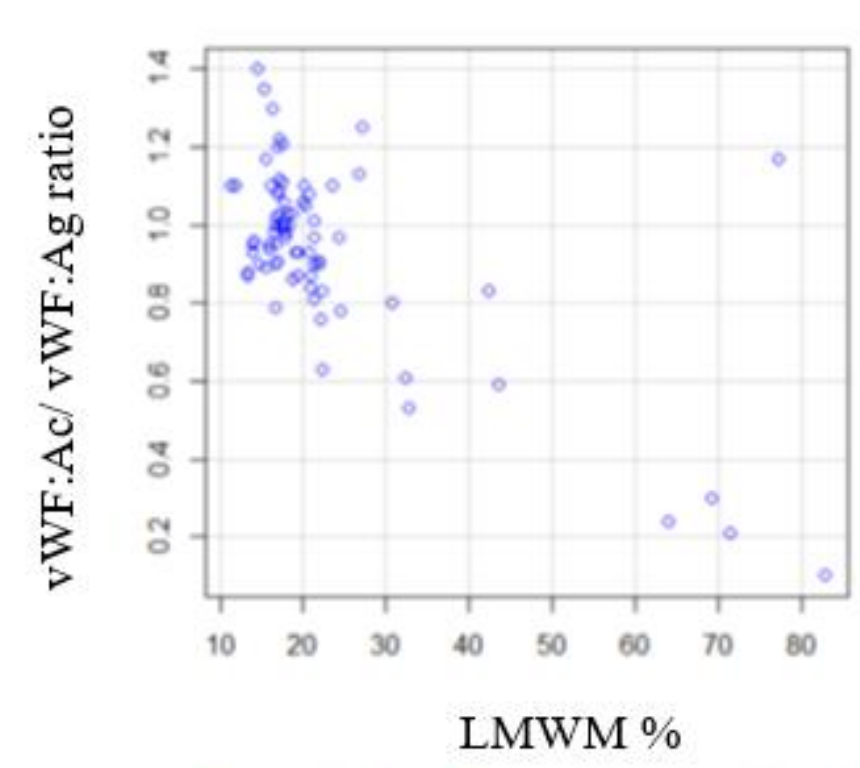
Graph 2. Correlation between LMWM and vWF:Ac

p = 0,0012
r = -0,3496



Graph 3. Correlation between LMWM and vWF:Ag

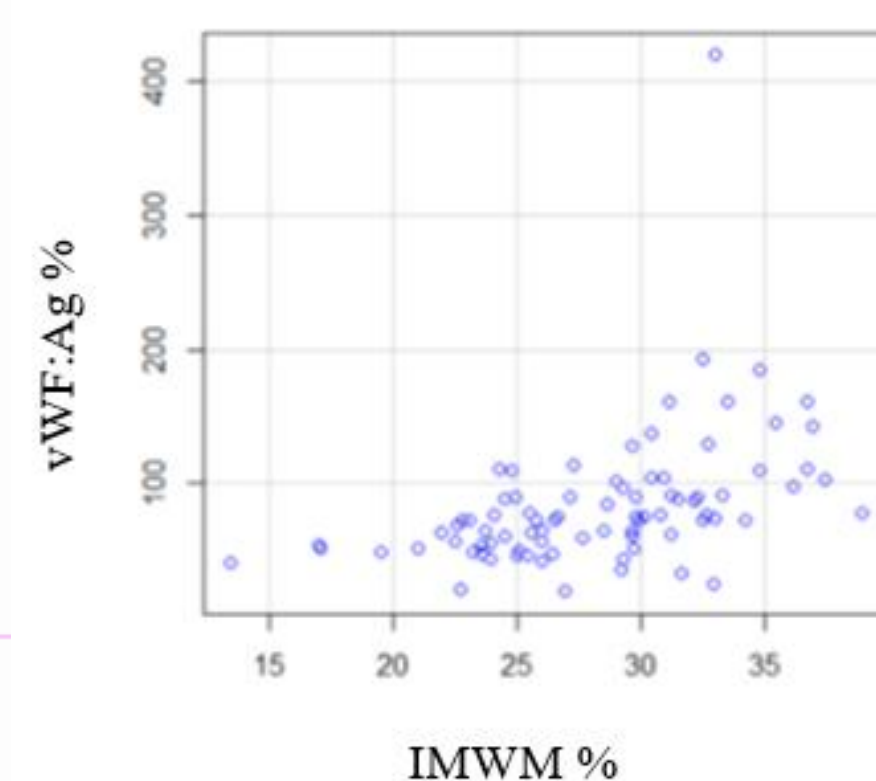
p = <0,01
r = -0,486



Graph 4. Correlation between LMWM and vWF:Ac/vWF:Ag

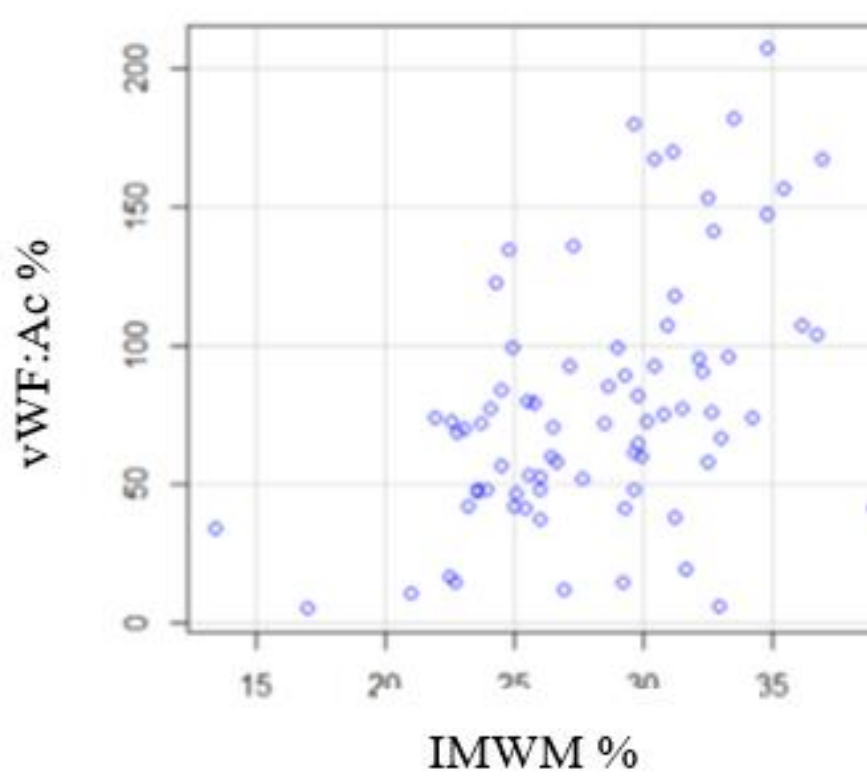
p = 0,003
r = -0,413

To evaluate whether there is a correlation between the concentration of IMWM in the blood and the concentrations of vWF antigen and activity, a correlation analysis was performed (Graph 5 and 6). After identifying a relationship between the concentration of IMWM in the blood and vWF activity and antigen levels, a further correlation analysis was conducted with the vWF:Ac/vWF:Ag ratio to determine whether changes in this ratio affect the concentration of IMWM in the blood (Graph 7).



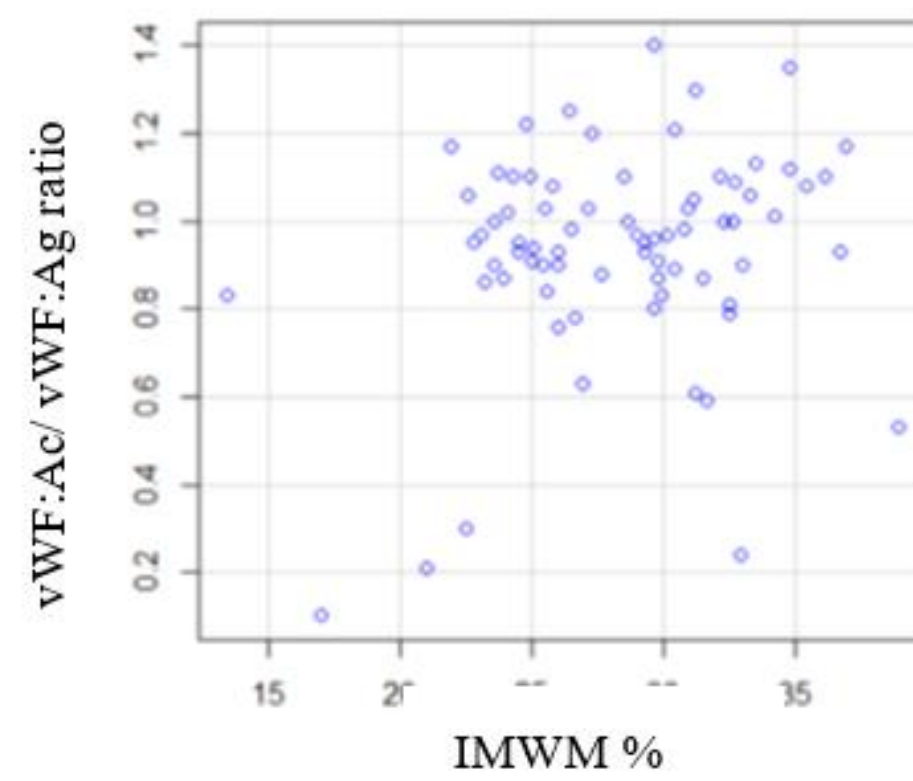
Graph 5. Correlation between IMWM and vWF:Ac

p = <0,001
r = 0,5834



Graph 6. Correlation between IMWM and vWF:Ag

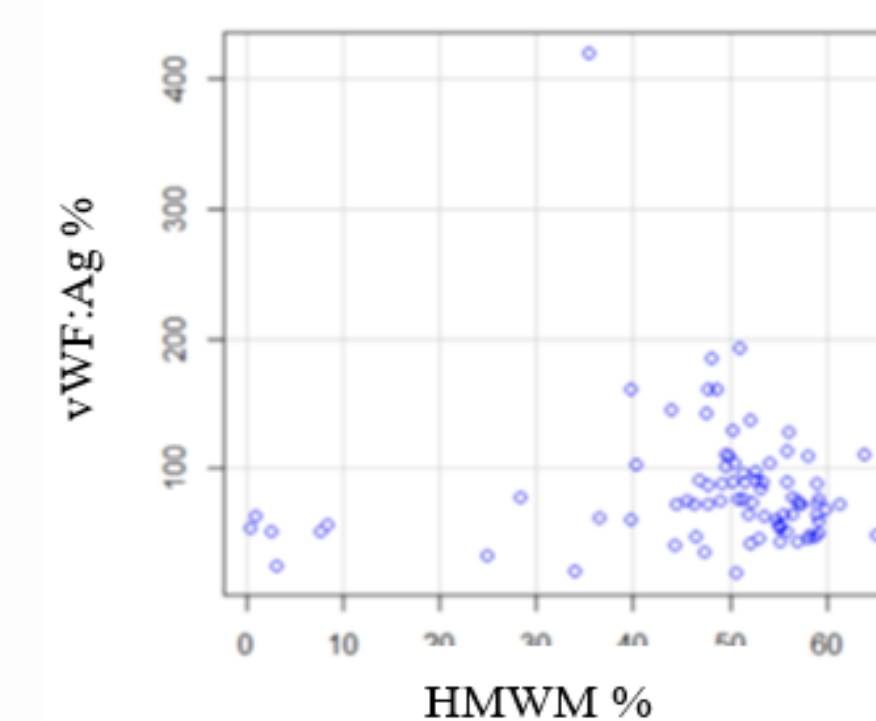
p = <0,001
r = 0,461



Graph 7. Correlation between IMWM and vWF:Ac/vWF:Ag

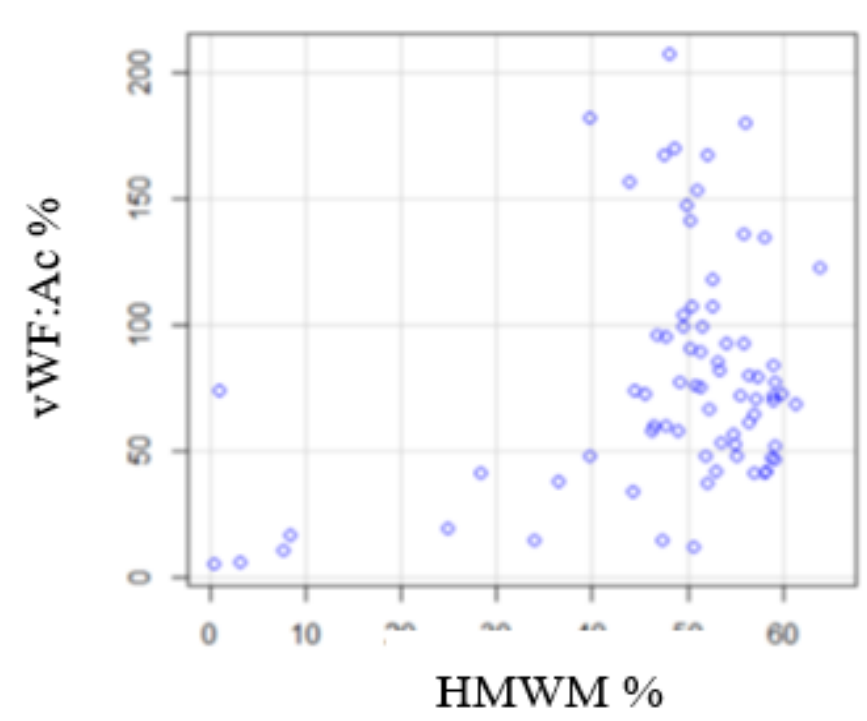
p = <0,1799
r = 0,1576

To assess whether there is a correlation between the concentration of HMWM in the blood and the concentrations of vWF antigen and activity, a correlation analysis was conducted (Graph 8 and 9). Upon finding no significant relationship between HMWM concentration in the blood and either the antigen or activity levels, a comparison was made with the vWF:Ac/vWF:Ag ratio (Graph 10).



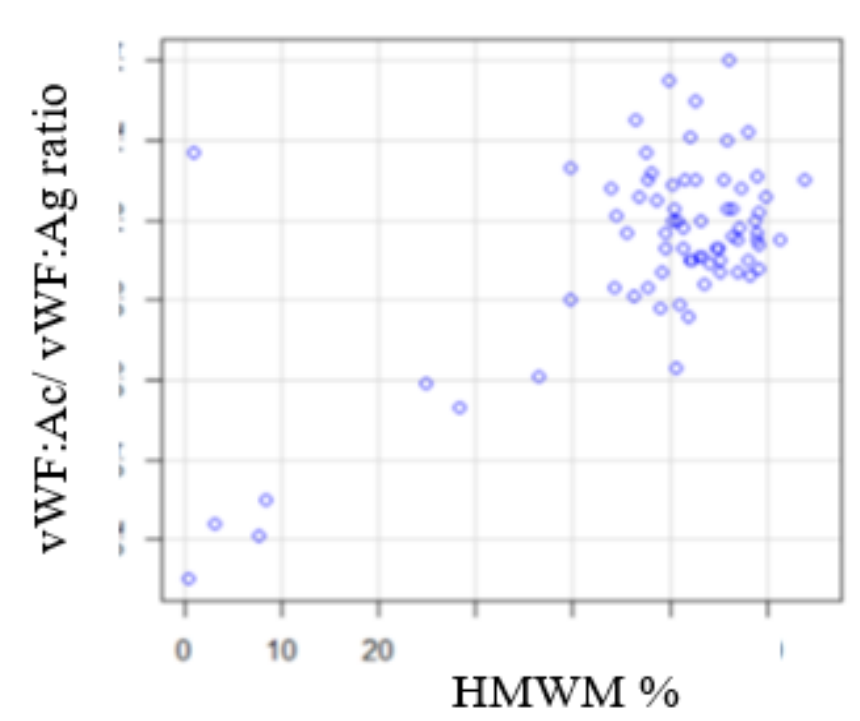
Graph 8. Correlation between HMWM and vWF:Ac

p = 0,6934
r = -0,0439



Graph 9. Correlation between HMWM and vWF:Ag

p = 0,2529
r = 0,1328

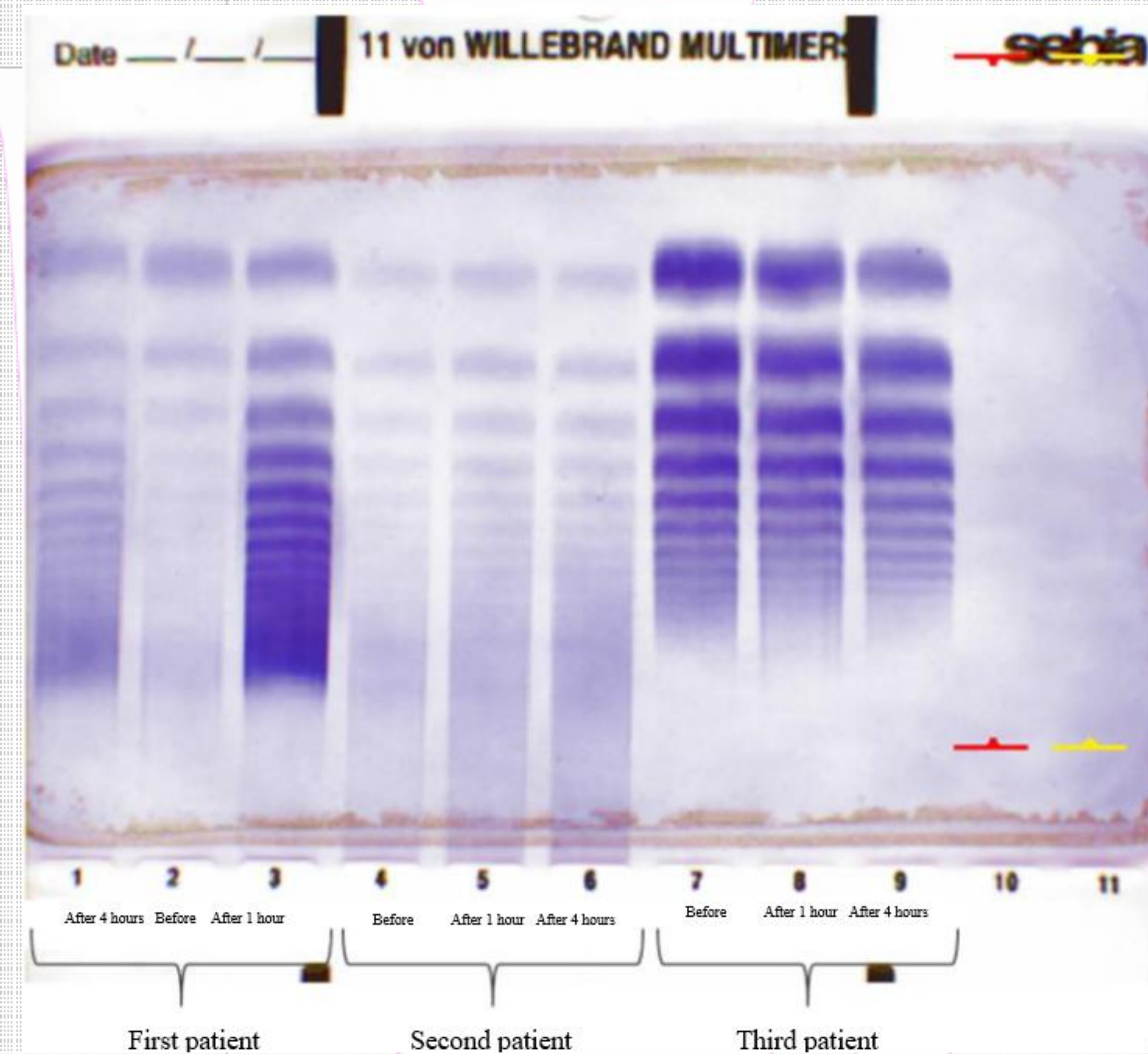


Graph 10. Correlation between HMWM and vWF:Ac/vWF:Ag

p = 0,0422
r = 0,2368

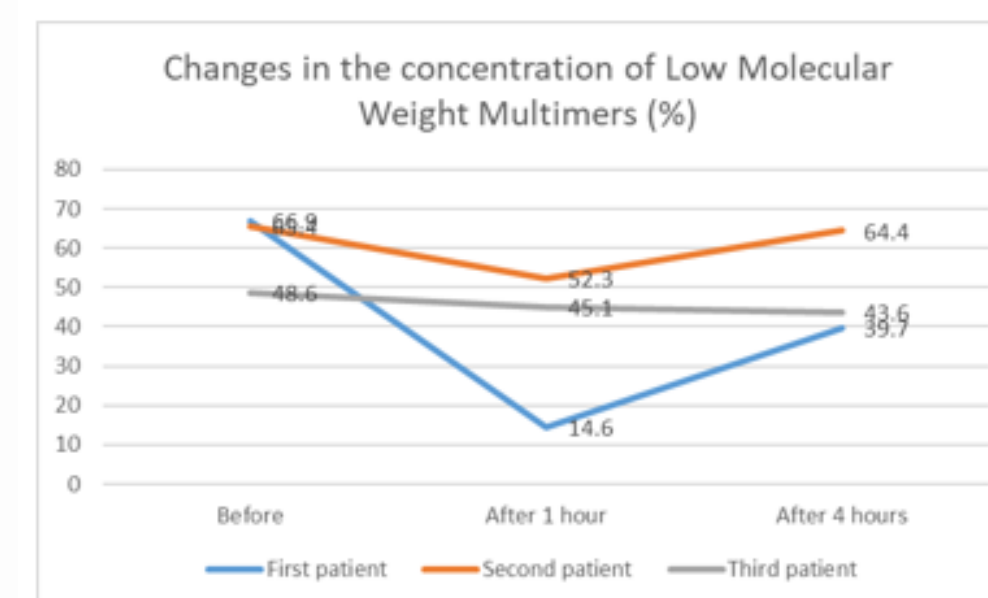
vWF:MM and desmopressin

The effect of desmopressin (DDAVP) on three patients diagnosed with von Willebrand disease was observed by conducting a vWF multimer electrophoresis test. The goal was to assess changes in the intensity of multimer fraction peaks before DDAVP administration, and 1 hour and 4 hours after the drug injection. Following the vWF analysis, the results for the first patient are shown in columns 1-3, for the second patient in columns 4-6, and for the third patient in columns 7-9 (Graph 11). The gel analysis clearly demonstrated an increase in HMWM 1 hour after DDAVP injection (columns 3, 5, and 7). In columns 1, 6, 8, and 9, the gel visually shows a fading of HMWM and a more pronounced appearance of IMWM fractions, corresponding to blood samples taken 4 hours after DDAVP injection. Columns 8 and 9 were analyzed using the same patient sample but at different dilutions: 1:6 (column 8) and 1:10 (column 9).

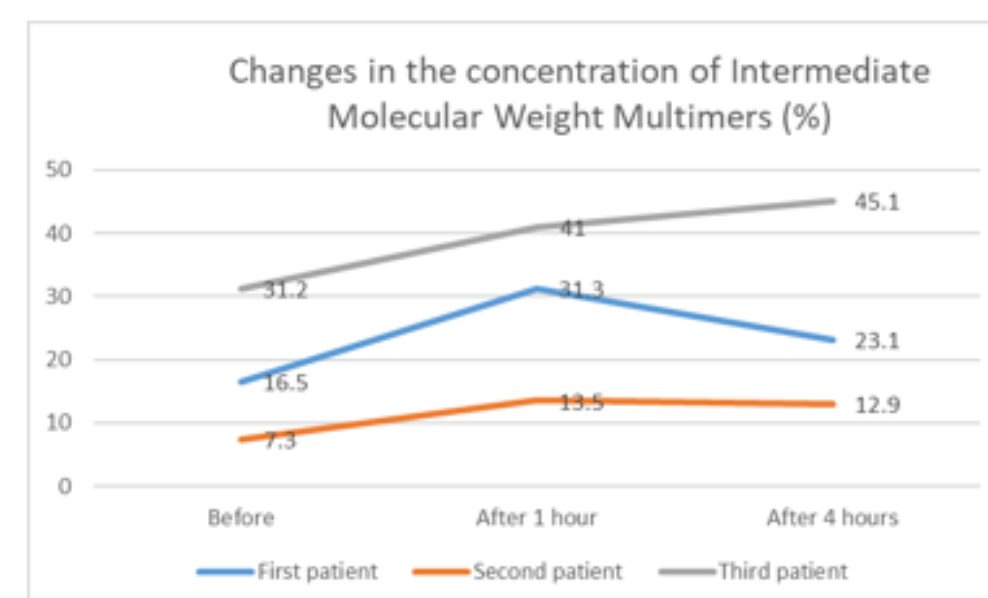


Graph 11. Agarose gel assay of vWF:MM

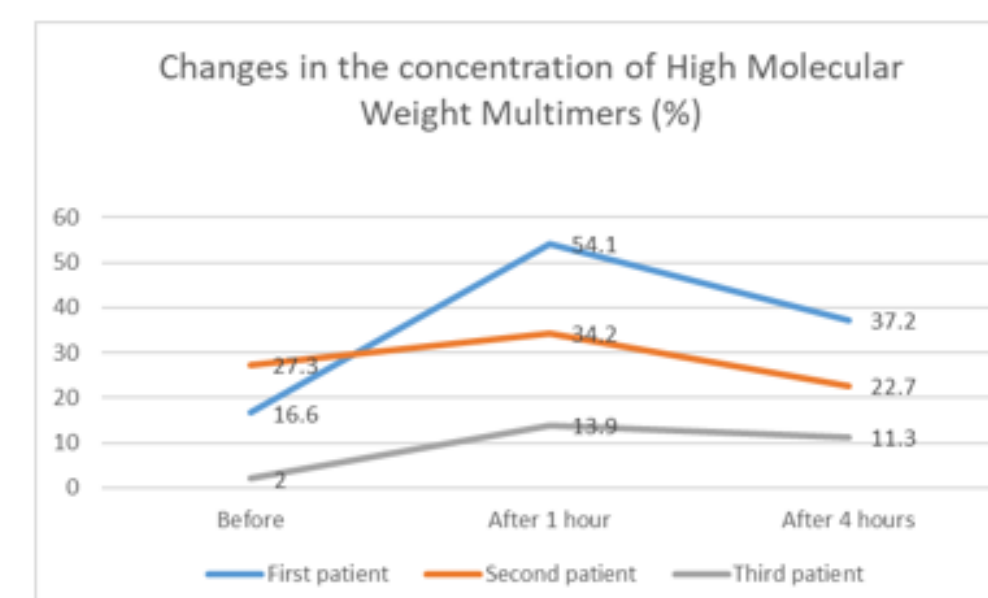
The patient responses to DDAVP were analyzed by categorizing them according to multimer classes (LMWM, IMWM, and HMWM) and the time points before the injection, 1 hour after the injection, and 4 hours after the injection. The distribution of multimers among the three patients is illustrated in Graphs 12, 13, and 14.



Graph 12. LMWM distribution of 3 patients before, 1 hour, and 4 hours after DDAVP injection. p = 0,285



Graph 13. IMWM distribution of 3 patients before, 1 hour, and 4 hours after DDAVP injection. p = 0,593



Graph 14. HMWM distribution of 3 patients before, 1 hour, and 4 hours after DDAVP injection. p = 0,109

CONCLUSIONS

1. Von Willebrand multimer testing is often ordered without considering guidelines or coagulation test results. However, this is not an excessive use of resources, as out of 83 patients who underwent the test and had no altered coagulation test results, 7 patients (8,4 %) were found to have altered multimer fractions, suggesting a diagnosis of von Willebrand disease.
2. The concentration of low and medium molecular weight multimers in the blood depends on the factor antigen and activity, while these indicators do not affect the concentration of high molecular weight multimers.
3. The von Willebrand factor multimer test allows for monitoring the effect of desmopressin on patients.