

Abstract

Physiological anticoagulants play a significant regulatory role in hemostasis. Due to numerous functions not only in hemostasis regulation, we focused specifically on protein S (PS) in this work, which we divided into three parts. The aim of the first part was to identify causal mutations in the *PROSI* gene in a cohort of patients with inherited PS deficiency using Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). PS deficiency is a significant known risk factor for venous thrombosis; however, considering the structure of PS, we hypothesized that mutations in certain parts of PS would carry larger thrombotic risk than in others. Therefore, we further analyzed the influence of mutation position on thrombosis risk in this cohort of patients with inherited PS deficiency. In the second part of the work, our goal was to describe the observed acquired PS deficiencies in a cohort of patients from the Institute of Hematology and Blood Transfusion in Prague with hematological malignancies. Finally, in the third part, the aim was to verify the stability of PS and other coagulation parameters in frozen plasma conditions, which has important implications for both daily laboratory practice and the diagnosis of PS deficiency.

Causal mutations in the *PROSI* gene were found in 73/79 (92%) patients. A total of 34 different mutations were identified, 15 of which had not been described previously. The distribution of mutations does not show clustering in any region of the gene. We identified a large deletion using the MLPA method in 3 families (7%), corresponding to the occurrence of deletions described in the literature. Furthermore, we found that the mutation location in the sex-hormone binding globulin (SHBG) domain of PS is an independent risk factor for thrombosis, and patients with these mutations also experienced thrombosis at a younger age. In patients with acquired deficiency, PS activity significantly differed between groups according to diagnosis, with the highest proportion of pathological values observed in patients with acute myelomonocytic leukemia. In the last part of the work, we demonstrated sufficient stability of all monitored parameters, including protein S, if stored at least at -80°C and snap-frozen. Under these conditions, the monitored parameters remained stable for 6 months, except for short-term anomalies at the beginning of storage, when APTT, PS activity, and free PS antigen were elevated above the method's uncertainty and

eventually returned to the level of results from fresh plasma (only samples stored at -80°C or lower).

This work confirms the significance of PS and its molecular diagnostics for better thrombosis risk estimation in patients with inherited deficiency and indicates future directions for studies of this multifunctional physiological coagulation inhibitor.