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Association of Acquired Coagulation Changes and Genetic Polymorphisms on Microvascular Free Flap Thrombosis in Reconstructive Surgery

Summary of the Doctoral Thesis for obtaining the scientific degree "Doctor of Science (*PhD*)"

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Abbreviations used in the Thesis

APC	Activated protein C
APCR	Activated protein C resistance
APTT	Activated Partial Thromboplastin Time
AT	Antithrombin
AUC	Area under the curve
FGG	Fibrinogen gamma chain gene
FII	Coagulation factor II, prothrombin
FV	Coagulation factor V
FV Leiden	Coagulation factor V Leiden
MTHFR	Methylene tetrahydrofolate reductase
ns	Nonsignificant
PCR	Polymerase chain reaction
PT	Prothrombin
ROC	Receiver operating characteristic
SERPINC1	Serine protease inhibitor 1 gene
Δt	Temperature difference

Introduction

Microvascular free flap surgery represents a cornerstone technique in reconstructive surgery, with successful outcomes dependent on meticulous restoration of blood flow through small-vessel anastomoses. Despite advances in microsurgical techniques and perioperative management, thrombotic complications remain the primary cause of flap failure, occurring in 2–9 % of cases (Bowman, 2011; Friedman, 2010). These complications represent a complex pathophysiological process governed by Virchow's triad: hypercoagulability, endothelial dysfunction, and haemodynamic alterations (Kumar, 2010).

While technical and mechanical aspects have been extensively studied, the role of genetic factors in thrombotic complications has received relatively less attention in microvascular surgery (Khansa, 2011). Recent advances in molecular genetics have highlighted the potential influence of inherited factors on thrombotic events (Friedman, 2010). Single nucleotide variants (SNVs) in genes regulating coagulation pathways may contribute significantly to individual thrombotic risk variation.

Five genetic variants have been implicated in thrombotic disorders: **Factor V Leiden (rs6025)**, the most prevalent inherited thrombophilia in Caucasians (2–15 % frequency), conferring 3–8-fold increased venous thrombosis risk through resistance to activated protein C (Rees, 1995; Rosendaal, 1995; Segers, 2007); **Prothrombin G20210A (rs1799963)**, occurring in 1–3 % of Caucasians with 2–3-fold increased risk due to elevated plasma prothrombin levels (Poort, 1996; Simone, 2013); **Fibrinogen Gamma Chain (rs2066865)**, affecting clot structure and stability, with fibrinogen elevation during inflammatory states, trauma, and malignancy (de Willige, 2005; Grünbacher, 2007; van Hylckama Vlieg, 2003); **SERPINC1 (rs2227589)**, affecting antithrombin with 10–20-fold increased thrombosis risk when deficient (Zöller, 1999); and **MTHFR C677T (rs1801133)**, influencing homocysteine metabolism with associated thrombotic events (Goyette, 1998; Liew, 2015).

While extensive epidemiological data support associations between these variants and thrombotic risk in general populations, their specific relevance to microvascular surgical outcomes remains inadequately described. Previous investigations demonstrated Factor V Leiden carriers exhibit 3–7-fold increased venous thrombosis risk (Kujovich, 2011), while prothrombin G20210A carriers show 2–3-fold elevated risk (Simone, 2013). However, limited studies have focused specifically on genetic contributions to thrombosis in microvascular free flap surgery. The unique haemodynamic environment of microvascular anastomoses, combined with surgical trauma-induced prothrombotic states, may amplify the clinical significance of genetic thrombophilic variants. Identification of genetic markers associated with increased

thrombotic risk could revolutionise preoperative risk assessment and guide personalised anticoagulation strategies, potentially improving surgical outcomes.

Aim of the Thesis

The aim of the Thesis was to determine whether specific genetic polymorphisms associate with increased thrombotic complications in microvascular free flap surgery with goal of developing genetic risk stratification parameters for individualised perioperative care.

Tasks of the Thesis

1. To identify patients with single nucleotide variants: rs6025 in *FV* gene; rs1799963 in prothrombin gene; rs2066865 in *FGG*; rs2227589 in *SERPINC1* and rs1801133 in *MTHFR*.
2. To examine and evaluate the association between determined single nucleotide variants and microvascular free flap thrombosis incidence.
3. To evaluate the impact of acquired thrombophilia factors on microvascular free flap thrombosis incidence.
4. To estimate the thrombosis risk in patients carrying combined factors (i. e. single nucleotide variants and acquired thrombophilia factors).

Hypotheses of the Thesis

Patients carrying variants in genes affecting haemostatic function (rs6025 in FV Leiden; Prothrombin rs1799963; rs2066865 in *FGG*; rs2227589 in *SERPINC1*; and/or rs1801133 in *MTHFR*) would demonstrate increased incidence of flap thrombosis.

Novelty of the Thesis

This study presents a novel approach to understanding thrombotic complications in microvascular free flap surgery by establishing an assessment model that combines genetic polymorphism analysis with standardised perioperative protocols. The main aspect lies in the systematic evaluation of both inherited thrombophilic traits and acquired coagulation changes, providing a foundation for personalised risk assessment and targeted prophylactic strategies in reconstructive microsurgery. This integrated approach offers insight into the multifactorial nature of flap thrombosis and presents potential pathways for improving surgical outcomes through genetically informed clinical decision-making.

1 Materials and methods

1.1 Study design and patient enrolment

This observational prospective case series study was conducted from December 2016 to July 2019 at the Centre of Plastic and Reconstructive Microsurgery of Latvia. Following application of stringent inclusion and exclusion criteria, 155 adult patients scheduled for microvascular free flap surgery were enrolled. The study protocol and informed consent form, including genetic material donation request, received approval from the Latvian Central Ethics Committee (No 1/28-11-16), ensuring adherence to international ethical guidelines and patient rights protection.

Inclusion criteria comprised all adult patients undergoing microvascular free flap surgery with signed informed consent.

Exclusion criteria were established to mitigate participant risk and enhance study reliability:

- **Pregnancy and peripartum period:** Excluded due to confounding gestational and postpartum physiological alterations.
- **Recent transfusions:** Patients receiving allogeneic blood components and/or coagulation factors within 72 hours prior to surgery were excluded to prevent confounding variables affecting outcomes.
- **Cardiac conditions:** Individuals with proven left ventricular failure were excluded to avoid complications interfering with surgical success and recovery.
- **Bone marrow and liver transplantations:** Patients with allogenic bone marrow or liver transplantation were excluded due to complex recovery factor interplay and potential outcome impact.
- **End-stage kidney diseases:** Excluded due to confounding systemic alterations.
- **Recent medication use:** Direct oral anticoagulants required cessation 72 hours before sample collection. Vitamin K antagonists were halted 4–5 days preoperatively based on INR levels. High thrombosis risk patients were switched to low-molecular-weight heparin, discontinued 12 hours prior to surgery.

Standardised interviews conducted by the same clinician collected patient history including previous thrombotic events, antithrombotic medication use, regular medications (including oral contraceptives), family history of thrombotic events, and previously diagnosed inherited thrombophilias. Tissue injury factors were recorded, with recent trauma defined as occurring within 30 days for trauma or polytrauma aetiology. Positive thrombosis history included any arterial or venous thrombotic event.

1.2 Genotyping

Blood samples were collected preoperatively prior to anaesthetic induction and crystalloid administration, then transported to the Latvian Biomedical Research and Study Centre for analysis.

DNA extraction and quality assessment: Genomic DNA was extracted from peripheral blood leukocytes using standardised phenol-chloroform extraction protocol. Extracted DNA underwent spectrophotometric quality assessment (260/280 ratio) and concentration determination. DNA integrity was verified through gel electrophoresis.

SNV genotyping: Single nucleotide variant genotyping employed TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA) on ViiA 7 Real-Time PCR system (Applied Biosystems). Specific assays included: C_11975250_10 for Factor V Leiden (rs6025), C_1799963_10 for prothrombin gene (rs1799963), C_11503414_10 for Fibrinogen Gamma (rs2066865), C_16180170_10 for *SERPINC1* (rs2227589), and C_1202883_20 for *MTHFR* (rs1801133).

PCR protocol: Reaction mixture comprised 12.5 µL TaqMan Universal PCR Master Mix, 0.625 µL SNP Genotyping Assay (40×), 2 µL DNA template (10 ng/µL), and 9.875 µL nuclease-free water (total volume 25 µL). Thermal cycling conditions included: initial AmpErase UNG activation (50 °C, 2 minutes), polymerase activation (95 °C, 10 minutes), 40 cycles of denaturation (95 °C, 15 seconds) and annealing/extension (60 °C, 1 minute), concluding with final extension (72 °C, 1 minute).

Quality control: Concurrent analysis of known homozygous wild-type, heterozygous, and homozygous variant controls alongside no-template controls. Genotype determination through allelic discrimination plots and cluster analysis. Validation criteria: minimum 95 % call rate, Hardy-Weinberg equilibrium assessment, and control sample concordance verification. Internal quality assurance maintained through duplicate testing of 10 % of samples and cross-platform verification where applicable. All procedures followed manufacturer's recommendations with comprehensive documentation of reaction conditions, reagent lot numbers, and equipment calibration status. Data analysis utilised manufacturer's software (Applied Biosystems) with standardised analytical parameters.

1.3 Clinical laboratory investigation

Blood samples were drawn on surgery day prior to anaesthesia induction and crystalloid infusion, with all tests processed within one hour at "NMS-Laboratorija" Ltd facility.

Fibrinogen: Total plasma fibrinogen concentration measured by Clauss method (Mackie, 2003) in citrated plasma using STA-R COMPACT (Diagnostika Stago, Asnières-sur-Seine, France). Reference range: 2–4 g/L.

Activated protein C resistance: Modified APC-R test with patient plasma diluted 1:4 with FV-deficient plasma. Parallel testing with/without APC using APTT-based clotting time measurement. Reference ranges: normal > 2.1, borderline 1.8–2.1, abnormal < 1.8, heterozygous FVL typical 1.5–1.8, homozygous FVL typical < 1.5.

Prothrombin time: Reagent containing thromboplastin and calcium chloride mixed with patient plasma; time to clot formation measured photo-optically. Reference range: 70–130 %.

Homocysteine: Concentration investigated with Chemiluminescent Immunoassays (CLIA). Reference range: 5–12 μmol/L.

Antithrombin: Chromogenic assay with reference range 75–125 %.

1.4 Anaesthesia and intraoperative patient monitoring

Three anaesthesia types were conducted based on patient needs: standardised general anaesthesia, regional anaesthesia, or combination of regional and general anaesthesia.

Standardised general anaesthesia protocol

***Preoperative phase*:** Pre-medication with Midazolam 0.02–0.04 mg/kg IV (maximum 2 mg). Prophylactic antibiotics per institutional guidelines. Antiemetic prophylaxis: Dexamethasone 8 mg IV + Ondansetron 4 mg IV.

Induction phase: Pre-oxygenation with 100 % O_2 for 3 minutes via face mask. Induction medications: Fentanyl 1–2 mcg/kg IV, Propofol 1.5–2.5 mg/kg IV, Cisatracurium 0.15–0.2 mg/kg IV.

Maintenance phase: Inhalational agent: Sevoflurane MAC 0.8–1.2 in O_2/Air mixture (FiO_2 0.4–0.5). Analgesia: Fentanyl 0.5–1 mcg/kg/h continuous infusion or Remifentanil 0.05–0.2 mcg/kg/min continuous infusion. Muscle relaxation: Cisatracurium 0.1–0.2 mg/kg/h maintaining TOF 1–2 twitches.

Standardised regional anaesthesia protocol

For upper and lower extremities: Long-acting agents Ropivacaine 0.2–0.5 % or Bupivacaine 0.25–0.5 %, volume 20–30 ml with maximum dosage calculation. Adjuvants: Dexamethasone 4–8 mg. Neural blockade performed utilising dual guidance: real-time ultrasonographic visualisation and peripheral nerve stimulation (Stimuplex, B. Braun, Melsungen, Germany) ensuring precise anatomical localisation.

Fluid management and monitoring

Fluid management: Maintenance with balanced crystalloid solution 4–6 mL/kg/h and additional boluses based on clinical assessment. Central catheter and indwelling urinary catheter placement for surgeries exceeding 2 hours.

Target parameters:

- Haemodynamic goals: Mean arterial pressure 70–80 mmHg, heart rate 60–80 beats/minute
- Central venous pressure: Optimal range 4–6 mmHg (not exceeding 10 mmHg due to venous congestion risk)
- Urinary output: 0.5–1.0 mL/kg/h
- Haematocrit: 30–35 %
- Temperature management: Core temperature (nasopharyngeal) 36.0–37.0 °C, Δt (core-peripheral) ≤ 1 °C, utilising fluid warmer and forced-air warming device

Multimodal analgesia: Paracetamol 1g IV or Metamizole 1g IV and Dexketoprofen 50 mg IV (if not contraindicated).

Recovery room monitoring: American Society of Anesthesiologists (ASA) standard monitoring, flap perfusion assessment, temperature monitoring, pain management.

1.5 Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) V.23 (IBM Korea, Seoul, Korea). The Kolmogorov-Smirnov test assessed normal distribution. Normally distributed continuous variables were presented as mean ± standard deviation (M ± SD) and categorical variables as percentages (%). Non-normally distributed values were presented as medians and interquartile ranges (IQRs). Odds ratios and 95 % confidence intervals evaluated factor impacts between groups. Comparisons between genotype groups employed Kruskal-Wallis H tests for nonparametric variables and ANOVA for parametric variables. Pearson's χ^2 correlation coefficient and p-values were calculated, with Spearman's rank correlation coefficient used where applicable. Statistical significance assumed two-tailed $p < 0.05$.

Thrombosis risk assessment: Primary analysis used logistic regression models for each SNV with subsequent calculation of odds ratios (OR), 95 % confidence intervals, and adjusted p-values. Allele distribution analysis employed Chi-square test for genotype frequencies, Fisher's exact test (when $n < 5$), and odds ratios for allele frequencies. Combined genetic risk analysis utilised multiple logistic regression and genetic risk score calculation with ROC curve analysis (AUC).

Risk prediction models: Multivariable logistic regression employed three predictor categories:

- **Genetic variants:** *FV* rs6025, *FII* rs1799963, *FGG* rs2066865, *SERPINC1* rs2227589, *MTHFR* rs1801133
- **Acquired coagulation changes:** Fibrinogen, homocysteine, APCR, prothrombin, antithrombin
- **Confounding factors:** Age, gender, smoking

Statistical power and sample size calculations for genetic association assumed $\alpha = 0.05$ and $\beta = 0.20$.

2 Results

2.1 Study population characteristics

Following application of inclusion and exclusion criteria, 162 consecutive patients were initially enrolled, with seven subsequently excluded due to incomplete data sets, resulting in a final study population of 155 subjects. The cohort comprised 118 males (76.1 %) and 37 females (23.9 %), with mean age 45.07 ± 14.94 years. Aetiologies including trauma (44.5 %), chronic inflammation (20 %), malignancy (18 %), and polytrauma (7.7 %). Defect localisation predominantly involved lower extremities (54.8 %), followed by upper extremities (23.2 %) and head/orofacial regions (18.7 %). Notable patient characteristics included smoking (41.3 %), recent trauma < 30 days (32.9 %), family history of thrombosis (13.5 %), alcohol abuse (11.6 %), metabolic disturbances (9.7 %), and personal history of thrombosis (6.5 %).

Flap thrombosis occurred in 14 patients (9 %), with complete flap loss in 8 cases (5.16 %) despite salvage attempts and partial flap loss in 5 cases (3.22 %), yielding overall microvascular flap survival success rate of 94.84 %. Venous thrombosis occurred more frequently than arterial thrombosis (71.4 % of cases either alone or combined with arterial complications). Despite aggressive surgical intervention, partial or complete flap necrosis occurred in 64.3 % of patients (n = 9), necessitating alternative reconstructive strategies including local flaps, split-thickness skin grafts (STSG), and negative pressure wound therapy (NPWT). Cases involving both arterial and venous thrombosis demonstrated higher flap necrosis rates, emphasising dual circulation compromise importance in determining outcomes.

Individual case series analysis of 14 patients with microvascular thrombosis revealed notable patterns. All cases carried at least one genetic variant, with *MTHFR* rs1801133 most prevalent (10/14, 71.4 %), followed by *FGG* rs2066865 (7/14, 50 %) and *SERPINC1* rs2227589 (5/14, 35.7 %).

Only one patient with thrombotic complications carried Factor V Leiden variant, also harbouring *FGG* variant with correspondingly elevated fibrinogen levels and reduced APCR values. Laboratory abnormalities were consistently present in patients carrying corresponding genetic variants, with elevated fibrinogen in all *FGG* variant carriers and elevated homocysteine in *most MTHFR variant carriers.*

2.2 Genetic variant frequencies

The most prevalent genetic variants were *MTHFR* rs1801133 (26.5 %), while Prothrombin G20210A (rs1799963) showed lowest frequency (1.3 %), consistent with population studies (Table 2.1).

Table 2.1

Comparison of genetic variant frequencies between study cohort and reference population

Gene, variant (legacy name)	Variant Allele Frequency in the large cohort* (n = 605)	Variant Allele Frequency in the study group, % (n = 155)	Frequency Fold change
Factor V, rs6025 (Factor V Leiden)	18/1210, 1.5 %	7/310, 2.1 %	1.33
Factor II, rs1799963 (Prothrombin G20210A)	15/1210, 1.2 %	4/310, 1.3 %	1.01
FGG, rs2066865	301/1210, 24.9 %	68/310, 22 %	0.93
SERPINC1, rs2227589	118/1210, 9.8 %	30/310, 9.7 %	1.01
MTHFR, rs1801133	356/1210, 29.4 %	82/310, 26.5 %	0.91

* Large cohort – Development of Latvian Population Genome Reference. European Recovery Fund funded project No 4.1.1.r.0/3/22/I/VM/001.

Comparison with large Latvian population reference cohort (n = 605) showed very similar allele frequencies without statistically significant differences, confirming genetic comparability and supporting study representativeness and validity. Frequency fold changes ranged from 0.91 (*MTHFR*) to 1.33 (Factor V Leiden). Rare variants like Factor V Leiden and G20210A had low frequencies, limiting power for rare variant analysis. Overall genetic consistency validates broader applicability of findings.

Frequency distribution of five thrombophilia-associated genetic variants analysed according to thrombosis status (Table 2.2) showed no statistically significant associations.

Table 2.2

Frequency of genetic variants by thrombosis status

Gene, variant (legacy name)	Variant allele frequency in thrombotic group, alleles/% (n, 14)	Variant allele frequency in no-thrombosis group, alleles/% (n, 141)	OR (CI, 95 %)	*p*
Factor V, rs6025 (Factor V Leiden)	1/28, 3.6 %	6/282, 2.1 %	0.84 (0.05–15.60)	1
Factor II, rs1799963 (Prothrombin G20210A)	1/28, 3.6 %	3/282,1.1 %	2.88 (0.26–32.30)	0.245
FGG, rs2066865	8/28, 28.6 %	60/282, 21.3 %	1.36 (0.67–2.76)	0.375
SERPINC1, rs2227589	5/28, 17.9 %	25/282, 8.9 %	1.46 (0.59–3.62)	0.448
MTHFR, rs1801133	11/28, 39.3 %	71/282, 25.2 %	1.28 (0.67–2.45)	0.5

FVL – Factor V Leiden; FII – prothrombin gene; *FGG* – fibrinogen gamma chain gene; MTHFR– methylene tetrahydrofolate reductase. Odds ratios (OR) with 95 % confidence intervals (CI) for five genetic variants. Statistical analysis was performed using Fisher's exact test, significance level $\alpha = 0.05$ (two tailed).

Prothrombin G20210A showed strongest association (OR = 2.88, 95 % CI: 0.26–32.30, p = 0.245), followed by *SERPINC1* rs2227589 (OR = 1.46, p = 0.448), *FGG* rs2066865 (OR = 1.36, p = 0.375), *MTHFR* rs1801133 (OR = 1.28, p = 0.500), and Factor V Leiden (OR = 0.84, p = 1.000). P-values ranged from 0.245 to 1.000, indicating insufficient statistical power to detect associations with thrombosis outcome. Wide confidence intervals across all variants reflect limited precision of effect estimates due to relatively small sample size, particularly for rare variants.

All examined variant genotype frequencies were in Hardy-Weinberg equilibrium, describing state where allele and genotype frequencies remain constant in studied population, validating genetic analysis quality.

2.3 Association of genetic variants and coagulation parameters

Genotype-phenotype correlation analysis (Table 2.3) encompassed all five targeted variants and associated proteins.

Significant associations: Factor V Leiden carriers (C/T genotype) demonstrated significantly reduced APCR values (1.19 ± 0.17) compared to wild-type (2.02 ± 0.32, p = 0.006), confirming functional impact on protein C resistance. *FGG* rs2066865 variant showed clear gene-dose effect, with fibrinogen levels progressively increasing from G/G carriers (4.08 ± 1.32 g/L) to G/A carriers (4.64 ± 1.74 g/L, p = 0.04) and A/A carriers (5.57 ± 1.81 g/L, p = 0.004 compared to G/G) (Table 2.3).

Table 2.3

Analysed SNVs and determined proteins in studied population (n, 155)

SNVs and genotype distribution in study group	Determined protein reference interval (min; -max) M (±SD)	*p*
rs6025 *FV* (C>T) C/C (n = 148) C/T (n = 7) T/T (n = 0)	APCR (<1.8) 2.02 (0.32) 1.19 (0.17) –	0.006
rs1799963 *F2* (G>A) G/G (n = 152) G/A (n = 2) A/A (n = 1)	PT (70–130 %) 94.38 (20.34) 95.50 (20.51) 106.00	*ns*
rs2066865 *FGG* (G>A) G/G (n = 92) G/A (n = 54) A/A (n = 9)	Fibrinogen (2–4 g/L) 4.08 (1.32)$^{\#}$ 4.64 (1.74)* 5.57 (1.81)$^{*\#}$	0.04* 0.004$^{\#}$
rs2227589 *SERPINC1* (C>T) C/C (n = 126) C/T (n = 28) T/T (n = 1)	AT (75–125 %) 89.57 (14.14) 97.89 (14.52) 92.10	*ns*

Table 2.3 continued

SNVs and genotype distribution in study group	Determined protein reference interval (min; -max) M (±SD)	*p*
rs1801133 *MTHFR* (G>A) G/G (n = 83) G/A (n = 62) A/A (n = 10)	Homocysteine (5–12 mkmol/L) 10.71 (4.17) 13.61 (5.44) 13.50 (5.8)	*ns*

Values are presented as mean, ± SD, sig. 2-tailed $p < 0.05$; SNV – single nucleotide variant; APCR – activated protein C resistance; FVL – Factor V Leiden; F2 – prothrombin gene PT – prothrombin; *FGG* – fibrinogen gamma chain gene; *MTHFR* – methylene tetrahydrofolate reductase gene; AT – antithrombin.

Non-significant associations: Prothrombin G20210A, *SERPINC1*, and *MTHFR* variants did not demonstrate statistically significant associations with respective protein products, possibly due to small sample sizes for variant carriers or acute-phase reaction influences. *MTHFR* rs1801133 showed non-significant association with plasma homocysteine and *SERPINC1* rs2227589 carriers showed no significant antithrombin activity differences.

Baseline coagulation parameters stratified by thrombosis status (Table 2.4) revealed only two statistically significant differences. APCR was significantly lower in patients developing thrombosis (1.83 vs 2.01, $p = 0.043$). Conversely, homocysteine levels were unexpectedly lower in thrombosis patients (7.9 vs 11.1 μmol/L, $p = 0.029$), contradicting established hyperhomocysteinemia-thrombotic risk associations, requiring cautious interpretation due to small thrombosis group sample size. No significant differences were observed in prothrombin time, fibrinogen concentration, or antithrombin activity.

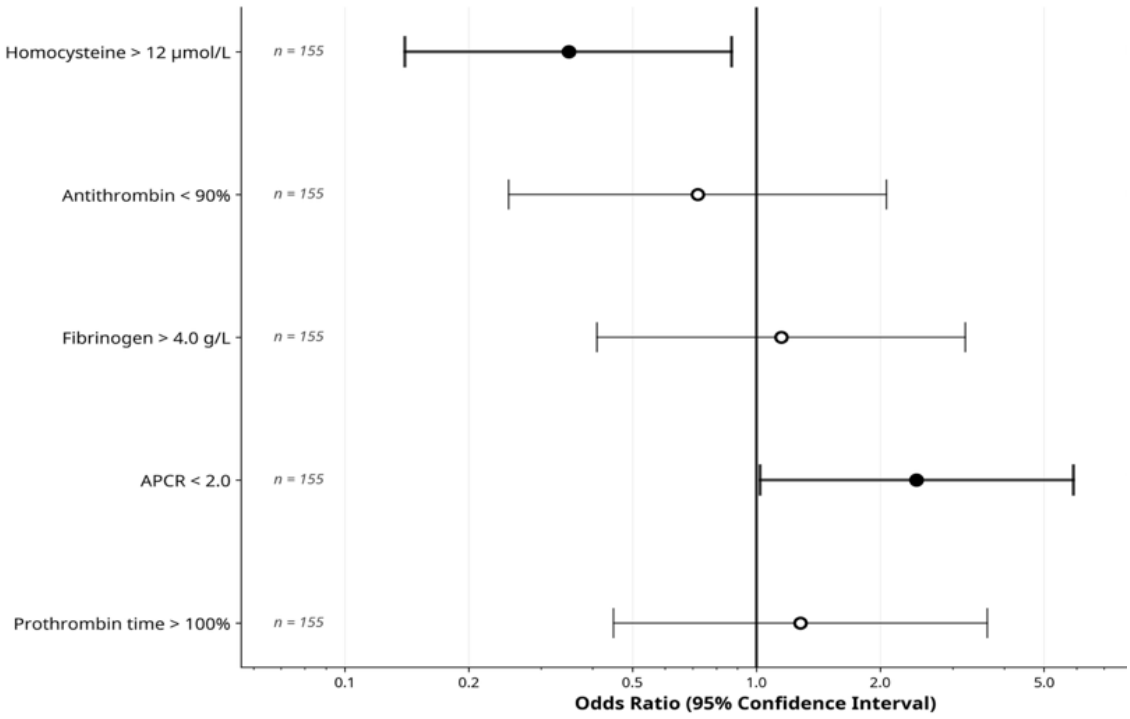
Table 2.4

Baseline coagulation parameters according to thrombosis status

Variable	No-Thrombosis group n, 141	Thrombosis group n, 14	*p*
PT, %	97.0 (85.0–109.0)	102.5 (89.3–116.8)	0.26
APCR	2.01 (1.75–2.22)	1.83 (1.69–1.9)	0.043
Fibrinogen, g/L	4.08 (3.18–5.32)	4.28 (3.78–4.78)	0.60
AT	89.1 (79.9–97.4)	96.2 (85.4–111.8)	0.231
Hmc, mkmol/L	11.1 (8.4–14.6)	7.9 (7.1–8.4)	0.0029

Data are presented as median (interquartile range). P were calculated using the Mann-Whitney U test. $p < 0.05$ was considered statistically significant. PT – prothrombin time, APCR – activated protein C resistance, AT – antithrombin, Hmc – homocysteine.

Forest plot analysis (Figure 3.1) evaluating five coagulation parameters using clinically relevant cut-off values revealed distinct patterns. APCR< 2.0 emerged as most clinically relevant biomarker (OR = 2.45, 95 % CI: 1.02–5.89, $p = 0.043$). Homocysteine > 12 μmol/L showed unexpected protective association (OR = 0.35, 95 % CI: 0.14–0.87, $p = 0.029$).



Parameter	OR (95% CI)	P-value	Sig.
Prothrombin time>100%	1.28 (0.45-3.64)	0.260	ns
APCR<2.0	**2.45 (1.02-5.89)**	**0.043**	*
Fibrinogen>4.0 g/L	1.15 (0.41-3.22)	0.600	ns
Antithrombin<90%	0.72 (0.25-2.07)	0.231	ns
Homocysteine>12 µmol/L	**0.35 (0.14-0.87)**	**0.029**	*

Figure 3.1 **Forest plot of coagulation parameters associated with free flap thrombosis**

Data points represent odds ratios with 95 % confidence intervals for coagulation parameters. Filled circles indicate statistically significant associations ($p < 0.05$), while hollow circles represent non-significant findings ($p \geq 0.05$). The vertical line at OR = 1.0 represents no effect. APCR < 2.0 was significantly associated with increased thrombosis risk (OR = 2.45, 95 % CI: 1.02–5.89, $p = 0.043$). Homocysteine > 12 µmol/L showed an unexpected protective association (OR = 0.35, 95 % CI: 0.14–0.87, $p = 0.029$). Sample size: n = 155 patients with 14 thrombotic events.

Traditional coagulation parameters including prothrombin time > 100 % (OR = 1.28, $p = 0.260$), fibrinogen > 4.0 g/L (OR = 1.15, $p = 0.600$), and antithrombin < 90 % (OR = 0.72, $p = 0.231$) showed no significant associations, suggesting routine coagulation screening may have limited predictive value.

2.4 Combined risk factor analyses

Three logistic regression models assessed predictive power of different factor sets (Figure 3.2). Genetic-only model for polymorphisms *FV* rs6025, *FII* rs1799963, *FGG* rs2066865, *SERPINC1* rs2227589, *MTHFR* rs1801133 showed pseudo R-squared 0.02327, suggesting genetic variants alone explain very small variance proportion in flap thrombosis. No individual genetic variants showed statistically significant association (all $p > 0.05$), indicating these factors are not strong flap thrombosis predictors in studied group.

Coagulation-only model showed pseudo R-squared 0.05983, modestly higher than genetic-only model, suggesting coagulation factors explain slightly larger variance proportion. APCR showed statistically significant negative association with flap thrombosis ($p = 0.035$), consistent with previous results confirming Factor Leiden functional impact on APCR.

Combined model with confounders showed highest pseudo R-squared (0.08160), suggesting including all genetic, coagulation, and confounding factors explains slightly larger but still limited variance proportion. In combined model, no genetic variants, coagulation factors, or confounding factors showed statistically significant association (all $p > 0.05$). Previously borderline significant homocysteine ($p = 0.075$) and APCR ($p = 0.071$) no longer reached the 0.05 threshold suggesting that, when controlling for other factors, their individual predictive power diminishes.

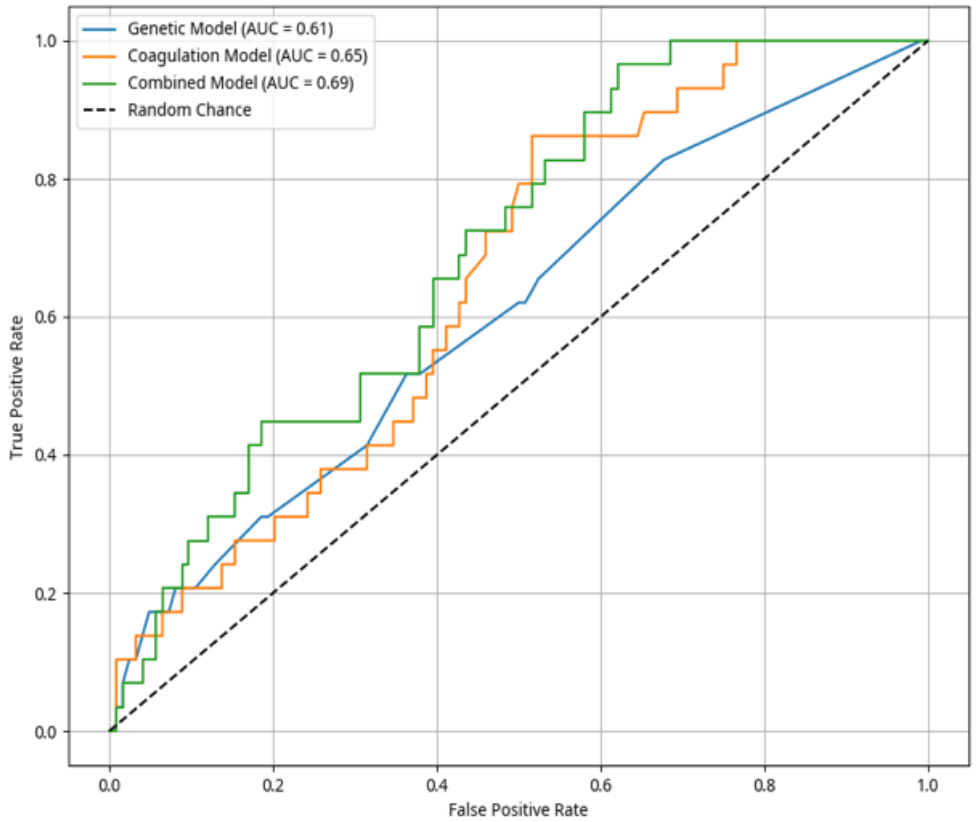


Figure 3.2 **ROC Curves for Logistic regression models for three categories of predictors**

Genetic model AUC = 0.61 (very poor discriminatory power, slightly better than random chance AUC = 0.50); Coagulation model AUC = 0.65 (slightly better discriminatory power, modest ability distinguishing patients with/without flap thrombosis); Combined model AUC = 0.69 (highest among three but still weak to moderate discriminatory power, indicating limited overall flap thrombosis prediction ability despite incorporating more information).

Forest plot analysis (Figure 3.3) examining genetic polymorphisms and thrombotic complications showed: Factor V Leiden OR = 0.84 (95 % CI: 0.05–15.60, $p = 0.762$), with one thrombotic event among 7 carriers; Prothrombin G20210A OR = 2.88 (95 % CI: 0.26–32.30, $p = 0.245$), strongest association among five variants; *FGG* rs2066865 OR = 1.36

(95 % CI: 0.67–2.76, p = 0.375), emerging as mild genetic predictor with relatively narrow confidence interval suggesting potential clinical relevance; *SERPINC1* rs2227589 OR = 1.46 (95 % CI: 0.59–3.62, p = 0.449); *MTHFR* rs1801133 OR = 1.28 (95 % CI: 0.67–2.45, p = 0.500). Wide confidence intervals reflect small sample size and lack of statistical power.

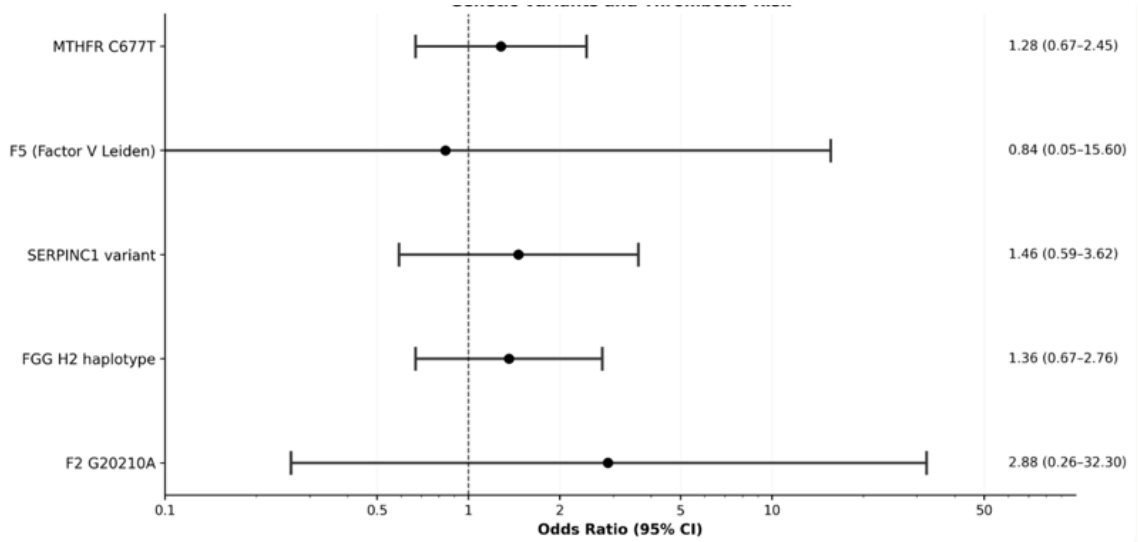


Figure 3.3. **Genetic variants associated with thrombosis risk: Forest plot analysis**

Odds ratios (OR) with 95 % confidence intervals (CI) for five genetic variants. Point estimates (squares) and confidence intervals (horizontal lines) are shown. Vertical dashed line indicates OR = 1.0. No associations reached statistical significance (all p > 0.05). Statistical analysis was performed using Fisher's exact test for categorical variables. Odds ratios and 95 % confidence intervals were calculated using logistic regression.

F2 – prothrombin gene; *FGG* – fibrinogen gamma chain gene; *MTHFR* – methylene tetrahydrofolate reductase gene; *SERPINC1* – serine protease inhibitor 1 (antithrombin) gene.

Cox proportional hazards regression analyses showed no variables achieving statistical significance at α = 0.05 level in either univariate or multivariate models. Age > 50 years showed elevated thrombotic risk (HR = 1.58, 95 % CI: 0.56–4.45, p = 0.386), with 8 events among 67 patients (11.9 % event rate). Active smoking demonstrated modest increase (HR = 1.24, 95 % CI: 0.45–3.42, p = 0.675), with 3 events among 29 smokers (10.3 % event rate). *FGG* variant and cumulative genetic burden (≥ 2 variants) showed most consistent associations across analytical approaches, with hazard ratios approaching 2.0 and p approaching significance, suggesting potential clinical relevance that may become apparent with larger sample sizes or higher-risk patient populations.

2.5 Survival analysis results: Timing and risk factors for flap thrombosis

Thrombosis-free survival analysis revealed characteristic biphasic pattern with distinct early and late phases. Survival curve demonstrated steep decline during initial 3-day postoperative period, with thrombosis-free survival probability decreasing from 100 % baseline to approximately 97 % at day 1, 94 % at day 2, stabilising at 91 % by day 3. Following this critical early period, survival curve plateaued with no additional thrombotic events through day

10 follow-up. This pattern indicates majority of thrombotic complications occurred within immediate postoperative window, after which event-free patients maintained stable thrombosis-free status. Final thrombosis-free survival probability 91 % represents proportion avoiding thrombotic complications throughout 10-day observation period, with 14 thrombotic events (9.0 %) occurring predominantly within first 72 hours post-surgery.

Kaplan-Meier analysis evaluating cumulative genetic variants impact revealed stepwise decrease in survival probability with increasing thrombophilic genetic variant numbers. Zero-variant group maintained highest thrombosis-free survival rate, stabilising at approximately 87 % after day 3. One-variant group showed lower survival probability, plateauing around 82 %. Two-variant and ≥ 3-variant groups exhibited progressively lower survival rates. Although clear trend suggested higher genetic burden associated with lower thrombosis-free likelihood, log-rank test for trend did not reach statistical significance ($\chi^2 = 2.95$, $p = 0.086$). This near-significant result is noteworthy given sample size limitations, suggesting biological effect that might reach statistical significance with larger cohorts. Similarity between two-variant and ≥ 3-variant groups suggests genetic risk relationship may not be strictly linear, potentially indicating ceiling effect where additional variants beyond two provide diminishing incremental risk, statistical power limitations preventing detection of further differences, or biological interaction effects modifying simple additive genetic models.

The Kaplan-Meier curves for gender showed some flap thrombosis-free survival probability differences, but log-rank test ($p = 0.1685$) indicated non-significant difference, suggesting gender alone may not be strong predictor of flap thrombosis timing. Patients with thrombosis history showed trend toward lower thrombosis-free survival compared to those without history. Log-rank test ($p = 0.2151$) did not reach statistical significance, but visual trend suggests thrombosis history might be important factor influencing future thrombotic event timing. Curves for patients receiving thromboprophylaxis versus those who did not showed some visual differences, but log-rank test did not indicate statistically significant survival time difference.

3 Discussion

Microvascular surgery requires precise vascular anastomosis to re-establish circulation in small-calibre vessels, with tissue viability critically depending on adequate perfusion. While technical factors contribute significantly to surgical success, thrombotic complications remain a principal concern. This comprehensive investigation examined relationships between genetic polymorphisms affecting haemostatic function and thrombotic complications in microvascular free flap surgery, providing important insights into complex pathophysiology underlying flap failure while challenging several established assumptions regarding genetic thrombophilia in surgical contexts.

The pathogenesis of thrombotic events in microvascular surgery appears multifactorial, with genetic factors potentially playing substantial roles. Polymorphisms in genes regulating haemostatic pathways including coagulation cascades, platelet function, and fibrinolytic systems may predispose certain individuals to thrombotic complications. Elucidating these genetic influences could enhance perioperative risk assessment and facilitate individualised therapeutic approach development. The study population demonstrated demographic characteristics consistent with typical reconstructive surgery patients, with male predominance (76.1 %) and mean age 45.1 years.

3.1 Principal findings and clinical implications

The observed rate of 9 % of flap thrombosis aligns with previously reported complications in the range of 2–9 % in the published literature (Bowman, 2011; Friedman, 2010), validating the representativeness of the study population. The 94.84 % overall flap success rate demonstrates excellent clinical outcomes consistent with contemporary microsurgical practice standards. This success rate, while impressive, underscores continued clinical significance of thrombotic complications, as even small failure percentages can result in devastating functional and aesthetic consequences.

Contrary to initial hypothesis, individual genetic polymorphisms demonstrated no statistically significant association with flap thrombosis risk. This finding challenges direct extrapolation of genetic thrombophilia research from general populations to specific microvascular surgery contexts. Absence of significant associations between Factor V Leiden (rs6025), Prothrombin G20210A (rs1799963), and other examined genetic variants suggests pathophysiological mechanisms underlying spontaneous venous thromboembolism may differ substantially from those governing microvascular anastomotic thrombosis. The multifactorial nature of thrombotic events in microsurgery necessitates comprehensive analysis of both inherited and acquired risk factors.

3.2 Genetic variants

Genetic variant prevalence in our study population generally corresponded to established population frequencies, with *MTHFR* rs1801133 showing highest frequency (26.5 %) and Prothrombin G20210A lowest (1.3 %). These distributions are consistent with European population studies (Rosendaal, 1995; Kujovich, 2011), confirming appropriate population sampling and validating our cohort as representative of broader surgical population rather than highly selected thrombophilic group. The variant allele frequencies demonstrated excellent concordance with the large Latvian population reference cohort (n = 605), with fold changes ranging from 0.91 to 1.33 across all the loci examined, confirming the absence of significant selection bias in the recruitment of patients.

3.2.1 rs6025 in FV gene

Factor V Leiden remains most prevalent inherited thrombophilia in Caucasian populations, with heterozygote frequencies 3–7 % in Northern European populations and near absence in African and Asian populations (Rees, 1995; Ridker, 1997). The variant arose from single founder mutation approximately 21 000–24 000 years ago, with persistence suggesting potential evolutionary advantages, possibly related to improved haemostasis during childbirth or trauma (Lindqvist, 2001; Zivelin, 2006). The Arg506Gln substitution eliminates one of three activated protein C cleavage sites in factor Va, resulting in approximately 10-fold prolonged cofactor activity and sustained thrombin generation (Segers, 2007; Castoldi, 2010).

Despite extensive literature supporting 3–7-fold increased thrombotic risk in carriers (Kujovich, 2011), our study revealed no significant association with flap thrombosis (OR = 0.84, p = 0.762). The relatively small number of carriers (n = 7, 2.1 %) certainly limited our ability to detect modest effects, particularly given low baseline thrombosis rate (9 %). Post-hoc power calculations indicate detecting 3-fold increased risk with 7 carriers and 9 % baseline thrombosis rate would require sample sizes exceeding 800 patients. Second, the haemodynamic environment of microvascular anastomoses, characterised by low flow states, small vessel calibres (1–3 mm), and significant surgical trauma, may create thrombotic conditions fundamentally different from spontaneous venous thromboembolism (Khouri, 1998).

Approximately 5 % of APCR cases arise through alternative mechanisms unrelated to Factor V Leiden (Segers, 2007), including elevated factor VIII levels, lupus anticoagulants, pregnancy, and oral contraceptive use (Castoldi, 2010). One patient exhibiting APCR (ratio 1.21) with Factor V Leiden heterozygosity underwent two microvascular flap procedures within two years, both resulting in thrombosis. This case illustrates clinical relevance of functional APCR measurement beyond genetic testing, as phenotypic expression of resistance influenced by both genetic and acquired factors may better predict thrombotic risk than genotype alone

(Vos, 2006). Absence of demonstrable Factor V Leiden association with microsurgical thrombosis aligns with limited prior literature in reconstructive surgery (Khansa, 2011; Biban, 2019).

3.2.2 rs1799963 variant in prothrombin gene

Prothrombin G20210A represents second most common inherited thrombophilia in European populations, occurring with frequencies 1–3 % and conferring approximately 2–3-fold increased venous thrombosis risk (Simone, 2013; Bertina, 1998). Unlike Factor V Leiden affecting protein function, G20210A variant resides in 3' untranslated region, resulting in elevated mRNA stability and increased protein synthesis without altering prothrombin's enzymatic properties (Poort, 1996; Jadaon, 2011). Heterozygous carriers typically exhibit prothrombin levels 30 % above population means, though considerable overlap with normal ranges complicates phenotypic identification (Poort, 1996).

In our cohort, one patient carrying prothrombin gene variant experienced free flap thrombosis, yielding non-significant odds ratio 2.88 (95 % CI: 0.26–32.30, $p = 0.245$). While this aligns with data conferring patients carrying prothrombin gene variant have 2–3-fold increased thrombosis risk in population studies (Simone, 2013), the extremely wide confidence interval reflects profound statistical uncertainty attributable to variant's rarity. The 1.3 % variant frequency was consistent with typically reported (1–3 %), confirming appropriate population sampling without selection bias. The clinical significance of prothrombin G20210A in microsurgical populations remains controversial. The variant's rarity combined with modest effect sizes renders adequately powered clinical trials impractical for this specific question.

3.2.3 rs2066865 in fibrinogen gamma chain gene

The association between *FGG* rs2066865 and elevated plasma fibrinogen concentrations represents the most significant genetic finding. A/A genotype carriers demonstrated markedly elevated fibrinogen levels (5.57 ± 1.81 g/L) compared to G/G carriers (4.08 ± 1.32 g/L), with statistically significant gene-dose effects ($p = 0.001$ for A/A vs. G/G; $p = 0.04$ for G/A vs. G/G). This strong genotype-phenotype correlation validates functional relevance of rs2066865 as quantitative trait locus modulating fibrinogen expression, consistent with previous reports from Leiden Thrombophilia Study and subsequent investigations (de Willige, 2005; Grünbacher, 2007; El-Galaly, 2013).

Earlier investigations established significant correlations between advancing age, elevated fibrinogen levels, and increased thrombotic events, particularly within venous system (Rosendaal, 2009). Fibrinogen, as both essential coagulation factor and acute-phase reactant, occupies unique position in surgical haemostasis. Baseline genetic predisposition to elevated levels may be amplified during inflammatory states, trauma, and malignancy, creating

prothrombotic milieu compounding surgical risk (van Hylckama Vlieg, 2003; Schlimp, 2016). Fibrinogen also contributes to blood viscosity, particularly relevant in low-flow microvascular systems where elevated viscosity may impair perfusion (Eber, 1993).

Despite A/A genotype subjects exhibiting both higher mean age and elevated plasma fibrinogen concentrations, this did not translate to statistically significant increase in flap thrombosis incidence (OR = 1.36, p = 0.375). The significant correlation between genetic variants and phenotypic expression suggests *FGG* rs2066865 may serve as biomarker for identifying patients at risk for hypercoagulable states. However, absence of direct correlation between elevated fibrinogen levels and flap thrombosis in regression analysis indicates that genetic predisposition to elevated fibrinogen may be necessary but not sufficient for thrombotic complications, suggesting additional factors – whether technical, mechanical, or patient-specific – must converge to precipitate clinical thrombosis (Khouri, 1998; Pattani, 2010).

3.2.4 rs2227589 in *SERPINC1* gene

Antithrombin deficiency, first described by Egeberg in 1965, represents one of earliest recognised inherited thrombophilias and among most clinically significant, conferring 10–20-fold increased thrombosis risk – substantially higher than Factor V Leiden or prothrombin G20210A (Zöller, 1999; Patnaik, 2008). This serpin superfamily member serves as principal physiological inhibitor of multiple coagulation proteases, with activity dramatically enhanced (up to 1000-fold) by heparin and endogenous heparan sulphate (Pike, 2005). The critical importance of antithrombin is underscored by embryonic lethality in homozygous-deficient mice, suggesting that complete deficiency is incompatible with mammalian life (Zöller, 1999).

Inherited antithrombin deficiency follows autosomal dominant pattern with variable penetrance, classified into type I (quantitative deficiency, 10–15 % of cases) and type II (qualitative defects, subdivided into reactive site, heparin-binding site, and pleiotropic effect variants) (Kumar, 2015; Perry, 1996). The rs2227589 variant in *SERPINC1* (c.41+141G>A) represents relatively common polymorphism whose functional significance and thrombotic risk association remain incompletely characterised, with conflicting evidence from population studies (Jiang, 2017).

Previous research demonstrated reduced antithrombin concentrations do not invariably result in thrombotic events (van der Meer, 1973). In our studied population, no significant differences in plasma antithrombin activity were observed between patients carrying heterozygous or homozygous variants of rs2227589, regardless of free flap thrombosis status. All measured antithrombin levels remained within normal reference ranges (75–125 %),

providing no phenotypic evidence this particular variant substantially impairs antithrombin function. Notably, two of five patients presenting with heterozygous genotype and subsequent free flap thrombosis were under 50 years, consistent with data indicating initial thrombotic events typically manifest by age 30 (Patnaik, 2008; Hart, 2022).

3.2.5 rs1801133 in *MTHFR* Gene

MTHFR C677T variant demonstrated highest prevalence in our cohort (26.5 %), consistent with European population frequencies where homozygote rates range from 10–14 % (Wilcken, 1996; Botto, 2000). This variant results in alanine-to-valine substitution at position 677 of methylenetetrahydrofolate reductase, catalysing conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate – the methyl donor for homocysteine remethylation to methionine (Goyette, 1998). Homozygous variant carriers exhibit approximately 70 % reduction in enzyme activity, with heterozygotes showing intermediate reduction (Rozen, 1997).

Primary biochemical consequence of C677T homozygosity is mild to moderate hyperhomocysteinemia, particularly in individuals with suboptimal folate status (Klerk, 2002). Homocysteine has been implicated in both arterial and venous thrombotic disorders through multiple proposed mechanisms including endothelial dysfunction, oxidative stress, impaired nitric oxide bioavailability, and enhanced platelet reactivity (Welsch, 1997; Liew, 2015). However, the causal relationship between hyperhomocysteinemia and thrombosis remains controversial, with meta-analyses yielding conflicting conclusions (Ray, 2002; Clarke, 2012).

Among all patients with free flap thrombosis, ten were identified as *MTHFR* gene rs1801133 G/A genotype carriers, with more than half (n = 8) exhibiting elevated serum homocysteine levels (> 12 μmol/L). Three patients presented with isolated *MTHFR* gene variants, with one experiencing arterial free flap thrombosis and two developing venous thrombosis. This distribution involving both arterial and venous circulations aligns with literature suggesting hyperhomocysteinemia, unlike classical venous thrombophilias, may predispose to thrombosis in both vascular beds (Falcon, 1994; Pathare, 2004).

The clinical implications of *MTHFR* C677T for microsurgical practice remain uncertain. Given the variant's high population prevalence (approximately one-quarter of Europeans carry at least one variant allele), the absence of strong thrombotic associations in our study, and conflicting evidence from broader literature, routine *MTHFR* genotyping cannot currently be recommended for preoperative risk assessment. Future research should focus on whether homocysteine-lowering interventions improve microsurgical outcomes in genetically or

biochemically high-risk patients, though negative results of cardiovascular trials suggest limited potential for benefit (Bonaa, 2006).

3.3 Coagulation parameters

The coagulation parameter findings yield several important clinical implications for microsurgical practice, while revealing complex pathophysiology underlying free flap thrombosis. The significant association between reduced activated protein C resistance (APCR) and thrombosis risk (OR = 2.45, p = 0.043) represents the most clinically actionable finding. APCR values below 2.0, particularly observed median 1.83 in thrombosis patients versus 2.01 in successful cases, suggest preoperative APCR testing could potentially identify high-risk patients who might benefit from enhanced thromboprophylaxis or more intensive postoperative monitoring (Dahlbäck, 1993; Castoldi, 2010).

The protein C anticoagulant pathway plays critical role in regulating thrombin generation through inactivation of factors Va and VIIIa (Segers, 2007). In microvascular surgery context, where small vessel calibre and low flow states create inherently prothrombotic conditions, even modest impairment of this regulatory mechanism may shift haemostatic balance toward thrombosis (Furie, 2008). The finding that reduced APCR correlates with increased flap thrombosis risk, independent of Factor V Leiden genotype status, suggests APCR measurement captures functional anticoagulant capacity more comprehensively than genetic testing alone. APCR can be influenced by acquired factors including pregnancy, oral contraceptive use, inflammatory states, and elevated factor VIII levels – all conditions that may be present in surgical populations (Castoldi, 2010).

Clinical utility of routine APCR screening in microsurgical candidates warrants careful consideration. While 2.45-fold increased thrombosis risk associated with APCR <2.0 appears substantial, absolute risk increase must be embedded within overall 9 % thrombosis rate observed in this cohort. Absence of current consensus regarding thromboprophylaxis protocols in microsurgery (Khansa, 2013; Pan, 2014) further complicates translation of APCR findings into clinical practice, as optimal management strategies for identified high-risk patients remain undefined.

Absence of significant associations between thrombosis risk and other coagulation parameters including prothrombin time, fibrinogen concentration, and antithrombin activity highlights complex and multifactorial nature of microsurgical thrombosis. This finding contrasts with well-established associations between these parameters and systemic venous thromboembolism, where elevated fibrinogen levels (Rosendaal, 2009), reduced antithrombin activity (Patnaik, 2008), and prothrombin elevation (Poort, 1996) constitute recognised risk factors.

Unlike spontaneous venous thromboembolism, where systemic hypercoagulability drives thrombotic risk through Virchow's classical triad of hypercoagulability, endothelial injury, and stasis (Kumar, 2010), microsurgical thrombosis may be more dependent on local factors at anastomotic site. These include technical precision of vessel approximation, anastomotic tension, vessel wall trauma, calibre mismatch between donor and recipient vessels, and unique haemodynamic environment created by surgical reconstruction (Khouri, 1998; Pattani, 2010). Small vessel calibre typical of microsurgical anastomoses (1–3 mm) creates flow conditions distinct from larger venous systems, potentially minimising impact of systemic coagulation abnormalities while amplifying importance of local mechanical and haemodynamic factors.

The fibrinogen findings merit particular discussion given robust genetic association between *FGG* rs2066865 and plasma fibrinogen levels. Despite A/A genotype carriers exhibiting significantly elevated fibrinogen concentrations (5.57 ± 1.81 g/L vs. 4.08 ± 1.32 g/L in G/G carriers, $p = 0.004$), this genetic predisposition to hyperfibrinogenaemia did not translate to statistically significant increases in clinical thrombosis risk. Fibrinogen exists as both substrate for thrombin (generating fibrin) and acute-phase reactant responsive to surgical stress (van Hylckama Vlieg, 2003). All patients underwent major surgery with associated inflammatory responses, potentially elevating fibrinogen levels universally and obscuring baseline genetic differences. Timing of measurement (preoperatively) may not capture peak fibrinogen elevations occurring during critical postoperative period when most thromboses develop (Schlimp, 2016; Tang, 2010).

The unexpected inverse association between homocysteine elevation (> 12 μmol/L) and thrombosis risk (OR = 0.35, $p = 0.029$) represents the most paradoxical finding requiring careful interpretation and validation. This observation directly contradicts established literature linking hyperhomocysteinemia to both arterial and venous thrombotic events (Falcon, 1994; Pathare, 2004; Liew, 2015), though meta-analyses have yielded conflicting conclusions regarding strength and causality of this relationship (Ray, 2002; Clarke, 2012). The small sample size for homocysteine measurements in thrombosis patients ($n = 14$) creates substantial potential for chance findings and Type I error. The *MTHFR* C677T polymorphism, while associated with elevated homocysteine in some populations, demonstrates variable penetrance dependent on folate status (Klerk, 2002; Rozen, 1997).

Results suggest comprehensive thrombophilia screening, beyond APCR measurement, may provide limited clinical value for predicting microsurgical thrombotic complications in unselected patient populations. This challenges intuitive approach of extensive preoperative coagulation testing advocated by some practitioners (Biban, 2019; Pannucci, 2015) and

supports more selective, targeted screening strategies. Poor discriminatory power of our combined coagulation-based predictive model (AUC = 0.65) reinforces this conclusion, indicating laboratory parameters alone inadequately capture complex, multifactorial determinants of flap success.

3.4 Predictive model and clinical utility

Relatively poor discriminatory power of all three predictive models (AUC: 0.61–0.69) highlights multifactorial nature of flap thrombosis. Genetic-only model performed barely better than random chance (AUC = 0.61), while coagulation-only model showed modest improvement (AUC = 0.65). Combined model, incorporating genetic, coagulation, and confounding factors, achieved highest but still limited discriminatory power (AUC = 0.69). These findings suggest thrombotic complications in microvascular surgery result from complex interactions between multiple factors extending beyond genetic predisposition and laboratory parameters. Technical factors, surgical experience, anastomotic tension, vessel quality, and postoperative management likely play equally or more important roles in determining outcomes.

3.5 Study limitations and methodological considerations

Several important limitations must be acknowledged. First, relatively small sample size (n = 155) may have limited statistical power to detect modest genetic effects, particularly for rare variants. With only 14 thrombotic events, the study lacks sufficient power to detect modest genetic effects, particularly for rare variants such as Factor V Leiden (n = 7 carriers) (Rosendaal, 1995; Kujovich, 2011) and Prothrombin G20210A (n = 3 carriers). Third, timing of coagulation parameter measurement in relation to surgery may have influenced results. Acute-phase reactions following surgical trauma can significantly alter coagulation parameters, potentially obscuring baseline genetic effects (van Hylckama Vlieg, 2003; Schlimp, 2016). The 40 % salvage rate after late recognition of complications emphasises critical importance of standardised monitoring protocols. Despite standardised treatment protocols and procedures performed by experienced microsurgeons, technical surgical variables remain significant limiting factor (Vanags, 2020). It is important to note that our study cohort (N = 155, patients recruited between 2016 and 2019) overlaps in both time and setting with the cohort analysed in the doctoral thesis of J. Stepanovs (2025) (N = 103, trauma patients, 2016–2019), both studies having been conducted at the Latvian Centre for Reconstructive and Microsurgery. Although the present study encompassed a broader range of surgical indications – including oncological reconstruction, osteomyelitis, and traumatic injuries – with a primary focus on genetic thrombophilic factors, the work of Stepanovs (2025) concentrated specifically on trauma

patients and examined the role of surgical variables and rotational thromboelastometry (RTE) in predicting thrombotic risk. His findings convincingly demonstrated that surgical duration exceeding 240 minutes was the principal risk factor for free flap thrombosis in patients undergoing early post-traumatic reconstruction, whereas in late reconstructions, thrombogenic comorbidities and RTE-detected hypercoagulability emerged as the predominant determinants (Stepanovs, 2025). This context is critically important for the interpretation of the present results. Future investigations should incorporate an integrated analysis combining genetic data with detailed surgical parameters and RTE measurements from a single, unified patient cohort, in order to develop a comprehensive and clinically applicable risk prediction model. Finally, focus on specific genetic polymorphisms may have overlooked other relevant variants or epigenetic factors influencing thrombotic risk, supporting characterisation of VTE as multigenetic and multifactorial disorder (Dahlbäck, 2005).

3.6 Future research

Despite absence of strong genetic associations, this study provides valuable insights for personalised approaches development to microvascular surgery. Identification of fibrinogen polymorphisms as modulators of plasma fibrinogen levels suggests potential therapeutic targets for risk modification. Larger multicentre studies are needed to validate findings and provide adequate statistical power for detecting modest genetic effects. Genome-wide association studies may identify novel genetic variants associated with microvascular thrombotic complications (de Haan, 2012). Functional studies examining mechanistic relationships between genetic variants and microvascular thrombosis could provide insights into therapeutic targets. Development of comprehensive risk prediction models incorporating genetic, clinical, technical, and laboratory factors represents important goal for personalised surgical care.

3.7 Strengths and limitations

Study strengths: Prospective observational design with standardised protocols minimised potential recall bias. All microvascular procedures were performed by highly experienced microsurgeons at specialised centre, reducing technical variability. Comprehensive assessment approach integrating genetic analysis of five thrombophilia-associated variants with corresponding phenotypic measurements enabled robust genotype-phenotype correlation analyses, as evidenced by significant association between *FGG* rs2066865 and plasma fibrinogen concentrations (de Willige, 2005; Grünbacher, 2007).

Study limitations: Single-centre design limits generalisability across different surgical practices, patient populations, and healthcare systems. Candidate gene approach may have overlooked novel or less-studied genetic contributors to microsurgical thrombosis. Technical

and surgical variables remained incompletely characterised and controlled. Relatively short follow-up period (10 days) captured only early thrombotic complications. Absence of validation cohort prevents external verification of findings. These limitations collectively suggest negative findings should be interpreted as insufficient evidence to exclude genetic associations rather than definitive proof of their absence.

Conclusions

1. Successful identification of patients carrying the targeted single nucleotide variants across all five genetic loci: rs6025 in *FV* (2.1 %), rs1799963 in *FII* (1.3 %), rs2066865 in *FGG* (22 %), rs2227589 in *SERPINC1* (9.7 %), and rs1801133 in *MTHFR* (26.5 %) was performed. Variant frequencies demonstrated excellent concordance with established European population distributions (fold change 0.91–1.33), confirming appropriate population sampling without selection bias. All genotype distributions maintained Hardy-Weinberg equilibrium, validating technical accuracy and population representativeness.
2. The study aim was achieved by determining that no individual genetic variant demonstrated a statistically significant association with thrombotic outcomes (all $p > 0.05$). This null finding is a significant scientific conclusion, suggesting that the thrombotic risk conferred by these common polymorphisms is of limited clinical penetrance in the context of microsurgery, where local haemodynamic, technical, and acquired factors may predominate.
3. Among acquired coagulation parameters, only activated protein C resistance (APCR) below 2.0 demonstrated significant association with thrombosis (OR = 2.45, 95 %CI: 1.02–5.89, $p = 0.043$). Conventional parameters including prothrombin time, fibrinogen concentration, and antithrombin activity showed no significant associations (all $p > 0.05$). Acquired thrombophilia factors, as traditionally measured preoperatively, demonstrate limited standalone predictive utility except for functional APCR assessment.
4. Integrated risk assessment models incorporating genetic variants, coagulation parameters, and confounders yielded insufficient discriminatory power for clinical application (AUC: 0.61–0.69). The overall flap success rate of 94.84 % with 9.0 % thrombosis incidence concentrated within 72 hours postoperatively aligns with contemporary microsurgical standards. These results indicate that the examined combination of common genetic variants and standard coagulation parameters cannot reliably predict individual thrombotic risk, highlighting the multifactorial nature of flap thrombosis where technical, mechanical, and local haemodynamic factors may predominate over systemic genetic predisposition.

Proposals

The limitations identified in this study point toward several important research priorities:

1. Multi-centre prospective studies with standardised protocols are warranted to validate these findings and provide adequate statistical power for detecting modest genetic effects, particularly for less common genetic variants like Factor V Leiden.
2. Longitudinal studies could be considered to track patients over time and assess the long-term impact of genetic and acquired factors on thrombotic outcomes and overall flap success.
3. Investigation of genetic variants affecting anticoagulant drug metabolism and efficacy could guide personalised anticoagulation strategies in high-risk patients.
4. Because our predictive models showed insufficient discriminatory power (AUC 0.61–0.69), the development of predictive models using machine learning approaches may better capture complex interactions between genetic, clinical, technical, and laboratory factors than traditional statistical methods.
5. Future research should also consider the ethical implications of genetic testing in surgical contexts, including patient consent, genetic discrimination, and the psychological impact of genetic risk information on patients and their families.

While this study did not demonstrate strong genetic associations with thrombotic complications, it emphasises the importance of continued research into the complex factors governing microvascular surgical outcomes. The pursuit of personalised medicine in reconstructive surgery remains a worthy goal, albeit one that requires more comprehensive approaches than genetic testing alone.

List of publications on the topic of the Thesis

Publications:

1. Drizlionoka, K., Zariņš, J., Ozoliņa, A., Ņikitina-Zake, L., Mamaja, B. 2019. Polymorphism rs2066865 in the Fibrinogen Gamma Chain (FGG) gene increases plasma fibrinogen concentration and is associated with an increased microvascular thrombosis rate. *Medicina*, 55, 563
2. Ozoliņa, A., Vanags, I., Ņikitina-Zaķe, L., Mamaja, B. 2021. Inherited Thrombophilias in Thrombosis Advancement in Microvascular Flap Surgery. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences*. Vol. 75, No. 2 (731), 113–120–567.

Presentations at international conferences

1. Drizlionoka, K., Stepanovs, J., Ozoliņa, A., Ņikitina-Zaķe, L., Mamaja, B. 2019. Markers for thrombosis prediction in free flap surgery. Euroanaesthesia, European Congress of European Society of Anaesthesiology, Vienna, Austria, 01.–03. June 2019.
2. Drizlionoka, K., Stepanovs, J., Ozoliņa, A., Ņikitina-Zaķe, L., Mamaja, B. 2019. Assessment of rotational thromboelastometry and standard coagulation profile in predicting thrombosis in microvascular flap surgery. Rīga Stradiņš University international conference on medical and health care sciences. 04.–05. April 2019. Riga, Latvia. Abstract Book 2019, 433
3. Drizlionoka, K., Stepanovs, J., Krustiņš, L., Ozoliņa, A., Ņikitina-Zaķe, L., Mamaja, B. 2018. The association of hereditary thrombophilia with clinically relevant hypercoagulation state and free flap thrombosis in microvascular surgery. Congress of International Society of Thrombosis and Haemostasis. ISTH SSC Dublin, Ireland, 17.–21. July 2018.
4. Drizlionoka, K., Ozoliņa, A., Ņikitina-Zaķe, L., Mamaja, B. 2018. Plasma fibrinogens increase due to polymorphism rs2066865 in FGG gene as a risk factor for thrombosis in microvascular surgery. Euroanaesthesia, Congress of European Society of Anaesthesiology, Copenhagen, Denmark, 02.–04. June 2018.
5. Drizlionoka, K., Stepanovs, J., Ozoliņa, A., Ņikitina-Zaķe, L., Mamaja, B. 2018. The association of increased plasma fibrinogen concentration due to polymorphism in FGG gene with free flap thrombosis in microvascular surgery. Rīga Stradiņš University Scientific Congress 16.–17. March 2018, Rīga, Latvia. https://www.rsu.lv/sites/default/files/imce/Zinātnes %20departaments/2018/RSU_zinatnis
6. Drizlionoka, K., Stepanovs, J., Ņikitina-Zaķe, L., Mamaja, B. 2017. Does inherited thrombophilia contributes thrombosis in microvascular free flap surgery: a pilot study. Euroanaesthesia, Congress of European Society of Anaesthesiology. Geneva, Switzerland, 03.–05. June 2017.
7. Drizlionoka, K., Stepanovs, J., Ņikitina-Zaķe, L., Mamaja, B. 2017. Genetic contribution towards thrombosis in free flap surgery: a pilot study. Rīga Stradiņš University Scientific Congress Rīga, Latvia, 5 April 2017, Abstracts, http://www.rsu.lv/images/ stories/zk_2017/genetic_contribution_thrombosis_ free_flap_surgery.pdf
8. Drizlionoka, K., Stepanovs, J., Ņikitina-Zaķe, L., Mamaja, B. 2016. The role of inherited thrombophilia in patients undergone free flap surgery: a systemic review of the literature. 8th International Baltic Congress of Anaesthesiology and Intensive Care. 1–3 December 2016. Tallinn, Estonia.

References

1. Adams, J., Charlton, P. (2003). Anaesthesia for microvascular free tissue transfer. *British Journal of Anaesthesia*, 3(2), 33–37.
2. Ahmad, S.S., London, F. S., Walsh, P.N. (2003). The assembly of the factor X-activating complex on activated human platelets. *Journal of Thrombosis and Haemostasis*, 1(1), 48–59.
3. Autin, L., Miteva, M. A., Lee, W. H., Mertens, K., Radtke, K. P., Villoutreix, B. O. (2005). Molecular models of the procoagulant factor VIIIa-factor IXa complex. *Journal of Thrombosis and Haemostasis*, 3(9), 2044–2056.
4. Bertina, R. M. (1998). The prothrombin 20210 G to A variation and thrombosis. *Current Opinion in Hematology*, 5(5), 339-342.
5. Biban, J. A., Atmodiwirjo, P. (2019). Free flap thrombosis in patients with hypercoagulability: a systematic review. *Archives of Plastic Surgery*, 46(6), 572–579.
6. Bonaa, K., Njolstad, I., Ueland, P., Schirmer, H., Tverdal, A., Steigen, T. (2006). Homocysteine lowering and cardiovascular events after acute myocardial infarction. *New England Journal of Medicine*, 354(15), 1578–1588.
7. Bonde, C., Khorasani, H., Hoejvig, J., Kehlet, H. (2017). Cyclooxygenase-2 inhibitors and free flap complications after autologous breast reconstruction: a retrospective cohort study. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 70(11), 1543–1546.
8. Botto, L., Yang, Q. (2000). 5,10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review, *American Journal of Epidemiology*, 151(9), 862–877.
9. Bowman, K. G., Carty, M. J. (2011). Flap complications and thrombophilia: an evidence-based model and cost analysis for preoperative screening. *Open Access Journal of Plastic Surgery*, 11, 32–45.
10. Broze, G. J., Lange, G. W., Duffin, K. L., MacPhail, L. (1994). Heterogeneity of plasma tissue factor pathway inhibitor. *Blood Coagulation & Fibrinolysis*, 5(4), 551–559.
11. Bruegger, D., Bauer, A., Finsterer, U., Bernasconi, P., Kreimeier, U., Christ, F. (2002). Microvascular changes during anaesthesia: sevoflurane compared with propofol. *Acta Anaesthesiologica Scandinavica*, 46(5), 481–487.
12. Brummel-Ziedins, K., Mann, K. G. (2013). Molecular basis of blood coagulation, in Hoffman, R. et al. (eds.) *Hematology: Basic Principles and Practice*. 6th edn. Philadelphia: Elsevier, ch. 128, 1821–1841.
13. Castoldi, E., Rosing, J. (2010). APC resistance: biological basis and acquired influences. *Journal of Thrombosis and Haemostasis*, 8(3), 445–453.
14. Chalmers, A., Turner, M. W., Anand, R. (2012). Cardiac output monitoring to guide fluid replacement in head and neck microvascular free flap surgery – what is current practice in the UK? *British Journal of Oral and Maxillofacial Surgery*, 50(6), 500–503.
15. Chen, C. M., Ashjian, P., Disa, J. J., Cordeiro, P. G., Pusic, A. L., Mehrara, B. J. (2008). Is the use of intraoperative heparin safe? *Plastic and Reconstructive Surgery*, 121(3), 49e–53e.
16. Clarke, R., Bennett, D. A., Parish, S., Verhoef, P., Dötsch-Klerk, M., Lathrop, M. MTHFR Studies Collaborative Group. (2012). Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias. *PLoS Medicine*, 9(2), e1001177.
17. Collen, D., Lijnen, H. R. (1991). Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood*, 78(12), 3114–3124.
18. Dahlbäck, B., Carlsson, M., Svensson, P. J. (1993). Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proceedings of the National Academy of Sciences*, 90(3), 1004–1008.

19. Dahlbäck, B. (2005). Blood coagulation and its regulation by anticoagulant pathways: genetic pathogenesis of bleeding and thrombotic diseases. *Journal of Internal Medicine*, 257(3), 209–223.

20. Davie, E. W., Fujikawa, K., Kisiel, W. (1991). The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry*, 30(43), 10363–10370.

21. de Haan, H. G., Bezemer, I. D., Doggen, C. J., Reitsma, P. H., Rosendaal, F. R., le Cessie, S. (2012). Multiple SNP testing improves risk prediction of first venous thrombosis. *Blood*, 120(3), 656–663.

22. de Willige, S. U., de Visser, M. C., Houwing-Duistermaat, J. J., Rosendaal, F. R., Vos, H.L., Bertina, R.M. (2005). Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen γ′ levels. *Blood*, 106(13), 4176–4183.

23. Dizon-Towson, D., Nelson, L., Jang, H. (1997). The incidence of the factor V Leiden mutation in an obstetric population and its relationship to deep vein thrombosis. *American Journal of Obstetrics and Gynecology*, 176(4), 883–886.

24. Doh, G. H., Kim, B., Lee, D. Y., Yoon, J. S., Lim, S. A., Han, Y. S., Eo, S. R. (2021). Hemodynamic principles in free tissue transfer: vascular changes at the anastomosis site. *Archives of Hand and Microsurgery*, 26(4), 285–292.

25. Dort, J. C., Farwell, D. G., Findlay, M., Huber, G. F., Kerr, P., Shea-Budgell, M. A., Simon, C., Uppington, J., Zygogianni, A., Andresen, E., Breckenridge, A. (2017). Optimal perioperative care in major head and neck cancer surgery with free flap reconstruction: a consensus review and recommendations from the enhanced recovery after surgery society. *JAMA Otolaryngology – Head & Neck Surgery*, 143(3), 292–303.

26. Dunn, J. O. C., Mythen, M. G., Grocott, M. P. (2016). Physiology of oxygen transport. *BJA Education*, 16(10), 341–348.

27. Eber, B., Schumacher, M. (1993). Fibrinogen: its role in the haemostatic regulation of atherosclerosis. *Seminars in Thrombosis and Hemostasis*, 19(2), 104–107.

28. Edgington, T. S., Mackman, N., Brand, K., Ruf, W. (1991). The structural biology of expression and function of tissue factor. *Thrombosis and Haemostasis*, 66(1), 67–79.

29. El-Galaly, T. C., Severinsen, M., Overvad, K., Steffensen, R., Vistisen, A. K., Tjønneland, A., Kristensen, S.R. (2013). Single nucleotide polymorphisms and the risk of venous thrombosis: results from a Danish case-cohort study. *British Journal of Haematology*, 160(6), 838–841.

30. Esmon, C. T. (2006). Inflammation and the activated protein C anticoagulant pathway. *Seminars in Thrombosis and Hemostasis*, 32(1), 49–60.

31. Falcon, C. R., Cattaneo, M., Panzeri, D., Martinelli, I., Mannucci, P. M. (1994). High prevalence of hyperhomocystinemia in patients with juvenile venous thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 14(7), 1080–1083.

32. Farrell, D. H. (2012). γ' Fibrinogen as a novel marker of thrombotic disease. *Clinical Chemistry and Laboratory Medicine*, 50(11), 1903–1909.

33. Friedman, T., Coon, D. B., Michaels, J. V., Bontempo, F., Young, V. L., Clavijo, J. A., Rubin, J. P. (2010). Hereditary coagulopathies: practical diagnosis and management for the plastic surgeon. *Plastic and Reconstructive Surgery*, 125(5), 1544–1552.

34. Fu-Chan, W., Mardini, S. (2017). Classification of flaps, in *Flaps and Reconstructive Surgery*. 2nd edn. Philadelphia: Elsevier, ch. 2.

35. Furie, B., Furie, B. C. (2008). Mechanisms of thrombus formation. *New England Journal of Medicine*, 359(9), 938–949.

36. Goyette, P., Pai, A., Milos, R., Frosst, P., Tran, P., Chen, Z., Chan, M., Rozen, R. (1998). Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). *Mammalian Genome*, 9(8), 652–656.

37. Grünbacher, G., Weger, W., Marx-Neuhold, E., Pilger, E., Köppel, H., Wascher, T., März, W., Renner, W. (2007). The fibrinogen gamma (FGG) 10034C>T polymorphism is associated with venous thrombosis. *Thrombosis Research*, 121(1), 33–36.

38. Hagau, N., Longrois, D. (2008). Anaesthesia for free vascularized tissue transfer. *Microsurgery*, 28(3), 161–167.

39. Hart, C., Rott, H., Heimerl, S., Linnemann, B. (2022). Management of antithrombin deficiency in pregnancy. *Hamostaseologie*, 42(5), 320–329.

40. Hill, J. B., Patel, A., Del Corral, G. A. (2012). Preoperative anemia predicts thrombosis and free flap failure in microvascular reconstruction. *Annals of Plastic Surgery*, 69(4), 364–367.

41. Hosein, R. C., Cornejo, A., Wang, H. T. (2016). Postoperative monitoring of free flap reconstruction: a comparison of external Doppler ultrasonography and the implantable Doppler probe. *Plastic Surgery*, 24(1), 11–19.

42. Jadaon, M. M. (2011). Epidemiology of prothrombin G20210A mutation in the Mediterranean region. *Mediterranean Journal of Hematology and Infectious Diseases*, 3(1), e2011037.

43. Jiang, J., Liu, K., Zou, J., Ma, H., Yang, H., Zhang, X., Jiao, Y. (2017). Associations between polymorphisms in coagulation-related genes and venous thromboembolism. *Medicine (Baltimore)*, 96(13), e6537.

44. Joshi, G. P., Kehlet, H., Lobo, D. N. (2025). Nonsteroidal anti-inflammatory drugs in the perioperative period: current controversies and concerns. *British Journal of Anaesthesia*, 134(2), 294–296.

45. Kamikubo, Y., Mendolicchio, G. L., Zampolli, A., Marchese, P., Rothmeier, A. S., Orje, J. N., Gale, A. J., Krishnaswamy, S., Gruber, A., Østergaard, H., Petersen, L. C., Ruf, W., Ruggeri, Z. M. (2017). Selective factor VIII activation by the tissue factor-factor VIIa-factor Xa complex. *Blood*, 130(14), 1661–1670.

46. Khansa, I., Colakoglu, S., Curtis, M. S. (2011). Postoperative monitoring and management of the reconstructive microsurgery patient. *Seminars in Plastic Surgery*, 27(1), 62–69.

47. Khansa, I., Colakoglu, S., Haider, S., Craft, R., Curtis, M. S. (2013). Evidence for pharmacologic thromboprophylaxis in microvascular breast reconstruction. *Microsurgery*, 33(5), 338–343.

48. Khouri, R. K., Cooley, B. C., Kunselman, A. R., Landis, R. J., Yeramian, P., Ingram, D., Natarajan, N., Benes, C. O., Wallemark, C. (1998). A prospective study of microvascular free-flap surgery and outcome. *Plastic and Reconstructive Surgery*, 102(3), 711–721.

49. Kim, M. J., Woo, K. J., Park, B. Y. (2018). Effects of transfusion on free flap survival: searching for an optimal hemoglobin threshold for transfusion. *Journal of Reconstructive Microsurgery*, 34(8), 610–615.

50. Kim, P. Y., Tieu, L. D., Stafford, A. R., Fredenburgh, J. C., Weitz, J. I. (2012). A high affinity interaction of plasminogen with fibrin is not essential for efficient activation by tissue-type plasminogen activator. *Journal of Biological Chemistry*, 287(7), 4652–4661.

51. Klerk, M., Verhoef, P., Clarke, R., Blom, H. J., Kok, F. J., Schouten, E. G., MTHFR Studies Collaboration Group. (2002). MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA*, 288(16), 2023–2031.

52. Koeppen, B. M., Stanton, B. A. (2017). *Berne & Levy Physiology*. 7th edn. Philadelphia: Elsevier, ch. 17, 386–410.

53. Kohlert, S., Quimby, A. E., Saman, M. (2019). Postoperative free-flap monitoring techniques. *Seminars in Plastic Surgery*, 33(1), 13–16.

54. Kujovich, J. L. (2011). Factor V Leiden thrombophilia. *Genetics in Medicine*, 13(1), 1–16.

55. Kumar, D. R., Hanlin, E., Glurich, I., Mazza, J. J., Yale, S. H. (2010). Virchow's contribution to the understanding of thrombosis and cellular biology. *Clinical Medicine & Research*, 8(3–4), 168–172.

56. Kumar, R., Dawson, J. E., Chan, A. K., Forman-Kay, J. D., Kahr, W. H., Williams, S. (2015). c.1058C>T variant in the *SERPINC1* gene is pathogenic for antithrombin deficiency. *British Journal of Haematology*, 170(1), 123–125.

57. Kuri, M., Nakagawa, M., Tanaka, H., Hasuo, S., Kishi, Y. (2005). Determination of the duration of perioperative smoking cessation to improve wound healing after head and neck surgery. *Anesthesiology*, 102(5), 892–896.

58. Larsen, J. B. and Hvas, A. M. (2020). Fibrin clot formation and lysis in plasma. *Methods and Protocols*, 3(4), 67.

59. László, I., Janovszky, Á. and Lovas, A. (2019). Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery: a randomised clinical trial. *European Journal of Anaesthesiology*, 36(8), 592–604.

60. Lawson, J. H., Kalafatis, M., Stram, S. and Mann, K. G. (1994). A model for the tissue factor pathway to thrombin. I. An empirical study. *Journal of Biological Chemistry*, 269(37), 23357–23366.

61. Leung, J. S., Seto, A. and Li, G. K. (2017). Association between preoperative nutritional status and postoperative outcome in head and neck cancer patients. *Nutrition and Cancer*, 69(3), 464–469.

62. Leyte, A., Verbeet, M. P. and Brodniewicz-Proba, T. (1989). The interaction between human blood-coagulation factor VIII and von Willebrand factor. Characterization of a high-affinity binding site on factor VIII. *Biochemical Journal*, 257(3), 679–683.

63. Liew, S. C. and Gupta, E. D. (2015). Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *European Journal of Medical Genetics*, 58(1), 1–10.

64. Lighthall, J. G., Cain, R. and Gidley, P. W. (2013). Increased hematocrit is not associated with decreased free flap failure. *Laryngoscope*, 123(12), 2963–2967.

65. Lindqvist, P. G., Zöller, B. and Dahlbäck, B. (2001). Improved hemoglobin status and reduced menstrual blood loss among female carriers of factor V Leiden – an evolutionary advantage? *Thrombosis and Haemostasis*, 86(4), 1122–1123.

66. Mackie, I. J., Kitchen, S., Machin, S. J., Lowe, G. D., Haemostasis, Thrombosis Task Force of the British Committee for Standards in Haematology. (2003). Guidelines on fibrinogen assays. *British Journal of Haematology*, 121(3), 396–404.

67. Mak, Q. H., Chan, H. T., Irwin, M. G. (2020). Anaesthesia for plastic and reconstructive surgery. *Anaesthesia & Intensive Care Medicine*, 22(1), 64–69.

68. Menu, P., Bleeker, W., Longrois, D., Caron, A., Faivre-Fiorina, B., Muller, S., Labrude, P., Stoltz, J. F. (2000). In vivo effects of Hb solutions on blood viscosity and rheologic behavior of RBCs: comparison with clinically used volume expanders. *Transfusion*, 40(9), 1095–1103.

69. Moellhoff, N., Broer, P. N., Heidekrueger, P. I., Ninkovic, M., Ehrl, D. (2021). Impact of intraoperative hypothermia on microsurgical free flap reconstructions. *Journal of Reconstructive Microsurgery*, 37(2), 174–180.

70. Nelsestuen, G. L., Shah, A. M., Harvey, S. B. (2000). Vitamin K-dependent proteins. *Vitamins & Hormones*, 58, 355–389.

71. Neubauer, K., Zieger, B. (2022). Endothelial cells and coagulation. *Cell and Tissue Research*, 387(3), 391–398.

72. O'Reilly, M. S., Pirie-Shepherd, S., Lane, W. S., Folkman, J. (1999). Antiangiogenic activity of the cleaved conformation of the serpin antithrombin. *Science*, 285(5435), 1926–1928.

73. Oldenburg, J., Marinova, M., Müller-Reible, C. (2008). The vitamin K cycle. *Vitamins & Hormones*, 78, 35–62.

74. Pan, X. L., Chen, G. X., Shao, H. W., Han, C. M., Zhang, L. P., Zhi, L. Z. (2014). Effect of heparin on prevention of flap loss in microsurgical free flap transfer: a meta-analysis. *PLoS One*, 9(4), e95111.

75. Pannucci, C. J., Kovach, S. J., Cuker, A. (2015). Microsurgery and hypercoagulable state: a hematologist perspective. *Plastic and Reconstructive Surgery*, 135(4), 545–552.

76. Pathare, A., Alkindi, S., Albalushi, T., Bayoumi, R., Dennison, D., Muralitharan, S. (2004). Heterozygous methylene tetrahydrofolate reductase mutation with mild hyperhomocysteinemia associated with deep vein thrombosis. *Clinical and Laboratory Haematology*, 26(2), 143–146.

77. Patnaik, M. M., Moll, S. (2008). Inherited antithrombin deficiency: a review, *Haemophilia*. 14(6), 1229–1239.

78. Pattani, K. M., Byrne, P., Boahene, K. (2010). What makes a good flap go bad? A critical analysis of the literature of intraoperative factors related to free flap failure. *Laryngoscope*, 120(4), 717–723.

79. Pepe, G., Camacho, V. O., Giusti, B., Brunelli, T., Marcucci, R., Attanasio, M. (1998). Heterogeneity in world distribution of the thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. *American Journal of Human Genetics*, 63(3), 917–920.

80. Perry, D. J., Carrell, R. W. (1996). Molecular genetics of human antithrombin deficiency, *Human Mutation*, 7(1), 7–22.

81. Piety, N. Z., Reinhart, W. H., Stutz, J., Shevkoplyas, S. S. (2017). Optimal hematocrit in an artificial microvascular network. *Transfusion*, 57(9), 2257–2266.

82. Pike, R. N., Buckle, A. M., le Bonniec, B. F., Church, F. C. (2005). Control of the coagulation system by serpins. Getting by with a little help from glycosaminoglycans. *FEBS Journal*, 272(19), 4842–4851.

83. Poort, S. R., Rosendaal, F. R., Reitsma, P. H., Bertina, R. M. (1996). A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*, 88(10), 3698–3703.

84. Pottinger, B. E., Read, R. C., Paleolog, E. M., Higgins, P. G., Pearson, J. D. (1989). von Willebrand factor is an acute phase reactant in man. *Thrombosis Research*, 53(4), 387–394.

85. Rahemtullah, A., Van Cott, E. M. (2007). Hypercoagulation testing in ischemic stroke. *Archives of Pathology & Laboratory Medicine*, 131(6), 890–901.

86. Ray, J. G., Shmorgun, D., Chan, W. S. (2002). Common C677T polymorphism of the methylene tetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies. *Pathophysiology of Haemostasis and Thrombosis*, 32(2), 51–58.

87. Rees, D., Cox, M., Clegg, J. (1995). World distribution of factor V Leiden. *Lancet*, 346(8983), 1133–1134.

88. Ridker, P. M., Glynn, R., Miletich, J. (1997). Age-specific incidence rates of venous thromboembolism among heterozygous carriers of factor V Leiden mutation. *Annals of Internal Medicine*, 126(7), 528–531.

89. Rijken, D. C., Lijnen, H. R. (2009). New insights into the molecular mechanisms of the fibrinolytic system. *Journal of Thrombosis and Haemostasis*, 7(1), 4–13.

90. Risman, R. A., Kirby, N. C., Bannish, B. E., Hudson, N. E., Tutwiler, V. (2023). Fibrinolysis: an illustrated review. *Research and Practice in Thrombosis and Haemostasis*, 7(2), e100070.

91. Rosendaal, F. R., Koster, T., Vandenbroucke, J. P., Reitsma, P. H. (1995). High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood*, 85(6), 1504–1508.

92. Rosendaal, F. R., Reitsma, P. H. (2009). Genetics of venous thrombosis. *Journal of Thrombosis and Haemostasis*, 7(Suppl. 1), 301–304.

93. Roshanov, P. S., Rochwerg, B., Patel, A., Salehian, O., Duceppe, E., Belley-Côté, E. P., Guyatt, G. H., Sessler, D. I., Le Manach, Y., Borges, F. K., Tandon, V., Worster, A., Thompson, A., Koshy, M., Devereaux, B., Spencer, F. A., Sanders, R. D., Sloan, E. N., Morley, E. E., Paul, J., Raymer, K. E., Punthakee, Z., Devereaux, P. J. (2017). Withholding versus continuing angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers before noncardiac surgery: an analysis of the vascular events in noncardiac surgery patients cohort evaluation prospective cohort. *Anesthesiology*, 126(1), 16–27.

94. Rozen, R. (1997). Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thrombosis and Haemostasis*, 78(1), 523–526.

95. Sakata, Y., Aoki, N. (1982). Significance of cross-linking of alpha 2-plasmin inhibitor to fibrin in inhibition of fibrinolysis and in hemostasis. *Journal of Clinical Investigation*, 69(3), 536–542.

96. Schlimp, C. J., Ponschab, M., Voelckel, W., Treichl, B., Maegele, M., Schöchl, H. (2016). Fibrinogen levels in trauma patients during the first seven days after fibrinogen concentrate therapy: a retrospective study. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*, 24(1), 29.

97. Schmaier, A. H. (2008). Assembly, activation, and physiologic influence of the plasma kallikrein/kinin system. *International Immunopharmacology*, 8(2), 161–165.

98. Schmid, M., Giger, R., Nisa, L. (2022). Association of multiprofessional preoperative assessment and information for patients with head and neck cancer with postoperative outcomes. *JAMA Otolaryngolog – Head & Neck Surgery*, 148(3), 259–267.

99. Scridon, A. (2022). Platelets and their role in hemostasis and thrombosis – from physiology to pathophysiology and therapeutic implications. *International Journal of Molecular Sciences*, 23(21), 12974.

100. Segers, K., Dahlbäck, B., Nicolaes, G. (2007). Coagulation factor V and thrombophilia: background and mechanisms. *Thrombosis and Haemostasis*, 98(3), 530–542.

101. Serletti, J. M., Higgins, J. P., Moran, S., Orlando, G. S. (2000). Factors affecting outcome in free-tissue transfer in the elderly. *Plastic and Reconstructive Surgery*, 106(1), 66–70.

102. Simone, B., De Stefano, V., Leoncini, E., Zacho, J., Martinelli, I., Emmerich, J., Rossi, E., Folsom, A. R., Almawi, W. Y., Scarabin, P. Y., Doggen, C. J., Palareti, G., Tenedios, F., Psaty, B. M., Tang, W., Buil, A., Lathrop, M., Souto, J. C., Hingorani, A. D. (2013). Risk of venous thromboembolism associated with single and combined effects of Factor V Leiden, Prothrombin 20210A and Methylenetetrahydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls. *European Journal of Epidemiology*, 28(8), 621–647

103. Slavik, L., Krcova, V., Hlusi, A., Prochazkova, J., Prochazka, M., Ulehlova, J., Indrak, K. (2009). Molecular pathophysiology of thrombotic states and their impact to laboratory diagnostics. *Biomedical Papers*, 153(1), 19–26.

104. Smith, S., Travers, R., Morrissey, J. (2015). How it all starts: initiation of the clotting cascade. *Critical Reviews in Biochemistry and Molecular Biology*, 50(4), 326–336.

105. Sou, W. K., Perng, C. K., Ma, H., Shih, Y. C. (2022). Perioperative myocardial infarction in free flap for head and neck reconstruction. *Annals of Plastic Surgery*, 88(1 Suppl 1), S56–S61.

106. Stanger, O., Herrmann, W., Pietrzik, K., Fowler, B., Giesel, J., Dierkes, J. (2004). Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. *Zeitschrift für Kardiologie*, 93(6), 439–453.

107. Stepanovs, J. (2025). Thromboelastometry for Microvascular Flap Thrombosis Risk Assessment in Trauma Patients Undergoing Reconstructive Surgery [Doctoral Thesis, Rīga Stradiņš University]. Rīga Stradiņš University. https://dspace.rsu.lv/jspui/handle/123456789/17143.

108. Svensson, P., Dahlbäck, B. (1994). Resistance to activated protein C as a basis for venous thrombosis. *New England Journal of Medicine*, 330(8), 517–522.

109. Tang, L., Liu, K., Wang, J., Wang, C., Zhao, P., Liu, J. (2010). High preoperative plasma fibrinogen levels are associated with distant metastasis and impacted prognosis after curative resection in patients with colorectal cancer. *Journal of Surgical Oncology*, 102(4), 428–432

110. van Hylckama Vlieg, A., Rosendaal, F. R. (2003). High levels of fibrinogen are associated with the risk of deep venous thrombosis mainly in the elderly. *Journal of Thrombosis and Haemostasis*, 1(12), 2677–2678.

111. Vanags, I., Stepanovs, J., Ozolina, A., Mukans, M., Bjertnaes, L. J., Mamaja, B. (2020). Thromboelastometry for assessing risks of free flap thrombosis in patients undergoing microvascular surgery. *Frontiers in Medicine*, 7, 294.

112. Versteeg, H. H., Heemskerk, J. W., Levi, M., Reitsma, P. H. (2013). New fundamentals in hemostasis. *Physiological Reviews*, 93(1), 327–358.

113. Vincent, A., Sawhney, R., Ducic, Y. (2019). Perioperative care of free flap patients. *Seminars in Plastic Surgery*, 33(1), 5–12.

114. Vos, H. L. (2006). Inherited defects of coagulation factor V: the thrombotic side. *Journal of Thrombosis and Haemostasis*, 4(1), 35–40.

115. Wayne, A. S., Kevy, S. V., Nathan, D. G. (1993). Transfusion management of sickle cell disease. *Blood*, 81(5), 1109–1123.

116. Welsch, G., Upchurch, G., Loscalzo, J. (1997). Hyperhomocysteinemia and atherothrombosis. *Annals of the New York Academy of Sciences*, 811(1), 48–58.

117. Westrick, R. J., Bodary, P. F., Xu, Z., Shen, Y. (2001). Deficiency of tissue factor pathway inhibitor promotes atherosclerosis and thrombosis in mice. *Circulation*, 103(25), 3044–3046.

118. Wilcken, D., Wang, X., Sim, A., McCredie, R. (1996). Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) C677T mutation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 16(7), 878–882.

119. Wolberg, A. S., Campbell, R. A. (2008). Thrombin generation, fibrin clot formation and hemostasis. *Transfusion and Apheresis Science*, 38(1), 15–23.

120. Woodruff, R. S., Sullenger, B., Becker, R. C. (2011). The many faces of the contact pathway and their role in thrombosis. *Journal of Thrombosis and Thrombolysis*, 32(1), 9–20.

121. Zheng, H., Ma, H. P., Xu, W. F., Zhang, Y. Q., Wu, J. J., Wang, J. (2014). Identification of a new *SERPINC1* mutation in a Kazak family that alters the heparin binding capacity of antithrombin. *Thrombosis Research*, 134(6), 1344–1349.

122. Zivelin, A., Mor-Cohen, R., Kovalsky, V. (2006). Prothrombin 20210G>A is ancestral prothrombotic mutation that occurred in whites approximately 24,000 years ago. *Blood*, 107(12), 4666–4668.

123. Zöller, B., Dahlbäck, B. (1994). Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet*, 343(8912), 1536–1538.

124. Zöller, B., Garcia de Frutos, P., Hillarp, A., Dahlbäck, B. (1999). Thrombophilia as a multigenic disease, *Haematologica*, 84(1), 59–70.

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Annexes

Research Ethics Committee and Ethics Committee approval

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„Rīgas Austrumu klīniskā universitātes slimnīca" SIA

Atzinums par pētījuma pieteikumu
„Iedzimto un iegūto trombofīliju nozīme
mikrovaskulārajā brīvo lēveru ķirurģijā"

Centrālā medicīnas ētikas komiteja 2016.gada 8.septembrī ir izskatījusi „Rīgas Austrumu klīniskā universitātes slimnīca" SIA iesniegto pētījuma pieteikumu *„Iedzimto un iegūto trombofīliju nozīme mikrovaskulārajā brīvo lēveru ķirurģijā".*

Pamatojoties uz Centrālās medicīnas ētikas komitejas 2016.gada 8.septembra sēdes protokola Nr.2016-4 punktu Nr.5 un iesniegtajiem papildinājumiem, tiek izsniegts atzinums, ka „Rīgas Austrumu klīniskā universitātes slimnīca" SIA iesniegtais pētījuma pieteikums *„Iedzimto un iegūto trombofīliju nozīme mikrovaskulārajā brīvo lēveru ķirurģijā"* nav pretrunā ar bioētikas normām.

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