Rihards Pēteris Ročāns

ORCID 0000-0002-3546-5612

Biomarkers for Predicting Complications in Microvascular Flap Surgery

Doctoral Thesis – set of publications – for obtaining the scientific degree "Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences

Sector – Clinical Medicine

Sub-Sector – Surgery

Supervisors of the Doctoral Thesis:

Dr. med., Professor Emeritus **Biruta Mamaja**, Rīga Stradiņš University, Latvia

Dr. med., Associated Professor **Simona Doniņa**, Rīga Stradiņš University, Latvia

Riga, 2026

Abstract

Microvascular flap surgery has secured its place in reconstructive surgery as an important technique for correcting various tissue defects. Despite substantial progress in surgical techniques and reduction of complication rates, flap loss remains a challenge. This research aimed to outline new avenues for improving outcomes and to examine novel nutritional, inflammatory, and immunological biomarker models for predicting complications in microvascular flap surgery.

The first part of the research involves a narrative literature review that analyses 80 studies in *PubMed*, *Scopus* and *Web of Science* published from 1 January 2007 to 1 July 2024. The aim of the first part was to outline novel avenues for best practice and provide an outlook for further research of anaesthesia and perioperative care concepts in microvascular flap surgery. The results of the narrative review indicated that the assessment of nutritional status, hypoalbuminemia, chronic inflammation, and hypercoagulability assessment currently lack sufficient data and are highly promising avenues for further research.

The first study of the prospective observational part includes 72 adult patients undergoing elective microvascular flap surgery. The aim of this study was to assess the predictive value of the Controlling Nutritional Assessment (CONUT) score for complications in microvascular flap surgery. Blood samples for full blood count, total plasma cholesterol, and albumin levels were obtained on the day of surgery prior to initiation of crystalloids. The serum albumin concentration, total peripheral lymphocyte count, and serum total cholesterol concentration were used to assign the CONUT score. The CONUT score had an AUC of 0.813 (0.659–0.967, $p = 0.012$) for predicting complications other than true flap loss due to vascular compromise. A CONUT score > 2 was indicated as optimal during cut-off analysis ($p = 0.022$). Patients with flap complications had a longer duration of hospitalisation (13.55, 10.99–16.11 vs. 25.38, 14.82–35.93; $p = 0.004$). Our findings indicated that the CONUT score has considerable predictive value for minor flap complications in microvascular flap surgery but was not predictive of true flap loss.

The second study of the prospective part aimed to evaluate the Fibrinogen-to-Albumin ratio (FAR) for predicting complications in microvascular surgery. It included 130 adult patients undergoing elective microvascular flap surgery. Preoperative blood draws for analysis of plasma fibrinogen and albumin were collected on the day of surgery prior to crystalloid infusion. Binary logistic regression revealed that patients with FAR < 0.08 and FAR < 0.06 had increased odds of flap haematoma or flap loss (OR 3.68, 1.04–13.03, $p = 0.044$ and 6.01, 1.71–21.08, $p = 0.005$). Patients with FAR > 0.10 had increased odds of minor flap complications (OR 5.47, 1.33–22.50, $p = 0.019$). Patients with FAR < 0.06 had increased odds

of flap complications (OR 4.71, 1.27–18.03, p = 0.021). FAR > 0.10 also increased the odds of flap complications (OR 3.09, 1.08–8.81, p = 0.035), implying a U-shaped link.

The third study of the prospective part evaluated the link between von Willebrand factor antigen (vWF:Ag) and flap complications in 88 adult patients undergoing elective microvascular flap surgery. Preoperative blood draws were collected on the day of surgery prior to crystalloid infusion. The plasma concentration of vWF:Ag, albumin, neutrophil-to-lymphocyte ratio (NLR), interleukin-6, C-reactive protein (CRP) and fibrinogen were determined. vWF:Ag levels were higher in true flap loss when compared to patients without complications (217.94 IU/dL, 137.27–298.45 vs. 114.14, 95.67–132.71, p = 0.001). Regression analysis revealed an association between vWF:Ag and true flap loss a cut-off point of 163.73 IU/dL (OR 70.22, 10.74–485.28, p = 0.043). Increased vWF:Ag concentrations were linked to increases in plasma fibrinogen (p < 0.001), CRP (p < 0.001), interleukin-6 (p = 0.032), and NLR (p = 0.019). The findings of this study indicate that preoperative plasma vWF:Ag concentration is linked to biomarkers of inflammation, and the selected cut-off provides a viable model for predicting complications in microvascular flap surgery.

The fourth study of the prospective part aims to evaluate the viability of tumor necrosis factor beta-1 (TGF-β1) for predicting microvascular flap complications. This prospective observational cohort study included 173 individuals who underwent elective reconstructive microvascular flap surgery. All patients who had true flap loss or secondary flap complications (n = 22) were included in the complication group. An equal number of patients with no complications (n = 22) were selected from the overall cohort using simple randomisation to form the 44 patient cohort to match the available sample count for laboratory analysis. Preoperative blood draws for analysis were collected on the day of surgery before crystalloid infusion. Postoperative blood draws were collected after surgery before leaving the operating room. Preoperative and postoperative plasma concentration of TGF-β1 as well as preoperative parameters such as full blood count, albumin, interleukin-6, total protein, triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, CRP, and fibrinogen, were determined. Increased postoperative TGF-β1 was positively linked to preoperative plasma fibrinogen (p = 0.020), plasma CRP (p = 0.021), haematocrit (p = 0.039) and haemoglobin (p = 0.009). Postoperative change of TGF-β1 was higher in patients with true flap loss when compared to patients without complications and patients with minor flap complications (0.403 log10 of ng/ml, 0.024–0.782 vs. 0.157, 0.029–0.285 vs. −0.089, −0.233–0.056, p = 0.002). Multivariate regression analysis revealed that an increase in postoperative change of TGF-β1 increases the odds of true flap loss (OR 2.028, 1.185–3.471, p = 0.009).

This research has developed models of nutritional, inflammatory, coagulation and immunological biomarkers to predict complications in microvascular flap surgery. It has also uncovered important new insights into the pathophysiology of these complications and expanded our understanding of preoperative assessment. These advancements may improve outcomes for patients undergoing microvascular flap surgery.

Keywords: microvascular flap complications; reconstructive surgery; microvascular flap surgery; flap loss; true flap loss; microvascular flap thrombosis; controlling nutritional status; CONUT; fibrinogen-to-albumin ratio; FAR; von Willebrand factor antigen; vWF:Ag; neutrophil-to-lymphocyte ratio; transforming growth factor beta-1; TGFB-1; TGF-β1

Anotācija

Biomarķieru modelis komplikāciju paredzēšanai brīvo lēveru mikroķirurģijā

Mikrovaskulāro lēveru ķirurģija ir plaši pielietota un nozīmīga metode dažādu defektu korekcijai. Neskatoties uz ievērojamu mikrovaskulārās tehnikas attīstību un kopējo komplikāciju biežuma samazināšanos, lēvera zudums joprojām ir klīnisks izaicinājums. Šī pētījuma mērķis bija atklāt jaunus virzienus komplikāciju novēršanai un izpētīt jaunus malnutrīcijas, iekaisuma un imūnmodulējošo biomarķieru modeļus, lai prognozētu komplikācijas mikrovaskulārajā lēveru ķirurģijā.

Pētījuma pirmā daļa ietver literatūras apskatu, kurā analizēti 80 pētījumi no *PubMed*, *Scopus* un *Web of Science* datubāzēm, kas publicēti no 2007. gada 1. janvāra līdz 2024. gada 1. jūlijam. Pirmās pētījuma daļas mērķis bija noteikt labākās prakses jaunos virzienus un sniegt ieskatu turpmākai pētniecībai par perioperatīvo aprūpi mikrovaskulāro lēveru ķirurģijā un iespējamajiem biomarķieriem komplikāciju paredzēšanai. Literatūras apskata rezultāti norādīja, ka nepietiekama uztura, hipoalbuminēmijas, hroniska iekaisuma un hiperkoagulācijas izvērtēšanai šobrīd trūkst pietiekamu datu, tomēr tie ir daudzsološi virzieni turpmākai pētniecībai.

Prospektīvās daļas pirmajā novērošanas tipa pētījumā, tika iekļauti 72 pilngadīgi pacienti, kuriem tika veikta plānveida mikrovaskulārā lēvera operācija. Pirmā pētījuma mērķis bija novērtēt uztura stāvokļa kontroles (CONUT, *Controlling Nutritional Status*) indeksa prognostisko vērtību attiecībā uz komplikācijām mikrovaskulāro lēveru ķirurģijā. Asins paraugi pilnas asinsainas, kopējā plazmas holesterīna un albumīna līmeņu noteikšanai tika iegūti operācijas dienā pirms pirmo kristaloīdu ievades. Serumā noteiktā albumīna koncentrācija, perifēro limfocītu skaits un kopējā holesterīna koncentrācija tika izmantoti, lai aprēķinātu CONUT indeksu. CONUT indeksa laukums zem līknes (AUC, *Area Under Curve*) nelielu lēvera komplikāciju prognozēšanai bija 0.813 (0.659–0.967, $p = 0.012$). CONUT vērtība > 2 tika norādīta kā optimāla robežvērtību analīzē ($p = 0.022$). Pacientiem ar lēvera komplikācijām bija ilgāks hospitalizācijas laiks (13.55, 10.99–16.11 pret 25.38, 14.82–35.93; $p = 0.004$). CONUT indeksam ir nozīmīga prognostiska vērtība nelielu lēvera komplikāciju paredzēšanai, taču tas neparedz lēvera zudumu.

Prospektīvās daļas otrā novērošanas tipa pētījuma mērķis bija novērtēt fibrinogēna un albumīna attiecības (FAR, *Fibrinogen-to-albumin ratio*) saikni ar pēcoperācijas komplikācijām. Tajā iekļauti 130 pilngadīgi pacienti, kuriem tika veikta plānveida mikrovaskulārā lēvera operācija. Pirmsoperācijas asins paraugi fibrinogēna un albumīna līmeņu

noteikšanai tika iegūti operācijas dienā pirms kristaloīdu ievades. Binārās loģistiskās regresijas analīze parādīja, ka pacientiem ar FAR < 0.08 un FAR < 0.06 bija paaugstināts lēvera hematomas vai lēvera zuduma risks (IA 3.68, 1.04–13.03, p = 0.044 un 6.01, 1.71–21.08, p = 0.005). Pacientiem ar FAR > 0.10 bija paaugstināts nelielu lēvera komplikāciju risks (IA 5.47, 1.33–22.50, p = 0.019). Gan pacientiem ar FAR < 0.06, gan ar FAR > 0.10 bija paaugstināta lēveru komplikāciju iespējamība (IA 4.71, 1.27–18.03, p = 0.021 un IA 3.09, 1.08–8.81, p = 0.035), kas liecina par U veida saistību.

Prospektīvās daļas trešā pētījuma mērķis bija izvērtēt von Villebranda faktora antigēna (vWF:Ag, *von Willebrand factor antigen*) saistību ar lēvera komplikācijām 88 pilngadīgiem pacientiem, kuriem tika veikta plānveida mikrovaskulārā lēvera operācija. Pirmsoperācijas asins paraugi tika savākti operācijas dienā pirms kristaloīdu ievadīšanas. Tika noteikta plazmas vWF:Ag, albumīna, neitrofilu-limfocītu attiecības (NLR, *Neutrophil-to-lymphocyte ratio*), interleikīna-6, C-reaktīvā olbaltuma (CRO, *C-reactive protein*) un fibrinogēna koncentrācija. vWF:Ag līmenis bija augstāks pacientiem ar lēvera zudumu, salīdzinot ar pacientiem bez komplikācijām (217.94 IU/dL, 137.27–298.45 vs. 114.14, 95.67–132.71, p = 0.001). Regresijas analīze atklāja saikni starp vWF:Ag un lēvera zudumu pie robežvērtības 163.73 IU/dL (IA 70.22, 10.74–485.28, p = 0.043). Paaugstinātas vWF:Ag koncentrācijas bija saistītas ar paaugstinātām fibrinogēna (p < 0.001), CRO (p < 0.001), interleikīna-6 (p = 0.032) un NLR (p = 0.019) koncentrācijām. Šīs daļas rezultāti liecina, ka pirmsoperācijas vWF:Ag koncentrācija plazmā ir saistīta ar iekaisuma biomarķieriem, un vWF:Ag > 163.73 IU/dL norāda uz palielinātu lēvera zuduma risku.

Prospektīvās daļas ceturtā pētījuma mērķis bija izvērtēt transformējošā augšanas faktora beta-1 (TGF-β1, *Transforming growth factor beta-1*) prognostisko vērtību attiecībā uz mikrovaskulāro lēveru komplikāciju risku. Šajā prospektīvajā novērošanas tipa pētījumā tika iekļauti 173 pacienti, kuriem tika veikta plānveida mikrovaskulārā lēvera operācija. Visi pacienti ar lēvera zudumu vai nelielām lēvera komplikācijām (n = 22) tika iekļauti komplikāciju grupā. Izmantojot vienkāršo randomizāciju, tika atlasīts vienāds skaits pacientu bez komplikācijām (n = 22), izveidojot 44 pacientu grupu laboratorisko analīžu veikšanai. Pirmsoperācijas asins paraugi tika savākti operācijas dienā pirms kristaloīdu ievadīšanas. Pēcoperācijas asins paraugi tika ievākti pēc operācijas beigām operāciju zālē. Plazmas TGF-β1 koncentrācijas plazmā tika noteiktas pirms un pēc operācijas. Pirmsoperācijas asins paraugos tika noteikta pilna asins aina, albumīns, interleikīns-6, kopējais olbaltums, triglicerīdi, kopējais holesterīns, augsta un zema blīvuma lipoproteīnu holesterīns, CRO un fibrinogēna līmenis. Paaugstināta pēcoperācijas TGF-β1 koncentrācija bija pozitīvi saistīta ar pirmsoperācijas fibrinogēna (p = 0.020), CRO (p = 0.021), hemoglobīna (p = 0.009) koncentrāciju kā arī

hematokrītu (p = 0.039). TGF-β1 pieaugums tūlītēji pēc operācijas bija izteiktāks pacientiem, kam sekojoši bija lēvera zudums, salīdzinājumā ar pacientiem bez komplikācijām vai nelielām lēvera komplikācijām (0.403 log10 ng/ml, 0.024–0.782 vs. 0.157, 0.029–0.285 vs. −0.089, −0.233–0.056, p = 0.002). Regresijas analīze atklāja, ka TGF-β1 pieaugums (ng/dl) pēc operācijas palielina tūlītēja lēvera zuduma iespējamību (IA 2.028, 1.185–3.471, p = 0.009).

Pētnieciskā darba gaitā izstrādāti nepietiekama uztura, iekaisuma, koagulācijas un imūnās atbildes biomarķieru modeļi komplikāciju paredzēšanai mikrovaskulāro lēveru ķirurģijā. Pētījumā atklātas nozīmīgas atziņas par atsevišķu komplikāciju tipu patoģenēzi un paplašināta izpratne par komplikāciju riska perioperatīvo laboratoro novērtēšanu.

Atslēgvārdi: mikrovaskulāro lēveru komplikācijas; rekonstruktīvā ķirurģija; mikrovaskulāro lēveru ķirurģija; lēvera zudums; lēvera tromboze; nepietiekams uzturs; CONUT; fibrinogēna un albumīna attiecība; FAR; fon Villebranda faktora antigēns; vWF:Ag; transformējošais augšanas faktors beta-1; TGF-β1.

Table of Contents

Abbreviations used in the Thesis

ANOVA	Analysis of variance
ASA	American Society of Anesthesiologists
AUC	Area under curve
BMI	Body mass index
CI95	95 % confidence interval
CONUT	Controlling Nutritional Status Score
CRP	C-reactive protein
CZ	Concordance probability method
FAR	Fibrinogen-to-albumin ratio
ICU	Intensive care unit
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	Interquartile range
NLR	Neutrophil-to-lymphocyte ratio
OR	Odds ratio
PNI	Prognostic nutritional index
RBC	Red blood cell
ROC	Receiver operator curve
TGF-β	Transforming growth factor beta
TGF-β1	Transforming growth factor beta-1
vWF	von Willebrand factor
vWF:Ag	von Willebrand factor antigen
YI	Youden's index

Introduction

Microvascular flap surgery has become a common method in reconstructive surgery for addressing a wide range of tissue defects (Lese et al., 2021). These defects may arise from causes such as trauma, cancer, chronic infections, or complex wounds of multiple aetiologies (Min et al., 2022). Compared to conventional surgical techniques, free flaps offer improved quality of defect correction, greater donor site flexibility, faster recovery, shorter hospital stays, and reduced treatment costs (Galviz Tabarez et al., 2025).

Despite advancements in surgical methods and decreasing rates of complications, flap loss remains a challenge (Lo et al., 2017). Complications are generally classified into three categories: true flap loss, minor flap complications, and flap haematoma (Lo et al., 2017; Vincent et al., 2019; Min et al., 2022). Throughout previous research, the overall complication rate ranges from 3–6 % (Hanasono et al., 2008; Dolan et al., 2012; Lese et al., 2021). Venous thrombosis is identified as the most common cause of true flap loss, while arterial thrombosis tends to be the primary contributor to early flap loss (Stevens et al., 2023). In venous flap thrombosis, pedicle kinking is a more prevalent cause of flap failure than anastomotic failure (Williams et al., 2004). Despite the technical complexity of microvascular flap transplantation, systemic responses to surgical trauma, including immune activation and coagulation processes, play a significant role in predicting the outcomes (Schuderer et al., 2023). Recent studies have highlighted the biological mechanisms underlying flap-related complications and have proposed the use of specific biomarkers for risk stratification before surgery (Chargi et al., 2022). These biomarkers may offer significant opportunities to evaluate the risk of true flap loss, improve our understanding of flap complication pathophysiology and refine perioperative care protocols.

Numerous studies have shown that malnutrition significantly contributes to complications across various surgical populations (Reed et al., 2024; Portuondo et al., 2020; Tobert et al., 2017). Malnourished patients are more prone to postoperative issues such as wound dehiscence, infection, and fistula formation, often requiring reoperation and increasing morbidity and hospital costs (Le et al., 2023; Quian et al., 2021). As nutritional status is a modifiable risk factor, early screening, assessment, and intervention can help reduce postoperative complications (Venianaki et al., 2021; Yu et al., 2020). Laboratory biomarkers, routinely included in preoperative assessments, offer a convenient means of malnutrition risk screening (Yu et al., 2020; Takagi et al., 2019). Given the complexity of microvascular flap reconstruction and the availability of effective nutritional intervention strategies, using biomarkers of malnutrition could improve surgical outcomes (Jaxa-Kwiatkowski et al., 2025; Yu et al., 2020). Common laboratory markers for malnutrition risk include serum albumin,

prealbumin, transferrin, and total lymphocyte count (Keller, 2019). The Controlling Nutritional Status (CONUT) score, a simple tool based on serum albumin, total cholesterol, and lymphocyte count, has proven effective in predicting postoperative outcomes across multiple surgical populations (Lo Buglio et a., 2024; Ulibarri et al., 2005). Due to its versatility, simplicity, and predictive value, the CONUT score may represent a promising tool for evaluating nutritional risk in microvascular flap surgery patients.

In addition to malnutrition, systemic inflammation plays a role in flap thrombosis and minor flap complications (Chargi et al., 2022). Low blood albumin levels are linked to elevated proinflammatory mediators and impaired tissue regeneration, while high fibrinogen levels are associated with inflammation and tissue repair, often serving as a predictor of complications (Eckart et al., 2020; Luyendyk et al., 2019). While individual biomarkers can predict surgical outcomes, recent studies show that combined measures enhance predictive accuracy and improve prognostic value (Ren et al., 2024; Zhang et al., 2021; Tomita et al., 2020). The fibrinogen-to-albumin ratio (FAR) is a promising combined measure that has demonstrated strong potential for assessing postoperative outcomes across various surgical populations (Altin et al., 2025; Park et al., 2022).

The detailed pathophysiology of true flap loss, although involving chronic inflammation, is currently not fully defined (Chargi et al., 2022). True flap loss has been associated with elevated fibrinogen, von Willebrand factor (vWF) function, and vWF antigen (vWF:Ag) (Rothweiler et al., 2021; Drizlionoka et al., 2019, Du et al., 2015; Handschel et al., 2013). Elevated vWF:Ag levels, often resulting from endothelial damage or inflammation, can promote platelet aggregation and adhesion, exacerbating thrombotic risks in reconstructive surgery (Rothweiler et al., 2021). The interaction between vWF and inflammatory biomarkers in the context of thrombotic events remains complex, though studies by Handschel et al. and Rothweiler et al. demonstrate the significant role of vWF:Ag in true flap loss (Rothweiler et al., 2021; Handschel et al., 2013). However, the full extent of vWF:Ag interactions with other risk factors, including chronic inflammation, remains elucidated.

It is generally accepted that microvascular flap surgery provokes a predominantly proinflammatory immunologic response (Schmidt et al., 2007, Zhang et al., 2006). Elevated levels of proinflammatory cytokines, including interleukin-6 (IL-6), interleukin-8 (IL-8), and macrophage colony-stimulating factor, have been associated with ischaemia-reperfusion injury in microvascular surgery (Finke et al., 2018). Nevertheless, there is a lack of studies specifically addressing the contribution of immunomodulatory cytokines to the pathophysiological mechanisms underlying microvascular flap complications. Although previous investigations have assessed cytokine levels within flap venous blood samples (Finke et al., 2018), no studies

evaluate the postoperative change in immunomodulatory cytokine concentrations in regular circulating blood samples. Transforming growth factor beta (TGF-β), a pleiotropic cytokine, is involved in multiple cellular processes, including immunoregulation, inflammatory resolution, and tissue repair (Deng et al., 2024). The principal isoform, transforming growth factor beta 1 (TGF-β1), has emerged as a potential biomarker, influencing thrombogenesis and thrombus stabilisation (Zhang et al., 2024). TGF-β1 is predominantly released from platelet alpha-granules and becomes bioactive under conditions of shear stress, which could have pathophysiological and predictive implications in true flap loss (Ahamed et al., 2008).

Despite growing interest in the use of biomarkers for risk stratification in microvascular flap surgery, there remains a lack of validated, clinically applicable models that integrate nutritional, inflammatory, and thrombosis-related markers with patient outcomes (Chargi et al., 2022; Vanags et al., 2020; Yu et al. 2020). This research aims to address this gap by investigating the prognostic value of combined biomarkers, including the CONUT score, FAR, as well as emerging individual biomarkers such as TGF-β1 and vWF:Ag, to enhance preoperative risk assessment and perioperative care in microvascular flap surgery.

Aim of the Thesis

The aim of this study is to establish novel biomarker models for the preoperative and early postoperative prediction of microvascular flap complications in reconstructive surgery.

Objectives of the Thesis

The following objectives are set to reach the aim of the Doctoral Thesis:

1. Outline novel avenues for best practice and provide an outlook for further research on preoperative risk assessment, anaesthesia, and perioperative care in microvascular flap surgery.
2. Assess the predictive value of the CONUT score for predicting complications in elective microvascular flap surgery.
3. Evaluate the predictive value of FAR in predicting various complication types in elective microvascular flap surgery.
4. Determine the predictive value of vWF:Ag for microvascular flap complications and investigate the relationship between markers of chronic inflammation and increased vWF:Ag in various complication types.
5. Assess the prognostic significance of postoperative TGF-β1 changes in relation to various microvascular flap complications and to examine the link between perioperative TGF-β1 levels and other biomarkers in microvascular flap surgery patients.

Hypotheses of the Thesis

- Increased preoperative CONUT score effectively predicts malnutrition and postoperative complication risk for microvascular flap surgery patients.
- Increased preoperative FAR predicts the risk of true flap loss in microvascular flap surgery.
- Increased preoperative plasma vWF:Ag is linked to a proinflammatory state and is predictive for complications in microvascular flap surgery.
- A significant postoperative increase in serum TGF-β1 relative to its preoperative level demonstrates early predictive value and is associated with true flap loss.

Novelty of the Thesis

The novelty of this research lies in the exploration of biomarker models for prediction of flap complications in reconstructive surgery. Many recent studies have the potential to improve perioperative care, assess the risk of flap loss, and understand the pathophysiology of microvascular flap complications using laboratory biomarkers. The development of biomarker models offers a valuable and cost-effective approach by integrating preoperative laboratory evaluations, combined measurements, and statistical modelling to enhance risk assessment before microvascular flap surgery. Despite significant advancements in this field, several knowledge gaps remain. It is increasingly evident that the factors influencing complications, and the success of free flap microvascular reconstructions are complex and multifactorial.

The research outlines the interplay between inflammatory and coagulation markers and how they can be combined to predict true flap loss. A key highlight of this research is the demonstration of the effectiveness of assessing the postoperative increase of the immunomodulating cytokine TGF-β1 for early prediction of impending true flap loss. Ultimately, this research represents a pioneering contribution to expanding our understanding of the pathophysiological patterns underlying various flap complication types, enhancing preoperative patient risk assessment, and improving perioperative care in microvascular flap surgery.

1 Materials

The research consisted of two parts:

1. Narrative literature review of 80 studies (95 articles screened, 80 articles included)
2. Prospective cohort studies:
   2.1. First study consisting of 72 patients (7 with other flap complications, 4 with true flap loss, 61 without complications) for evaluating the predictive capacity of the CONUT score.
   2.2. Second study consisting of 130 patients (16 with other flap complications, 7 with true flap loss, 107 without complications) for evaluating the predictive capacity of FAR.
   2.3. Third study consisting of 173 patients, of whom 88 patients were selected for analysis (14 with other flap complications, 10 with true flap loss, 64 without complications) for evaluating the predictive capacity of vWF:Ag.
   2.4. Fourth study consisting of 173 patients, of whom 44 patients were selected for analysis (12 with other flap complications, 10 with true flap loss, 22 without complications) for evaluating the predictive capacity of postoperative increase in TGF-β1.

The narrative literature review for the first part of the research was conducted using the *PubMed*, *Scopus*, and *Web of Science* databases. A predefined search strategy incorporating selected keywords was employed. Studies published between 1 January 2007 and 1 July 2024 were identified and included for analysis.

The prospective observational research with a total of 173 patients was conducted in Riga East University Hospital. The study period ranged from 1 October 2021 to 31 March 2024. Adult patients undergoing elective microvascular flap surgery who provided informed consent were included in the research. The study was observational in nature, all surgical, anaesthesia, and perioperative care decisions were made by a multidisciplinary team of attending physicians. The selection of flap type was based on the defect characteristics, pedicle length, patient positioning during surgery, patient body mass index (BMI), and adipose tissue distribution. All surgeries were performed by a team of experienced surgeons. All patients underwent general anaesthesia with monitoring of electrocardiography, pulse oximetry, blood pressure, end-tidal carbon dioxide levels, body temperature, and urine output. Peripheral nerve blocks with ultrasound and neurostimulation guidance were performed when indicated.

The following flap types were used in the research: anterolateral thigh flap; fibular flap; deep inferior epigastric artery perforator flap; radial free forearm flap, gracilis muscle flap, temporal artery flap, serratus anterior flap, and latissimus dorsi flap. Preoperative blood samples

were obtained on the day of surgery immediately upon the first arrival in the operating room before initiation of the first crystalloid infusion for all patients. Postoperative blood draws were obtained after the end of surgery before leaving the operating room. Attending surgeons closely monitored the microvascular flap for the first 3–5 post-operative days. Monitoring flap complications was done through clinical evaluation of perfusion, assessing parameters such as tissue colour, temperature, turgor, capillary refill, skin texture of the flap, absence of oedema, and response to the pinprick test.

Written and electronic medical records were reviewed to collect data on patient demographics, flap type, surgical site and duration, comorbidities, anaesthesia and postoperative care, outcomes at both the flap and donor sites, length of intensive care unit (ICU) stay, and total hospitalisation time. All data was collected in accordance with a previously defined protocol.

Preoperative clinical laboratory analysis for all patients included in the research was performed in Riga East University Hospital Laboratory Department. Full blood count analysis of the preoperative samples was performed using the XN-1500 system (Sysmex Europe SE, Norderstedt, Germany). Preoperative IL-6 concentrations were obtained by electrochemiluminescence immunoassay (Cobas e, Roche/Hitachi, Manheim, Germany). Preoperative C-reactive protein (CRP) concentrations were obtained using the method of immunoturbidimetry (Cobas C, Roche/Hitachi, Manheim, Germany). Preoperative fibrinogen concentrations were obtained using the CS 5100 system (Sysmex Corporation, Kobe, Japan). Preoperative levels of total protein, albumin, triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using the enzymatic colorimetric technique (Cobas c, Roche/Hitachi, Manheim, Germany).

Sample storage for the fourth and fifth parts of the research including 173 patients was conducted in Riga East University Hospital Centre of Oncology. The serum samples were preserved at −80 °C in a consistent temperature in screw-cap tubes appropriate for long term storage. vWF:Ag evaluation for the fourth part of the research was performed using the human vWF enzyme-linked immunoassay kit according to the manufacturer's protocol from Abcam (Cambridge, United Kingdom). TGF-β1 evaluation for the fifth part of the research was conducted using the TGF-β1 enzyme-linked immunoassay kit according to the manufacturers protocol from Merck (Darmstadt, Germany). All absorbance readings for enzyme-linked immunoassay analyses were performed on a Varioskan Lux microplate reader (Thermo Fisher Scientific, Massachusetts, United States of America) at the Rīga Stradiņš University Institute of Microbiology and Virology.

2 Methods

2.1 First Part. Narrative literature review of novel challenges and opportunities for anaesthesia and perioperative care in microvascular flap surgery

The study is described in the manuscript "Ojuva, A. M., Rocans, R. P., Zarins, J., Bine, E., Mahauri, I., Donina, S., Mamaja, B., & Vanags, I. (2024). Novel Challenges and Opportunities for Anaesthesia and Perioperative Care in Microvascular Flap Surgery: A Narrative Review. Clinics and Practice, 14(5), 2187–2201. https://doi.org/10.3390/clinpract 14050172".

The narrative literature review included 80 articles. The article search and screening process was carried out using the following algorithm and key terms: "preoperative risk", "risk factors", "biomarkers", "fibrinogen", "malnutrition", "comorbidities", "anaemia", "coagulation", "coagulology assessment", "inflammation", "regional anaesthesia", "general anaesthesia", "analgesia", "crystalloid", "vasopressor", "fluid", "monitoring", "anticoagulants", "antiaggregants", "intraoperative care", "preoperative care", "postoperative care", "continuous instrumental free flap monitoring" combined with "free flap failure", "true flap loss", "minor flap complications", "flap complications", "free flap thrombosis", "free flap surgery", or "microvascular flap surgery". Selection of studies was based on their relevance to routine clinical practice, with particular attention given to studies offering novel insights into risk prediction, personalised patient care, and outcome improvement. Priority was given to randomised clinical trials, large-scale observational studies, and the most recent publications. Article identification, screening and inclusion process used in this review is shown in Figure 2.1.

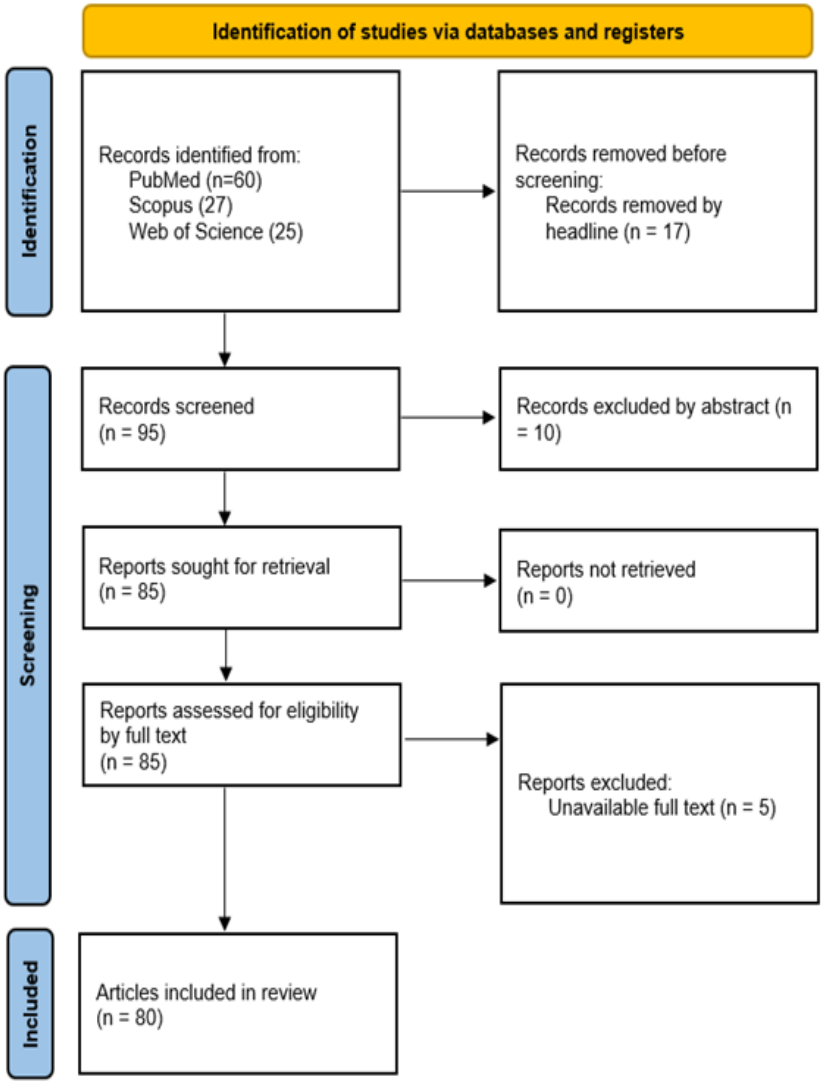

Figure 2.1. **Identification, screening, and inclusion of studies via databases and registers flowchart**

Definitions for complications used in the literature review: true flap loss is defined as the impairment of the flap blood flow due to venous or arterial anastomosed blood vessel dysfunction or thrombosis leading to complete loss of the transposed flap; flap haematoma is defined as the presence of a postoperative haematoma adjacent to the flap recipient site without interfering with the flap blood supply either due to surgical causes or insufficient coagulation function; minor flap complications are defined as the presence of flap recipient or donor site wound infection, slow or difficult flap wound healing, partial or marginal flap necrosis, or difficult healing at the donor site.

2.2 First prospective study of the second part. CONUT score for prediction of microvascular flap complications in reconstructive surgery

The study is described in the manuscript "Rocans, R. P., Zarins, J., Bine, E., Deksnis, R., Citovica, M., Donina, S., & Mamaja, B. (2023). The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery. *Journal of Clinical Medicine*, 12(14), 4794. https://doi.org/10.3390/jcm12144794".

2.2.1 Patient characteristics

This prospective cohort study included 72 patients undergoing elective microvascular flap transplantation surgery from the 1 October 2021 to the 31 January 2023. The exclusion criteria were patients with sepsis or severe systemic bacterial infection; patients with autoimmune disorders; patients with blood-borne viral infections (Hepatitis B; Hepatitis C and HIV); pregnant patients and patients during lactation period; and patients with congenital hypercoagulability or any clotting disorder. The patients' data is summarised in Table 2.1.

Table 2.1

Patient characteristics

Patient group	Overall n = 72	No complications n = 61	True flap loss n = 4	Any flap complications n = 11	p-value
Demographical data					
Mean age, years	55.3 (51.5–59.1)	56.9 (61.0–65.4)	65.0 (63.5–66.5)	49.6 (37.7–56.1)	0.057
Women, n (%)	32 (44.4)	25 (40.1)	2 (50)	5 (45.5)	0.418
Area of reconstruction					
Extremity, n (%)	15 (20.8)	12 (19.6)	–	3 (27.3)	0.289
ENT, n (%)	26 (36.1)	22 (36.1)	2 (50.0)	4 (36.4)	0.496
Head and Neck, n (%)	16 (22.2)	14 (30.0)	1 (25.0)	2 (18.2)	0.322
Breast, n (%)	15 (20.8)	13 (21.3)	1 (25.0)	2 (18.2)	0.457
Microvascular flap type					
ALT, (%)	32 (44.4)	27 (44.3)	2 (50)	5 (45.5)	0.828
Fibular flap, (%)	9 (12.5)	8 (13.1)	1 (25)	1 (9.1)	0.478
DIEP, n (%)	9 (12.5)	7 (11.5)	–	2 (18.2)	0.528
Radial artery flap, n (%)	6 (8.3)	6 (9.8)	–	–	–
Other, n (%)	16 (22.2)	13 (21.3)	1 (25)	3 (27.3)	0.413
Indication for surgery					
Trauma, n (%)	8 (11.1)	6 (10.1)	–	1 (9.1)	0.918
Oncology, n (%)	40 (55.6)	32 (58.2)	3 (75.0)	6 (54.5)	0.469
Defect, n (%)	19 (26.4)	11 (20.0)	1 (25.0)	4 (36.4)	0.511
Infection, n (%)	5 (6.9)	5 (8.2)	–	–	–
Comorbidities					
Coronary artery disease, n (%)	4 (5.6)	3 (4.9)	1 (25.0)	1 (9.1)	0.059
Diabetes mellitus, n (%)	5 (6.9)	4 (6.6)	–	1 (9.1)	0.691
Hypertension, n (%)	28 (38.8)	19 (31.1)	3 (75.0)	6 (54.5)	0.133
Dyslipidaemia, n (%)	16 (22.2)	13 (21.3)	1 (25.0)	3 (27.3)	0.624
Smoking history, n (%)	13 (18.1)	11 (18.0)	1 (25.0)	2 (18.2)	0.249
Obesity (BMI > 30 kg/m2), n (%)	12 (16.6)	8 (13.1)	2 (50.0)	5 (45.5)	0.010*
Cerebrovascular accident, n (%)	4 (5.6)	4 (6.6)	–	–	0.620

* Data is presented as mean (CI95) or count (percentage); BMI – Body mass index; ENT – Ear nose and throat surgery; DIEP – Deep Inferior Epigastric Artery Perforator flap; ALT – Anterolateral thigh flap.

2.2.2 Assignment of CONUT score

The serum albumin concentration, total peripheral lymphocyte count, and serum total cholesterol concentration were used to assign the CONUT score. As seen in Table 2.2, the CONUT score was determined by assigning laboratory values according to the tool first used by Ignacio de Ulíbarri and co-authors (Ulibarri et al., 2005). The CONUT score tool is summarised in Table 2.2.

Table 2.2

Evaluation of CONUT score tool as first described by Ignacio de Ulibarri and co-authors

Variable	Undernutrition degree			
	Normal	**Mild**	**Moderate**	**Severe**
Serum albumin (g/dL)	≥ 3.50	3.00–3.49	2.50–2.99	< 2.50
Score	0	2	4	6
Total lymphocyte count (count/mm3)	≥ 1600	1200–1599	800–1199	< 800
Score	0	1	2	3
Total cholesterol (mg/dL)	≥ 180	140–179	100–139	< 100
Score	0	1	2	3

2.3 Second prospective study of the second part. Evaluating FAR for predicting microvascular flap complications in reconstructive surgery

The study is described in the manuscript "Rocans, R. P., Zarins J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Vanags, I., Mamaja, B. (2025) Fibrinogen-to-albumin ratio (FAR) for predicting microvascular flap complications in reconstructive surgery, JPRAS Open, 44, 414–423, https://doi.org/10.1016/j.jpra.2025.03.022.".

2.3.1 Patient characteristics

This prospective cohort study included 130 patients from 1 October 2021 to 31 January 2024. The exclusion criteria were liver failure, kidney failure, disseminated tumor metastasis; preoperative neoadjuvant treatment (radiotherapy and chemotherapy); multiple concomitant malignancies; sepsis or severe systemic bacterial infection; autoimmune disorders; blood-borne viral infections (Hepatitis B, Hepatitis C, and HIV); pregnant patients and lactating women; or patients with congenital coagulation disorder. The patient characteristics data are summarised in Table 2.3.

Table 2.3

Patient characteristics

Patient group	Overall n = 130	No complications n = 107	True flap loss n = 7	Any flap complications n = 23	p-value
Demographical data					
Mean age, years	56.5 (53.9–59.1)	57.1 (54.4–59.8)	50.1 (45.9–55.5)	50.6 (47.7–53.1)	0.123
Women, n (%)	61 (46.9)	54 (50.5)	2 (28.6)	8 (34.7)	0.109
Area of reconstruction					
Extremity, n (%)	28 (21.5)	20 (18.7)	2 (28.6)	8 (34.8)	0.217
ENT, n (%)	59 (45.4)	50 (46.7)	3 (42.9)	9 (39.1)	0.795
Head and Neck, n (%)	24 (18.5)	21 (19.6)	1 (14.3)	3 (13.0)	0.731
Breast, n (%)	19 (14.6)	16 (15.0)	1 (14.3)	3 (13.0)	0.972
Microvascular flap type					
ALT, (%)	61 (46.9)	55 (51.4)	2 (28.6)	6 (26.1)	0.055
Fibular flap, (%)	12 (9.2)	8 (7.5)	2 (28.6)	4 (17.4)	0.093
DIEP, n (%)	15 (11.5)	13 (12.2)	–	2 (8.7)	0.639
Radial artery flap, n (%)	9 (6.9)	9 (8.4)	–	–	–
Other, n (%)	33 (25.4)	22 (20.6)	3 (42.9)	11 (47.8)	0.015*
Indication for surgery					
Trauma, n (%)	10 (7.7)	8 (7.5)	–	2 (8.7)	0.843
Oncology, n (%)	84 (64.6)	72 (67.3)	4 (57.1)	12 (52.2)	0.360
Defect, n (%)	23 (17.7)	18 (16.8)	3 (42.9)	5 (21.7)	0.220
Infection, n (%)	13 (10.0)	9 (8.4)	–	4 (17.4)	0.195
Comorbidities					
Coronary artery disease, n (%)	9 (6.9)	7 (6.5)	1 (14.3)	2 (8.7)	0.872
Diabetes mellitus, n (%)	8 (6.2)	6 (5.6)	–	2 (8.7)	0.578
Hypertension, n (%)	35 (26.9)	27 (25.2)	3 (42.8)	8 (34.7)	0.427
Dyslipidaemia, n (%)	20 (15.4)	14 (13.1)	1 (14.3)	6 (26.1)	0.624
Smoking history, n (%)	18 (13.8.1)	14 (13.1)	1 (14.3)	4 (17.4)	0.863
Obesity (BMI >30 kg/m2) n (%)	15 (11.5)	9 (8.4)	2 (28.6)	6 (26.1)	0.028*
Cerebrovascular accident, n (%)	6 (4.6)	6 (5.6)	–	–	–

* Data is presented as mean (CI95) or count (percentage); Abbreviations – BMI (Body mass index); ENT (Ear nose and throat surgery); DIEP (Deep Inferior Epigastric Artery Perforator flap); ALT (Anterolateral thigh flap).

2.3.2 Assignment of FAR

FAR was defined as the proportion of the plasma fibrinogen (g/l) to the plasma albumin level (g/l).

2.4 Third prospective study of the second part. vWF:Ag, biomarkers of inflammation, and microvascular flap thrombosis in reconstructive surgery

The study is described in the manuscript "Rocans, R. P., Zarins, J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Vanags, I., Gravelsina, S., Vilmane, A., Rasa-Dzelzkaleja, S., & Mamaja, B. (2024). Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery. *Journal of Clinical Medicine*, 13(18), 5411. https://doi.org/10.3390/jcm13185411".

2.4.1 Patient characteristics

This study included 88 patients undergoing elective microvascular flap transplantation surgery from the 1 October 2021 to the 31 March 2024. The study excluded patients with severe chronic liver or kidney diseases, with cardiovascular and autoimmune diseases, patients with pre-existing coagulopathies or any clotting and bleeding disorders, patients with inherited or acquired von Willebrand disease, patients receiving hormonal contraception or oestrogen therapy, patients after recent thrombotic or thromboembolic events, patients currently taking anticoagulants or antiplatelet agents, patients with active systemic infections or inflammatory conditions, pregnant patients and patients during the lactation period. The patients' data is summarised in Table 2.4.

Table 2.4

Patient characteristics

Patient group	Overall n = 88	No complications n = 64	Any flap complications n = 24	p-value
Demographic data				
Mean age, years	57.9 (54.8–61.0)	57.1 (54.4–59.8)	50.6 (47.7–53.1)	0.204
Women, n (%)	45 (51.1)	31 (48.4)	10 (41.67)	0.271
Location				
Extremity, n (%)	18 (20.5)	12 (18.8)	6 (25.0)	0.603
ENT, n (%)	40 (45.5)	30 (46.9)	10 (41.7)	0.787
Head and Neck, n (%)	17 (19.3)	12 (18.8)	5 (20.8)	0.857
Breast, n (%)	13 (14.8)	10 (15.6)	3 (12.5)	0.750
Flap type				
ALT, (%)	40 (45.5)	33 (51.6)	7 (29.2)	0.232
Fibular flap, (%)	9 (10.2)	5 (7.8)	4 (16.7)	0.279
DIEP, n (%)	10 (11.4)	8 (12.5)	2 (8.3)	0.622
Radial artery flap, n (%)	7 (8.0)	5 (7.8)	2 (8.3)	0.941
Other, n (%)	22 (25.0)	13 (20.3)	9 (37.5)	0.212
Indication				
Trauma, n (%)	9 (10.2)	7 (10.9)	2 (8.3)	0.106
Oncology, n (%)	55 (62.5)	42 (47.7)	13 (54.2)	0.629
Defect, n (%)	16 (18.2)	10 (11.4)	6 (25.0)	0.406

***** Data is presented as mean (CI95) or count (percentage); ENT – Ear nose and throat surgery; DIEP – Deep Inferior Epigastric Artery Perforator flap; ALT – Anterolateral thigh flap.

Additional definitions were used: trauma patients who underwent surgery within 30 days from injury were defined as early surgery, and patients who underwent surgery later than 30 days from injury were defined as late surgery. The patient's ABO blood group was determined from medical records reviewed during preoperative evaluation.

2.4.2 vWF:Ag ELISA analysis

All blood samples for vWF:Ag analysis were stored within 6 h from blood draw. Prior to storage, blood samples for vWF:Ag analysis (collected in citrate tubes) were spun at 3500×/g for 10 min. Plasma samples were then stored at −80 °C until analysis. vWF:Ag analysis was

performed after a single thaw using the human von Willebrand factor ELISA kit according to the manufacturer's protocol from Abcam (Cambridge, United Kingdom). All reagents, working standards, and samples were prepared as directed in the product protocol datasheet. The assay was performed at room temperature (20–25 °C) according to the manufacturer's protocol. The absorbance was read on a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 450 nm immediately after the stop solution was added. The obtained readings were grouped for further statistical analysis.

2.5 Fourth prospective study of the second part. Early postoperative increase in TGF-β1 predicts microvascular flap loss in reconstructive surgery

The study is described in the manuscript "Rocans, R. P., Zarins, J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Gravelsina, S., Vilmane, A., Rasa-Dzelzkaleja, S., Sabelnikovs, O., & Mamaja, B. (2025). Early Postoperative Increase in Transforming Growth Factor Beta-1 Predicts Microvascular Flap Loss in Reconstructive Surgery: A Prospective Cohort Study. *Medicina*, 61(5), 863. https://doi.org/10.3390/medicina61050863".

2.5.1 Patient characteristics

This prospective observational cohort study included 173 individuals who underwent elective reconstructive microvascular flap surgery from 1 October 2022 to 31 March 2024. All patients who had true flap loss or secondary flap complications (n = 22) were included in the complications group. To ensure optimal internal validity and comparability between cases over the study period, simple randomisation was used to select an equal number of patients without complications (n = 22) from the overall cohort (Dettori et al., 2019; Mann, 2012). This created a 44-patient cohort to match the available sample count for laboratory analysis.

To avoid confounding factors, patients with coagulation abnormalities, history of smoking, recent blood clotting, or thromboembolic complications, along with patients taking hormonal contraceptives or oestrogen therapy, were excluded, as these patient groups have been associated with flap thrombosis. Patients on anticoagulants or antiplatelet medications were excluded from the study to avoid confounders related to flap bleeding. Patients with active systemic infections, or autoimmune disorders were excluded, as these patient groups may have abnormal TGF-β1 levels (Deng et al., 2024). Pregnant patients, lactating patients were also excluded. The patient characteristics data is summarised in Table 2.5.

Table 2.5

Patient characteristics

Patient group	Overall n = 44	Complication group n = 22	Control group n = 22	p-value
Demographic data				
Mean age, years	57.9 (54.8–61.0)	59.5 (53.5–65.4)	54.4 (46.7–62.1)	0.389
Women, n (%)	20 (45.5)	10 (45.5)	10 (45.5)	–
Location of Reconstruction				
Extremity, n (%)	6 (13.6)	4 (18.2)	2 (9.1)	0.248
ENT, n (%)	26 (59.1)	14 (63.6)	12 (54.5)	0.539
Head and Neck, n (%)	6 (13.6)	2 (9.1)	4 (18.2)	0.248
Breast, n (%)	6 (13.6)	2 (9.1)	4 (18.2)	0.248
Flap type				
ALT, (%)	25 (56.8)	12 (54.5)	13 (59.1)	0.773
Fibular flap, (%)	5 (11.4)	3 (13.6)	2 (9.1)	0.635
DIEP, n (%)	5 (11.4)	1 (4.5)	4 (18.2)	0.154
Other, n (%)	9 (20.5)	6 (27.3)	3 (13.6)	0.262
Indication				
Trauma, n (%)	5 (11.4)	2 (9.1)	3 (13.6)	0.635
Oncology, n (%)	32 (72.7)	14 (63.6)	18 (81.8)	0.517
Defect, n (%)	7 (15.9)	6 (27.3)	1 (4.5)	0.099

* Data is presented as mean (CI95) or count (percentage); ENT – Ear nose and throat surgery; DIEP – Deep Inferior Epigastric Artery Perforator flap; ALT – Anterolateral thigh flap.

2.5.2 TGF-β1 ELISA analysis

All blood samples designated for TGF-β1 evaluation were frozen within 6 h after collection. Before storage, blood samples for TGF-β1 analysis were centrifuged at 3500× g for 10 min. All samples were centrifuged within 2 h after collection. Sample handling was conducted with meticulous care, and light or heat exposure was strictly avoided. The serum samples were preserved at a consistent temperature of −80 °C in screw-cap tubes appropriate for long-term storage. TGF-β1 evaluation was conducted following a single thaw cycle with the TGF-β1 ELISA kit, according to the manufacturer's protocol (Merck, Darmstadt, Germany). All reagents, calibration standards, and samples were prepared following the instructions outlined in the product's protocol guide. The assay was conducted at ambient temperature (20–25 °C), in accordance with the manufacturer's protocol. The absorbance reading was performed on a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm, immediately after the stop solution was added. The acquired measurements were collected for subsequent statistical analysis.

3 Statistical analysis

SPSS Statistics for Windows, Version 26.0. (IBM Corp. Armonk, NY, USA) was used to perform statistical analysis for the second study of the prospective part. GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA) and SPSS Statistics for Windows, Version 26.0. (IBM Corp. Armonk, NY, USA) were used to perform statistical analysis for the third, fourth and fifth study of the prospective part. GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA) was used to create graphic visualisations for the third, fourth and fifth study of the prospective part. The distribution of all variables was assessed for normality through visual examination of the Quantile-Quantile plot. Data conformity to a normal distribution was also evaluated using the Kolmogorov-Smirnov test. Analysis of nominal variable datasets was performed using the Chi-square test. Pearson correlation was applied to assess the relationship between parametric datasets. Spearman's Rho was used to evaluate non-parametric correlations. Variations in data distribution between the groups were analysed using the Mann-Whitney U test for non-parametric data. Independent t-test was used to compare the means of two independent groups conforming to normal distribution. Continuous variables following a normal distribution were presented as the mean with a 95 % confidence interval (CI95). Non-parametric variables were presented as the median and interquartile ratio (IQR). Statistical significance was defined as a two-tailed p-value less than 0.05.

3.1 First prospective study of the second part

Youden's Index (YI) and the Concordance Probability Method (CZ) were used for defining optimal CONUT cut-off values (Hassanzad et al., 2024; Unal, 2017). Binary logistic regression models were used to obtain odds ratios at specific CONUT cut-off points. The receiver operator curve (ROC) and area under curve (AUC) were used for evaluating the diagnostic ability of the binary classifier system.

3.2 Second prospective study of the second part

The patients were grouped in smaller cohorts according to their FAR level. The patients in each complication group were divided in quartiles according to their FAR levels (FAR < 0.6; FAR 0.6–0.8; FAR 0.8–1.0; FAR > 1.0). Binary logistic regression models were used to obtain odds ratios of complications with the three selected FAR cut-off levels. The models were adjusted for lymphocyte count and the presence of other flap types.

3.3 Third prospective study of the second part

Data sets for IL-6, CRP, fibrinogen, and neutrophil-to-lymphocyte ratio (NLR) were divided into four quartiles. The data distribution differences in vWF:Ag were further compared between quartiles using the Kruskal–Wallis H test. After obtaining the results of ROC analysis, YI and CZ were used to define optimal cut-off values for vWF:Ag concentration (Hassanzad et al., 2024; Unal, 2017). Binary logistic regression models were used to obtain odds ratios for specific variables. The models were adjusted for fibrinogen and the presence of other flap types.

3.4 Fourth prospective study of the second part

The datasets for CRP, fibrinogen, haemoglobin, and haematocrit were divided into quartiles. The interquartile differences in mean TGF-β1 concentrations were further compared using the analysis of variance (ANOVA) test. TGF-β1 concentrations were log transformed to reduce the effects of data skewness and permit the use of parametric tests (Olivier et al., 2008). ANOVA test comparisons were also performed for postoperative changes in the log10 transformations of TGF-β1 in different surgical outcome groups. Diagnostic performance was evaluated based on the ROC curve and the AUC of the log10-transformed postoperative TGF-β1 change. Cut-off values were determined using the CZ method (Hassanzad et al., 2024; Unal, 2017). Odds ratios (OR) for flap complications were calculated using binary logistic regression. The models were adjusted for age, sex, and preoperative plasma fibrinogen.

4 Results

4.1 First prospective study of the second part. CONUT score for predicting microvascular flap complications in reconstructive surgery

A total of 72 patients were included in the study, consisting of 40 men (55.6 %) and 32 women (44.4 %). The mean age was 55.3 years (95 % CI95 51.5–59.1). The overall complication rate was 15.2 % (n = 11). True flap loss with vascular compromise occurred in 5.6 % (n = 4), with two of these cases being late flap loss (> 72 h). In both instances of early true flap loss, urgent revision of the anastomosis was performed. The two cases of late flap loss were managed with repeated elective microvascular flap transplantation. Additional flap-related complications were observed in seven cases, including difficult healing or partial flap loss in 5.6 % of patients (n = 4), one case of flap infection, and two cases involving haematoma formation. The median number of revisions in patients with true flap loss was 1.5 (IQR 1). The median number of revisions in patients with other flap complications was 1 (IQR 0.75, p = 0.223).

There were no significant differences in age or gender distribution in patients with any flap complications or flap loss, and in patients without complications. No significant differences in true flap failure or other flap complications were found between different areas of reconstruction and different anatomical flap types. No significant differences in true flap failure or other flap complications were found between different indications for reconstruction. Of the included comorbidities, obesity was found to be more common in patients with any flap complications (p = 0.01). Only two patients had a BMI < 20 kg/m2, and there was no statistically significant link between decreased BMI and any flap complications. No statistically significant link was found between BMI and CONUT score. No significant differences in the rates of true flap failure or other flap complications were found in patients with other comorbidities. Flap complication rates by comorbidity type are summarised in Table 4.1.

Table 4.1

Flap Complication Rates by Comorbidity Type

Patient group	Overall n = 72	No complications n = 61	True flap loss n = 4	Any flap complications n = 11	p-value
Coronary artery disease, n (%)	4 (5.6)	3 (4.9)	1 (25.0)	1 (9.1)	0.059
Diabetes mellitus, n (%)	5 (6.9)	4 (6.6)	–	1 (9.1)	0.691
Hypertension, n (%)	28 (38.8)	19 (31.1)	3 (75.0)	6 (54.5)	0.133
Dyslipidaemia, n (%)	16 (22.2)	13 (21.3)	1 (25.0)	3 (27.3)	0.624
Smoking history, n (%)	13 (18.1)	11 (18.0)	1 (25.0)	2 (18.2)	0.249
Obesity (BMI > 30 kg/m2), n (%)	12 (16.6)	8 (13.1)	2 (50.0)	5 (45.5)	0.010*
Cerebrovascular accident, n (%)	4 (5.6)	4 (6.6)	–	–	0.620

* Data is presented as count (percentage); BMI – Body mass index.

No significant links were found between the duration of surgery and anaesthesia factors and any flap complications. A higher intraoperative haematocrit was associated with flap complications, with the highest intraoperative haematocrit found in cases with subsequent true flap loss ($p = 0.009$). Only one patient received intraoperative haemotransfusion, and five patients received haemotransfusion in the early postoperative period. There was no significant link between the presence of haemotransfusions and any flap complications. Intraoperative and anaesthesia considerations for the patient cohorts are summarised in Table 4.2.

Table 4.2

Intraoperative and anaesthesia considerations

Patient group	Overall n = 72	No complications n = 61	True flap loss n = 4	Any flap complications n = 11	p-value
Surgery duration, hours	6.39 (5.75–7.02)	6.33 (5.59–7.07)	7.63 (5.86–9.39)	6.66 (5.29–8.04)	0.235
Intraoperative crystalloid volume, ml	2345.83 (2141.39–2550.28)	2352.50 (2133.31–2571.69)	2875.00 (1681.58–4068.42)	2312.50 (1608.14–3016.86)	0.145
Intraoperative colloid volume, ml	506.25 (401.74–610.76)	482.50 (367.10–597.90)	500.00 (–)	625.00 (329.42–920.58)	0.471
Intraoperative colloid-to-crystalloid ratio	0.22 (0.17–0.27)	0.20 (0.15–0.25)	0.18 (0.10–0.27)	0.33 (0.09–0.56)	0.306
Intraoperative haematocrit, %	30.60 (29.20–32.00)	29.58 (27.70–31.45)	31.50 (25.15–37.85)	34.40 (30.32–38.48)	0.009*
Vasopressors/ sympathomimetics used, n (%)	41 (56.90)	36 (59.00)	2 (50.00)	6 (54.50)	0.549

* Data is presented as mean (CI95) or count (percentage).

Patients with any flap complications had a significantly lower plasma lymphocyte count ($p = 0.001$). Multivariate regression analysis revealed that an increase in lymphocyte count decreases the incidence of all complications (OR 0.998 CI95 0.996–0.999). Patients with any flap complications had a significantly lower plasma monocyte count ($p = 0.021$). No differences in plasma lymphocyte/monocyte ratio, plasma albumin, and total plasma cholesterol were found in patients with any flap complications. Results for biomarkers and nutritional systems for predicting flap complications are summarised in Table 4.3.

Table 4.3

Biomarkers and nutritional systems for predicting any flap complications

Patient group	Overall n = 72	No complications n = 61	Any flap complications n = 11	p-value
Biomarkers				
Lymphocyte count 10^9/L	1.59 (1.39–1.79)	1.71 (1.49–1.92)	0.97 (0.67–1.26)	0.001*
Monocyte count 10^9/L	0.55 (0.48–0.62)	0.58 (0.51–0.66)	0.37 (0.22–0.51)	0.021*
Lymphocyte/monocyte ratio	3.46 (2.91–4.02)	3.55 (2.90–4.20)	2.97 (2.28–3.65)	0.830
Mean plasma albumin, g/dl	3.94 (3.81–4.06)	3.96 (3.84–4.09)	3.79 (3.28–4.30)	0.631
Mean total plasma cholesterol, mg/dl	196.58 (185.21–207.95)	198.44 (186.43–210.45)	186.73 (147.93–225.53)	0.310
Nutritional assessment systems				
CONUT score (%)	2(2)	2 (3)	3 (6)	0.013*
CONUT score ≤ 2 (%)	50 (69.4)	46 (75.4)	4 (36.4)	0.009*

* Data is presented as mean (CI95) or count (percentage); CONUT – Controlling nutritional status.

The analysis on the predictive accuracy of CONUT score of other surgical complications found that CONUT score had an AUC of 0.813 (0.659–0.967, p = 0.012) (Figure 4.1).

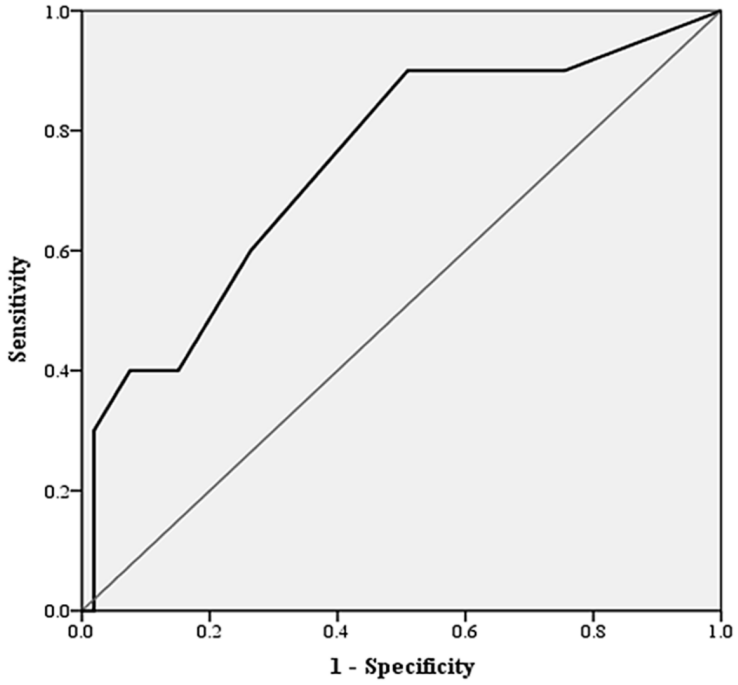

Figure 4.1. **ROC curve characteristics of CONUT score for predicting complications in microvascular flap surgery**

A CONUT score of > 2 was found to be optimal during cut-off analysis (sensitivity 21.1 %, specificity 95.6 %, positive predictive value 66.7 %, negative predictive value 74.1 %, p = 0.022). The CONUT score of > 2 increases the odds of other flap complications (OR 5.4, CI95 1.38–20.90, p = 0.015). Univariate regression revealed that any increase in CONUT score increased the odds of other flap complications (OR 1.43, 1.09–1.85). Patients with any flap

complications had a longer duration of hospitalisation (13.55, 10.99–16.11 vs. 25.38, 14.82–35.93; p = 0.004). There was no difference in duration of ICU stay between patients with flap complications and patients with no flap complications (1.13, 0.03–2.26 vs. 1.50, 1.00–2.00, p = 0.471).

4.2 Second prospective study of the second part. Evaluating FAR for predicting microvascular flap complications in reconstructive surgery

In total, 130 patients were included in the study, consisting of 69 men (53.1 %) and 61 women (46.9 %). The mean age was 56.5 years (CI95 53.9–59.1). The overall complication rate was 17.7 % (n = 23). True flap loss with vascular compromise occurred in 5.4 % (n = 7) with 3 of these cases being late flap loss (> 72 h). All 4 cases of early true flap loss underwent urgent anastomosis revision. Two cases of late flap loss underwent repeated microvascular flap transplantation, and 1 case underwent necrectomy and reconstruction with a regional flap. Other flap complications occurred in 16 cases (12.3 %), with flap haematoma occurring in 5 cases.

No significant differences in age or gender distribution were noted in patients with any flap complications and in patients without complications. No significant differences in the rates of true flap failure or any flap complications were found between different areas of reconstruction. Multiple logistic regression analysis revealed that using other flap types increased the odds of any flap complications (OR 5.23, 1.86–14.70, p = 0.002). No significant differences in true flap failure or any flap complications were found between different indications for reconstruction. Among the included comorbidities, obesity was found to be common in patients with any flap complications (p = 0.028). Results on comorbidities and intraoperative considerations are summarised in Table 4.4.

Table 4.4

Comorbidities and Intraoperative Considerations

Patient group	Overall n = 130	No complications n = 107	True flap loss n = 7	Any flap complications n = 23	p-value
Comorbidities					
Coronary artery disease, n (%)	9 (6.9)	7 (6.5)	1 (14.3)	2 (8.7)	0.872
Diabetes mellitus, n (%)	8 (6.2)	6 (5.6)	–	2 (8.7)	0.578
Hypertension, n (%)	35 (26.9)	27 (25.2)	3 (42.8)	8 (34.7)	0.427
Dyslipidaemia, n (%)	20 (15.4)	14 (13.1)	1 (14.3)	6 (26.1)	0.624
Smoking history, n (%)	18 (13.8.1)	14 (13.1)	1 (14.3)	4 (17.4)	0.863
Obesity (BMI >30 kg/m2), n (%)	15 (11.5)	9 (8.4)	2 (28.6)	6 (26.1)	0.028*
Cerebrovascular accident, n (%)	6 (4.6)	6 (5.6)	–	–	–

Table 4.4 continued

Patient group	Overall n = 130	No complications n = 107	True flap loss n = 7	Any flap complications n = 23	p-value
Anaesthesia and Surgical factors					
Surgery duration, hours	6.25 (5.74–6.76)	6.35 (5.76–6.94)	7.25 (6.11–8.39)	5.79 (4.67–6.91)	0.324
Intraoperative crystalloid volume, ml	2346.97 (2190.41–2450.00)	2340.74 (2167.75–2513.74)	2875.00 (1681.58–4068.42)	2375.00 (1943.96–2086.04)	0.845
Intraoperative colloid volume, ml	507.58 (426.18–588.77)	481.48 (390.71–572.25)	500.00 (–)	625.00 (427.53–822.46)	0.564
Intraoperative colloid-to-crystalloid ratio	0.22 (0.18–0.26)	0.20 (0.16–0.24)	0.22 (0.12–0.32)	0.30 (0.15–0.44)	0.364
Intraoperative haematocrit, %	30.94 (29.69–32.20)	31.02 (29.66–32.38)	33.00 (29.33–36.67)	30.55 (26.58–34.538)	0.032*
Vasopressors/ sympathomi-metics used, n (%)	67 (51.54)	51 (47.66)	5 (71.43)	16 (69.56)	0.095

* Data is presented as mean (CI95) or count (percentage); Abbreviations – BMI (Body Mass Index).

Higher intraoperative haematocrit was associated with flap complications, with the highest intraoperative haematocrit found in cases with subsequent true flap loss ($p = 0.032$; Table 4.4). No significant links were found between other perioperative factors and the rate of flap complications. Patients with any flap complications had a longer duration of hospitalisation (13.72, 11.79–16.15 vs. 23.83, 16.92–30.75, $p = 0.001$). There was no difference in the duration of ICU stay between patients with any flap complications and patients with no flap complications (1.17, 0.23–2.10 vs. 1.46, 0.96–1.96, $p = 0.537$). FAR was positively correlated with the duration of hospitalisation ($r = 0.422$, $p < 0.001$).

Patients with any flap complications had a significantly lower plasma lymphocyte count ($p = 0.001$) and significantly lower mean plasma albumin ($p = 0.045$). Multiple logistic regression analysis revealed that increased lymphocyte count decreases the odds of any flap complications (OR 0.39, 0.17–0.92, $p = 0.031$). Upon further analysis of the specific complication groups, patients with flap haematoma or flap loss had a lower mean fibrinogen than patients with no complications (2.75, 1.84–3.67 vs. 3.44, 3.25–3.64, $p = 0.014$). Patients with minor flap complications had significantly higher plasma fibrinogen than patients with no flap complications (4.19, 3.41–4.98 vs. 3.44, 3.25–3.64, $p = 0.043$). Patients with minor flap complications had lower mean albumin levels than patients with no complications (33.30, 30.18–36.42 vs. 39.34, 38.12–39.69, $p < 0.001$). No difference in plasma albumin level was found between patients with flap haematoma or flap loss and patients with no complications ($p = 0.394$). Increased FAR was specifically linked to minor flap complications when compared to patients with no flap complications (0.12, 0.09–0.15 vs. 0.09, 0.08–0.10, $p = 0.002$).

Decreased FAR was specifically linked to flap loss or flap haematoma when compared to patients with no flap complications (0.07, 0.04–0.09 vs. 0.09, 0.08–0.10, p = 0.046). Results on the biomarkers for predicting flap complications are summarised in Table 4.5.

Table 4.5

Laboratory values for predicting flap complications

Patient group	Overall n = 130	No complications n = 107	True flap loss n = 7	Any flap complications n = 23	p-value
Leukocyte count 10^9/L	6.31 (5.89–6.73)	6.42 (5.94–6.91)	6.16 (2.64–9.70)	5.77 (4.99–6.55)	0.379
Lymphocyte count 10^9/L	1.65 (1.52–1.77)	1.71 (1.58–1.84)	1.56 (0.43–2.71)	1.36 (1.08–1.63)	0.018*
Red blood cell count 10^9/L	4.08 (3.97–4.19)	4.08 (3.96–4.19)	4.24 (3.35–5.12)	4.10 (3.77–4.43)	0.717
Platelet count 10^9/L	244.97 (230.81–259.14)	244.00 (228.21–259.79)	202.00 (148.94–255.05)	249.52 (214.99–284.04)	0.770
Haemoglobin g/dl	12.31 (11.99–12.64)	12.31 (11.97–12.66)	13.06 (10.44–15.68)	12.30 (11.34–13.27)	0.632
Mean plasma albumin, g/l	38.90 (38.12–39.69)	39.34 (38.54–40.14)	41.16 (34.67–47.65)	36.91 (34.50–39.32)	0.045*
Mean plasma fibrinogen, g/l	3.47 (3.27–3.66)	3.44 (3.25–3.64)	2.66 (1.65–3.68)	3.58 (2.98–4.18)	0.892
Fibrinogen-to-albumin ratio	0.09 (0.08–0.10)	0.09 (0.08–0.10)	0.09 (0.05–0.13)	0.11 (0.09–0.14)	0.016*

* Data is presented as mean (CI95).

Logistic regression analysis revealed that patients with FAR<0.08 and FAR<0.06 had increased odds of flap haematoma or flap loss (OR 3.68, 1.04–13.03, p = 0.044 and 6.01, 1.71–21.08, p = 0.005). Patients with FAR > 0.08 had decreased odds of flap haematoma and flap loss (OR 0.22, 0.08–0.96, p = 0.044). Patients with FAR > 0.10 had increased odds of minor flap complications (OR 5.47, 1.33–22.50, p = 0.019). The results on the association between FAR and flap complication groups at various cut-off levels are summarised in Figure 4.2.

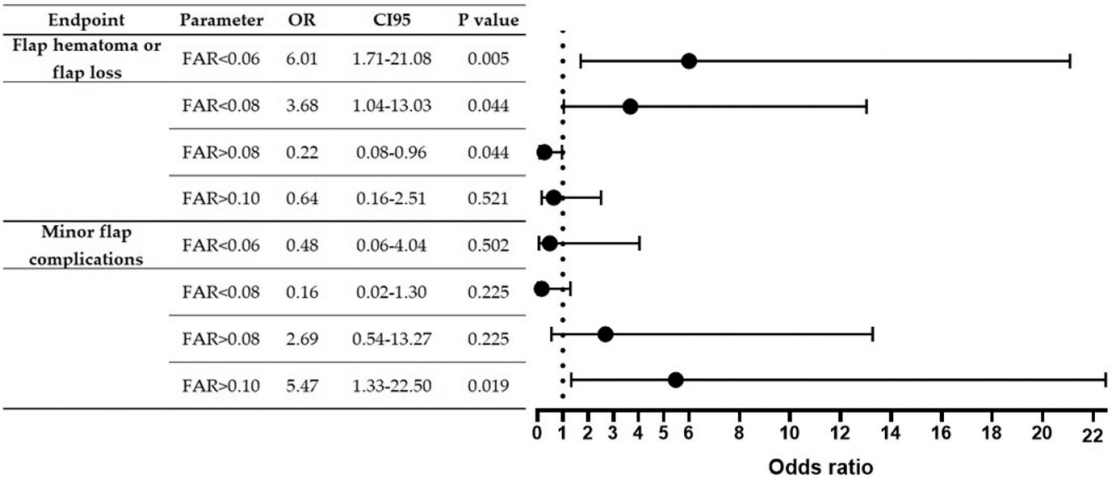

Figure 4.2. **Association between the fibrinogen-to-albumin ratio (FAR) and flap complication groups at various cut-off levels**

After adjustment for lymphocyte count and the presence of other flap types, multiple logistic regression analysis revealed that patients with FAR < 0.06 had increased odds of any flap complications (OR 4.71, 1.27–18.03, p = 0.021). Patients with FAR > 0.10 also had increased odds of any flap complications (OR 3.09, 1.08–8.81, p = 0.035). These results on the U-shaped association between FAR and any flap complications are summarised in Figure 4.3.

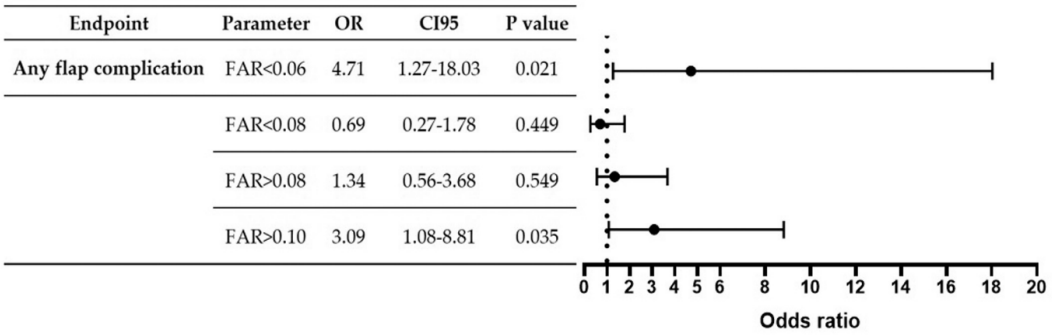

Figure 4.3. **U-shaped association between fibrinogen-to-albumin ratio (FAR) and flap complications**

4.3 Third prospective study of the second part. Von Willebrand factor antigen, biomarkers of inflammation, and microvascular flap thrombosis in reconstructive surgery

In total, 88 patients were included, 43 (48.9 %) men and 45 (51.1 %) women. The mean age was 57.9 years (95 % CI95 54.8–61.0). The overall rate of complication was 27.3 % (n = 24). True flap loss with vascular compromise occurred in 11.4 % (n = 10), with 4 of these cases being late flap loss (> 72 h). Minor flap complications occurred in 10 cases (11.4 %), and flap haematoma occurred in 4 (4.5 %) cases. All the cases of early true flap loss underwent urgent anastomosis revision. Three cases of late flap loss underwent repeated microvascular flap surgery, and one case underwent necrectomy and reconstruction with a rotated local flap. One patient received intraoperative haemotransfusion, while three patients received haemotransfusion during the early postoperative period. There was no significant relationship between the presence of haemotransfusion and vWF:Ag levels. The patient blood type and laboratory values for predicting flap complications are summarised in Table 4.6.

Table 4.6

Patient blood type and laboratory values for predicting flap complications

Patient group	Overall n = 88	No complications n = 64	Any flap complications n = 24	p-value
Blood type				
Blood type, O, n (%)	35 (39.8)	26 (40.6)	9 (37.5)	0.539
Laboratory values				
Leukocyte count 10^9/L	6.24 (5.76–6.73)	6.16 (5.56–6.75)	6.50 (5.62–7.38)	0.309
Lymphocyte count 10^9/L	1.74 (1.53–1.94)	1.83 (1.58–2.09)	1.41 (1.14–1.68)	0.020
Neutrophil count 10^9/L	3.66 (3.23–4.09)	3.54 (3.02–4.07)	4.06 (3.28–4.84)	0.109
NLR	2.57 (2.11–3.02)	2.32 (1.85–2.80)	3.4 (2.16–4.64)	0.006
Monocyte count 10^9/L	0.56 (0.51–0.61)	0.56 (0.50–0.61)	0.56 (0.46–0.66)	0.839
Red blood cell count 10^9/L	4.08 (3.97–4.19)	4.18 (4.03–4.32)	4.27 (4.06–4.49)	0.625
Platelet count 10^9/L	246.75 (229.25–264.24)	232.98 (216.00–249.96)	288.37 (238.35–338.40)	0.021
Haemoglobin g/dL	12.58 (12.22–12.94)	12.45 (12.03–12.87)	12.86 (12.13–13.61)	0.208
Mean total plasma protein g/L	64.72 (63.40–66.05)	64.84 (63.27–66.40)	64.08 (61.32–66.83)	0.543
Mean plasma albumin, g/L	38.90 (38.12–39.69)	39.21 (38.25–40.16)	36.58 (34.33–38.84)	0.049
Mean plasma bilirubin, mg/dl	0.47 (0.42–0.53)	0.48 (0.41–0.55)	0.43 (0.31–0.55)	0.447
Mean albumin-bilirubin score	−2.78 (−2.87– −2.69)	−2.80 (−3.02– −2.56)	−2.77 (−2.87– −2.67)	0.551
CRP, mg/L	7.10 (4.67–9.54)	6.76 (3.67–9.86)	7.93 (4.32–11.54)	0.058
Mean plasma fibrinogen, g/L	3.59 (3.35–3.84)	3.52 (3.24–3.81)	3.82 (3.30–4.35)	0.505
Interleukin-6, pg/mL	13.9 (10.09–16.29)	13.03 (9.33–16.7)	13.73 (7.26–20.20)	0.893
vWF:Ag, IU/dL	129.61 (111.93–147.27)	120.44 (99.43–141.36)	157.59 (123.62–191.61)	0.014

* Data is presented as mean (CI95) or count (percentage); Abbreviations – NLR (Neutrophil to Lymphocyte ratio); CRP (C reactive protein); vWF (von Willebrand factor).

Trauma was the indication for surgery in nine patients, and the mean time from trauma to surgery was 35.8 days. There was no correlation between time from trauma and vWF:Ag concentration ($r = -0.67$, $p = 0.865$). Regarding time from trauma, no significant differences in the rates of any flap complications were found between early surgery and late surgery groups ($p = 0.571$).

The mean duration of surgery was 6.41 (5.77–7.04) hours. There were no significant differences in the duration of surgery between patients with flap complications and patients without complications (6.68, 5.31–8.06 vs. 6.35, 5.61–7.09, $p = 0.135$). Patients with any flap complications had a significantly lower plasma lymphocyte count ($p = 0.020$); a lower mean plasma albumin ($p = 0.049$); a higher platelet count ($p = 0.021$); and a higher vWF:Ag

(p = 0.014). There was no link between albumin–bilirubin score and vWF:Ag concentration (r = 0.89, p = 0.430). There were no statistically significant differences in mean bilirubin concentration and albumin–bilirubin score between patients with flap complications and patients without complications (−2.77, −2.87–−2.67 vs. −2.80, −3.02–−2.56, p = 0.551).

When comparing different surgical indications, trauma had the highest preoperative vWF:Ag, followed by oncology, and patients with defects had the lowest vWF:Ag (138.62, 107.48–150.62 vs. 129.03, 107.52–150.63 vs. 89.92, 68.09–118.14, p = 0.029). As seen in Figure 4.4, vWF:Ag concentrations were positively linked to preoperative plasma fibrinogen (p < 0.001), plasma CRP (p < 0.001), plasma IL-6 (p = 0.032), and NLR (p = 0.019).

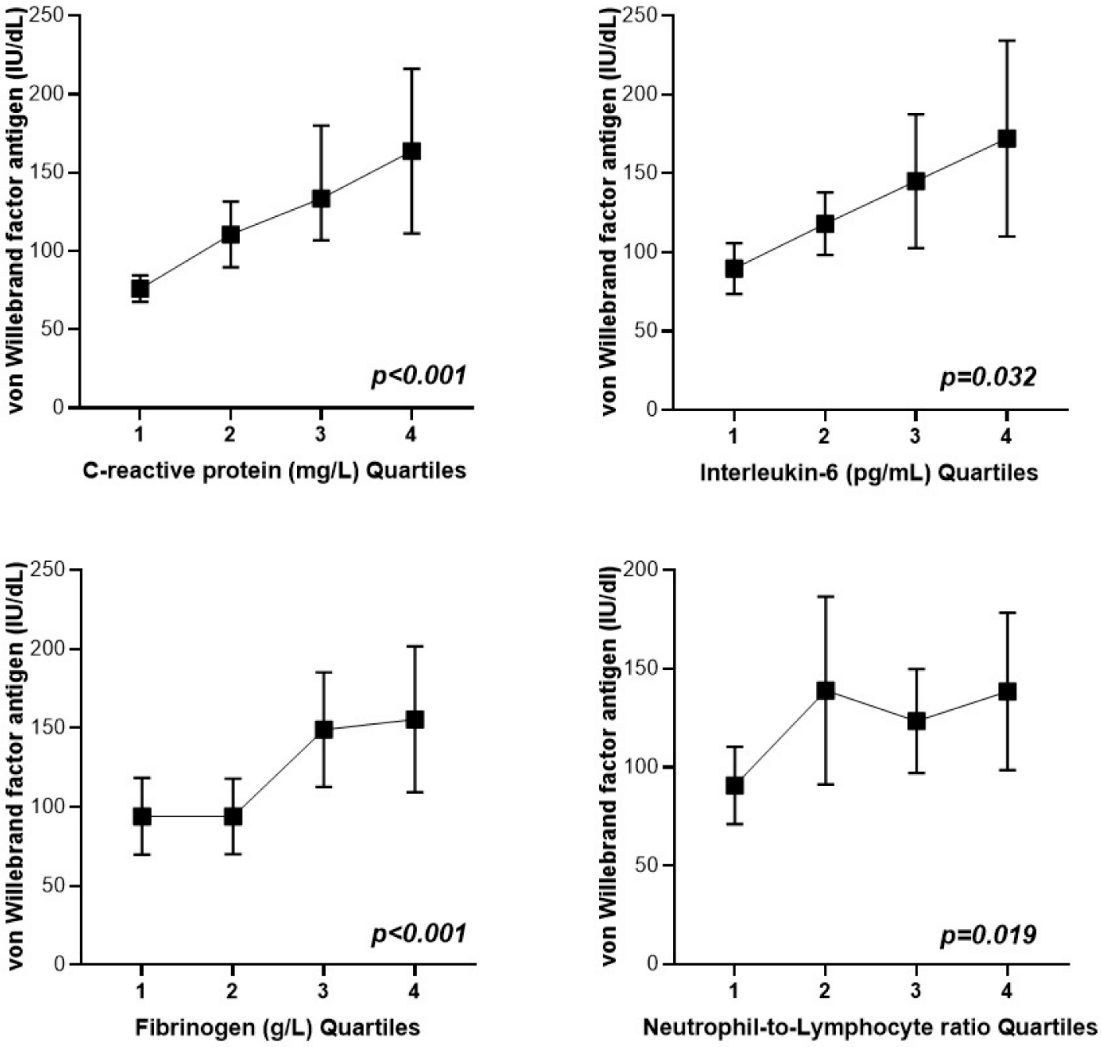

Figure 4.4. **Association between preoperative von Willebrand factor antigen and different preoperative markers of inflammation in microvascular flap surgery patients**

vWF:Ag levels were higher in true flap loss when compared to patients without complications (217.94, 137.27–298.45 vs. 114.14, 95.67–132.71, p = 0.001). Preoperative NLR was the highest in the patients with subsequent secondary flap complications when compared to patients without flap complications (4.36, 2.60–6.13 vs. 2.32, 2.03–2.32, p = 0.024).

Fibrinogen levels were higher in the patients with subsequent true flap loss compared to patients without complications (5.00, 4.29–5.70 vs. 3.46, 3.18–3.73, $p < 0.001$). The association between preoperative VWF:Ag, NLR, fibrinogen, and different flap complication types is summarised in Figure 4.5.

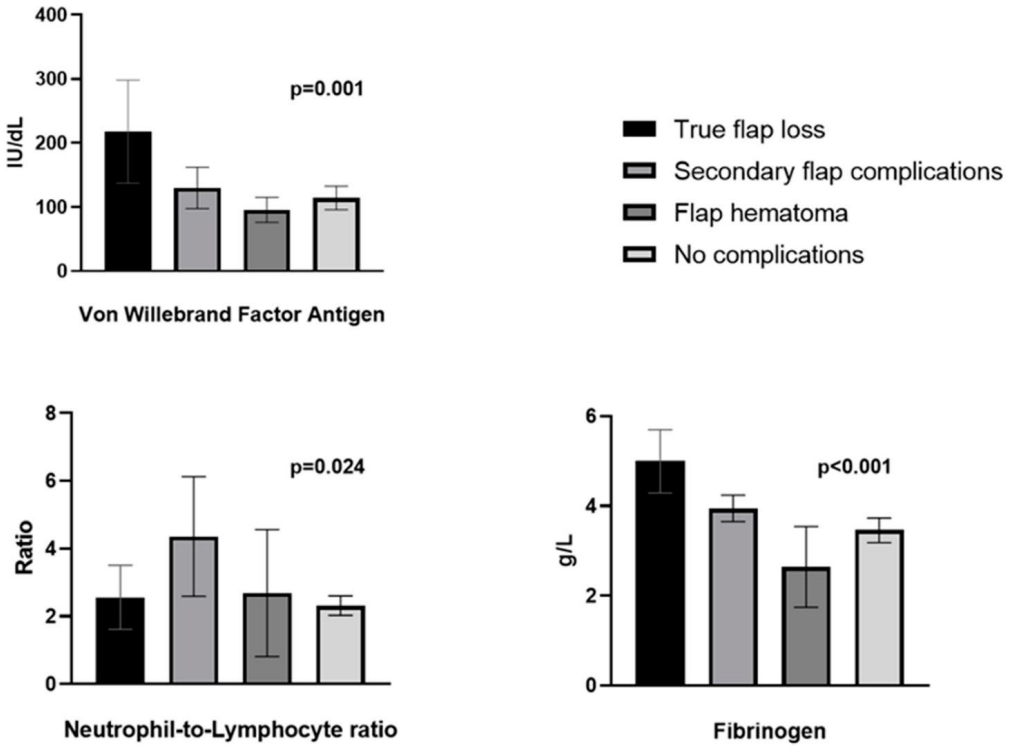

Figure 4.5. **Association between preoperative vWF:Ag, NLR, fibrinogen, and different flap complication types**

After adjustment for fibrinogen and the presence of other types of flaps, logistic regression analysis revealed the association between vWF:Ag and true flap loss at the selected cut-off level of 163.73 IU/dL (OR 70.22, 10.74–485.28, $p = 0.043$).

4.4 Fourth prospective study of the second part. Early postoperative increase in TGF-β1 predicts microvascular flap loss in reconstructive surgery

In total, 44 patients were included: 24 (59.1 %) men and 20 (40.9 %) women. Their mean age was 57.1 years (CI95 52.5–61.7). The complications group consisted of 22 patients, 12 of whom had minor flap complications, while 10 patients had true flap loss. Five of these cases had late flap loss (> 72 h). All patients with early true flap loss underwent urgent and successful anastomosis revision. Four cases of late true flap loss were treated with repeated microvascular flap reconstruction, while one case required necrectomy followed by rotated flap reconstruction. Out of all the analysed laboratory values, increased preoperative plasma fibrinogen was found to be associated with flap complications (4.04, 3.56–4.51 vs. 3.22, 2.69–3.75, $p = 0.044$). The laboratory values for predicting flap complications are summarised in Table 4.7.

Table 4.7

Laboratory values for predicting flap complications

Patient group	Overall n = 44	Complication group n = 22	Control group n = 22	p-value
Intraoperative and anaesthesia considerations				
Surgery duration, hours	6.03 (5.48–6.58)	5.93 (5.20–6.66)	6.18 (5.18–7.18)	0.739
Total intraoperative crystalloids, mL	2460.00 (2421.59–2498.41)	2480.00 (2434.76–2525.24)	2440.00 (2370.89–2509.11)	0.276
Total intraoperative colloids, mL	625.00 (521.04–728.94)	650.00 (477.22–822.78)	600.00 (449.19–750.81)	0.615
Intraoperative haematocrit, %	34.50 (33.30–35.70)	33.75 (31.76–35.74)	36.00 (34.16–37.84)	0.097
Vasopressors/ sympathomimetics used, n (%)	15 (34.1 %)	10 (45.5 %)	5 (22.7 %)	0.112
Laboratory values				
Red blood cell count, 10^9/L	4.13 (3.97–4.28)	4.28 (4.06–4.49)	4.00 (3.77–4.23)	0.084
White blood cell count, 10^9/L	6.36 (5.57–7.17)	6.50 (5.62–7.38)	6.25 (4.91–7.59)	0.418
Lymphocyte count, 10^9/L	1.67 (1.48–1.86)	1.63 (1.31–1.93)	1.71 (1.45–1.96)	0.497
Neutrophil count, 10^9/L	3.90 (3.13–4.67)	4.06 (3.28–4.84)	3.76 (2.44–5.09)	0.162
Monocyte count, 10^9/L	0.56 (0.50–0.63)	0.56 (0.47–0.66)	0.57 (0.47–0.66)	0.958
Platelet count, 10^9/L	258.95 (230.52–287.38)	288.37 (238.33–338.40)	233.55 (202.96–264.14)	0.092
Haemoglobin, g/dL	12.43 (11.90–12.95)	12.87 (12.13–13.61)	12.05 (11.28–12.81)	0.087
Haematocrit, %	38.81 (37.41–40.22)	40.12 (38.22–42.01)	37.69 (35.64–39.74)	0.065
Total plasma protein, g/L	63.79 (61.94–65.94)	64.08 (61.32–66.83)	63.51 (60.79–66.23)	0.794
Plasma albumin, g/L	38.77 (37.58–39.95)	39.00 (37.05–40.96)	38.55 (36.98–40.11)	0.668
CRP, mg/L	8.40 (3.90–12.91)	6.93 (3.25–10.61)	9.87 (1.23–18.51)	0.718
Plasma fibrinogen, g/L	3.61 (3.24–3.98)	4.04 (3.56–4.51)	3.22 (2.69–3.75)	0.044
Interleukin-6, pg/mL	14.62 (10.32–18.92)	11.73 (6.32–17.13)	17.37 (10.51–24.24)	0.262
HDL-C, mmol/l	1.27 (1.16–1.39)	1.17 (1.01–1.32)	1.37 (1.20–1.54)	0.094
LDL-C, mmol/l	2.89 (2.57–3.21)	2.84 (2.49–3.19)	2.93 (2.38–3.49)	0.950
Preoperative TGF-β1, ng/ml	2.64 (2.25–3.03)	2.68 (2.13–3.24)	2.60 (2.01–3.20)	0.771
Postoperative TGF-β1, ng/ml	3.12 (2.71–3.53)	3.48 (2.90–4.06)	2.77 (2.20–3.35)	0.072

* Data is presented as mean (CI95); CRP – C reactive protein; HDL-C – High density lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TGF-β1 – Transforming growth factor beta-1.

When evaluating different surgical indications, patients undergoing surgery for defects exhibited the highest postoperative TGF-β1 concentrations, followed by those treated for oncological reasons, while trauma patients had the lowest preoperative TGF-β1 levels (4.25 ng/mL, 3.51–4.98 vs. 2.99, 2.51–3.48 vs. 2.33, 1.02–3.64, p = 0.023). However, no significant differences were observed in preoperative TGF-β1, postoperative TGF-β1, or the postoperative change in TGF-β1 when comparing different reconstruction sites or flap types.

Postoperative changes in TGF-β1 were positively correlated with preoperative fibrinogen (r = 0.369, p = 0.021) and preoperative CRP (r = 0.333, p = 0.036). Additionally, postoperative TGF-β1 levels showed positive correlations with preoperative haemoglobin (r = 0.328, p = 0.029) and haematocrit (r = 0.341, p = 0.031). No significant associations were observed between preoperative TGF-β1 levels and any of the analysed preoperative biomarkers. The aforementioned correlation analyses are summarised in Figure 4.6.

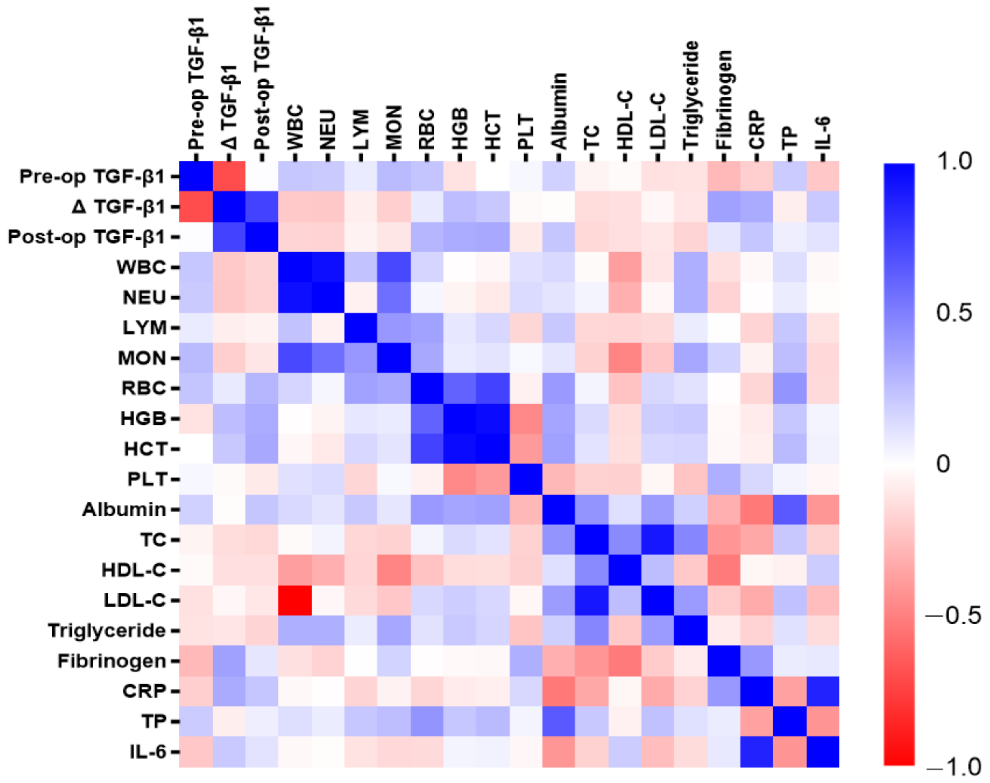

Figure 4.6. **Correlation analysis between transforming growth factor beta-1 and selected biomarkers in microvascular flap surgery patients**

* Pre-op – preoperative; TGF-β1 – transforming growth factor beta-1; Δ TGF-β1 – postoperative change in transforming growth factor beta-1; Post-op – postoperative; WBC – white blood cells; NEU – neutrophils; LYM – lymphocytes; MON – monocytes; RBC – red blood cells; HGB – haemoglobin; HCT – haematocrit; PLT – platelets; TC – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; CRP – C-reactive protein; TP – total protein); IL-6 – interleukin-6.

Postoperative changes in TGF-β1 concentrations were positively associated with preoperative plasma fibrinogen (p = 0.020) and plasma CRP (p = 0.021). Postoperative TGF-β1 concentrations were positively associated with preoperative haemoglobin (p = 0.009)

and haematocrit (p = 0.039). The associations between perioperative TGF-β1 and preoperative biomarkers are summarised in Figure 4.7.

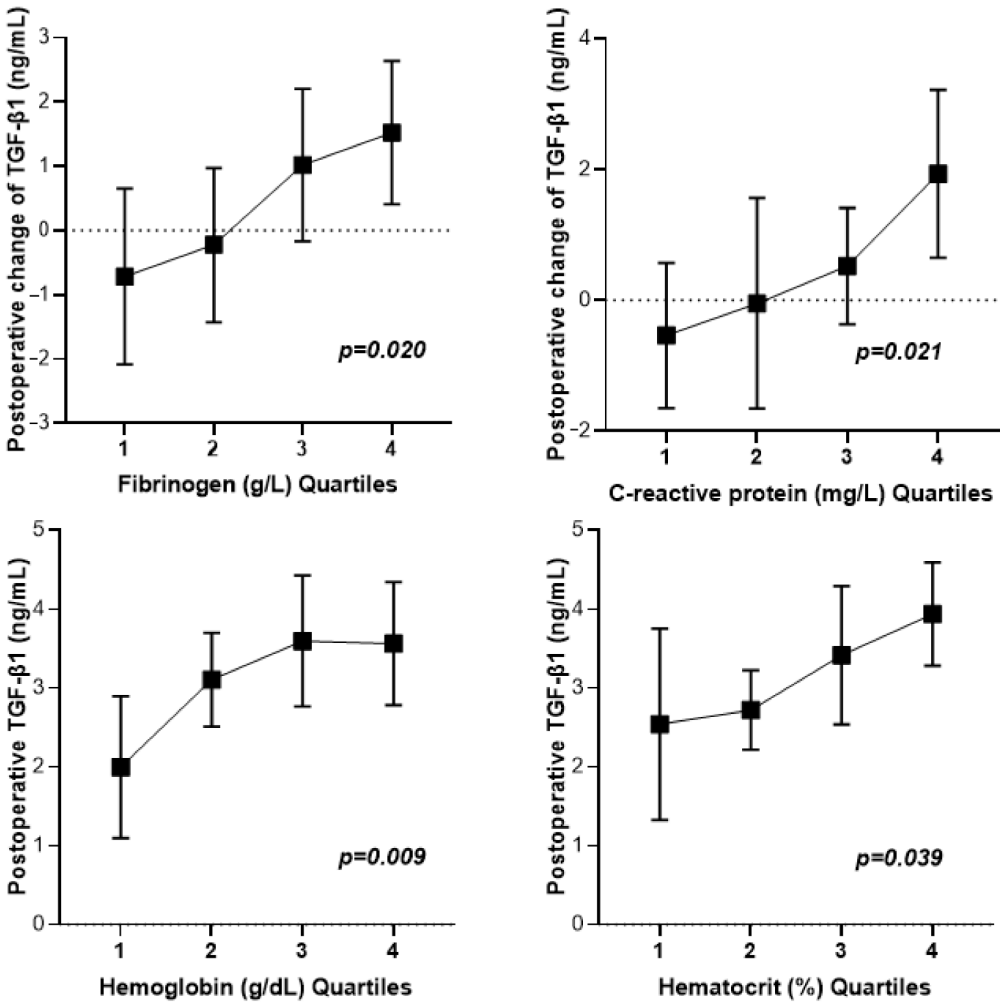

Figure 4.7. **Association between perioperative TGF-β1 and preoperative biomarkers in microvascular flap surgery patients**

*Quartiles of fibrinogen (g/L): Q1 < 2.31; Q2 2.32–3.78; Q3 3.79–4.34; Q4 > 4.35. Quartiles of C-reactive protein (mg/L): Q1 < 1.93; Q2 1.94–3.86; Q3 3.87–14.00; Q4 > 14.01. Quartiles of haemoglobin (g/dL): Q1 < 11.6; Q2 11.7–12.6; Q3 12.7–13.7; Q4 > 13.8. Quartiles of haematocrit (%): Q1 < 36.6; Q2 36.7–39.0; Q3 39.1–42.2; Q4 > 42.3. TGF-β1 – transforming growth factor beta-1. Data are presented as the mean (CI95).

The largest increase in the postoperative log10 of TGF-β1 (ng/mL) was found in cases with true flap loss (0.403, 0.024–0.782), followed by minor flap complications (0.157, 0.029–0.285). Patients without flap complications had the lowest postoperative change in the log10 of TGF-β1 (−0.089, −0.233–0.056, p = 0.002). Analysis of the predictive accuracy of postoperative changes in TGF-β1 for true flap loss found that the AUC for log10 of TGF-β1 was 0.797 (0.588–0.997, p = 0.005). A postoperative change in TGF-β1>1.00 ng/mL was determined to be optimal based on the cut-off analysis (specificity 79.4 %, sensitivity 80.0 %, positive predictive value 53.3 %, negative predictive value 93.1 %). When adjusted for age, sex, and preoperative plasma fibrinogen, multivariate regression analysis revealed that an increase in the postoperative change in TGF-β1 increases the odds of true flap loss

(OR 2.028, CI95 1.185–3.471, p = 0.009). Postoperative changes in log10 of TGF-β1 for different surgical outcomes are illustrated in Figure 4.8.

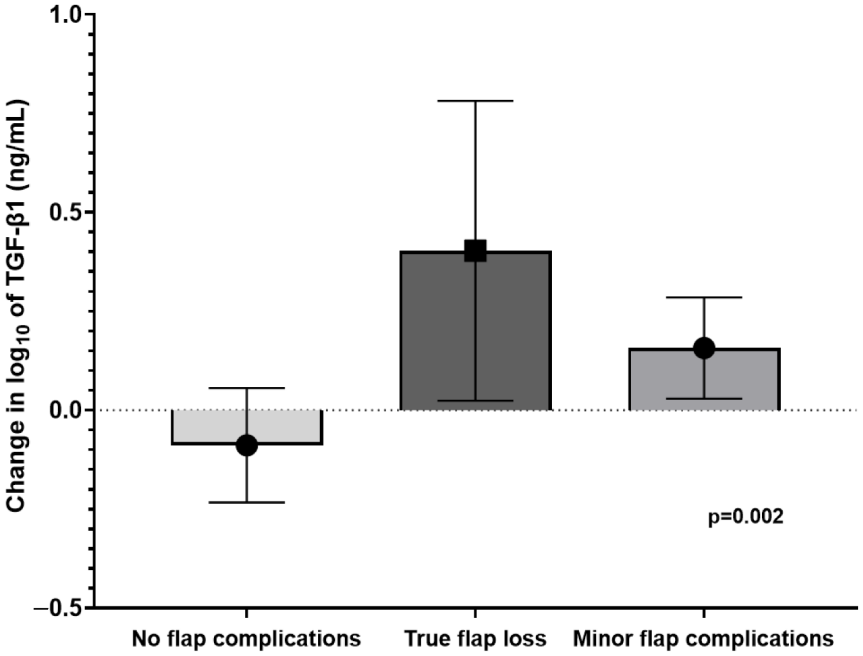

Figure 4.8. **Postoperative changes in log10 of TGF-β1 for different surgical outcomes: ANOVA test comparisons of postoperative changes in log10 of transforming growth factor beta-1 in different surgical outcome groups**

* TGF-β1 – Transforming growth factor beta-1.

5 Discussion

5.1 Challenges and advances in biomarker-based risk assessment and perioperative management in microvascular flap surgery (Narrative literature review)

Overall, our narrative literature review conducted during the initial phase of this research concluded that several perioperative factors are well-supported by previous studies. These include nutritional assessment, correction of preoperative anaemia, postoperative analgesia, and the use of peripheral nerve blocks, all of which are widely integrated into current clinical practice. (Koster et al., 2024; Go et al., 2022; Salibian et al., 2018; Hill et al., 2012). Nonetheless, further research is required to determine their optimal application and to develop standardised protocols. In the preoperative context, topics such as albumin supplementation, chronic inflammatory conditions, and assessments of hypercoagulability remain insufficiently explored and represent promising avenues for future investigation (Chargi et al., 2022; Vanags et al., 2020; Tomita et al., 2020; Drizlionoka et al., 2019). The narrative review outlines key issues across preoperative, intraoperative, and postoperative care, supporting a team-based, multidisciplinary approach to perioperative management in microvascular flap surgery, as seen in Figure 5.1.

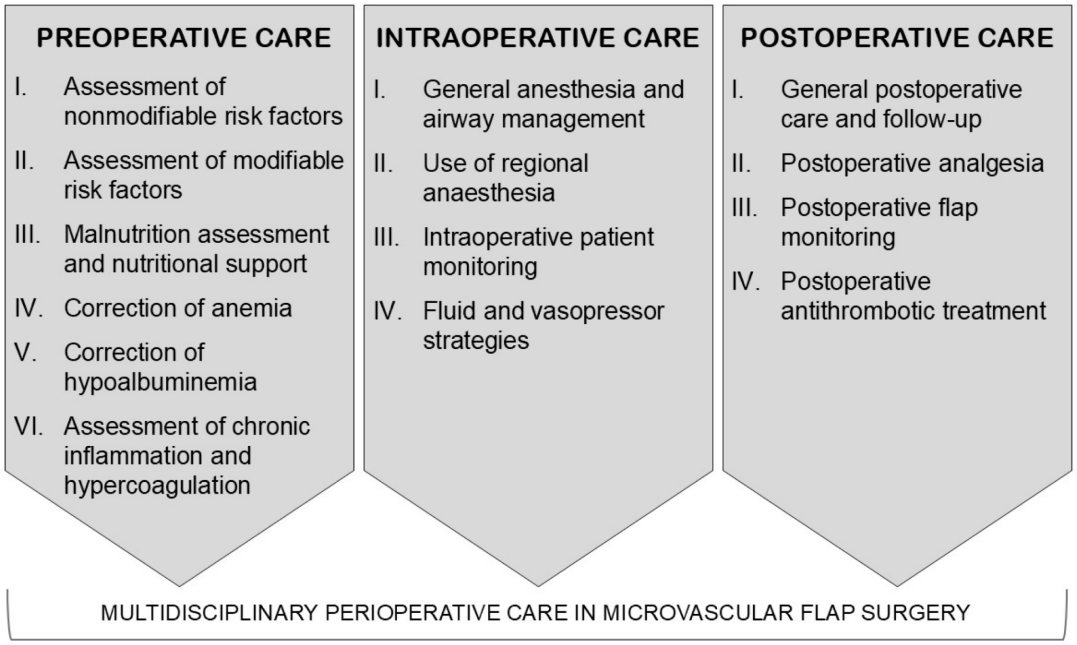

Figure 5.1. **Suggested multidisciplinary team-based approach framework for perioperative care in microvascular flap surgery**

The narrative review also identified key biomarker categories that may be significant in predicting complications. The main biomarkers of malnutrition identified are lymphocyte count, albumin and prognostic nutritional index (PNI) (Xiao et al., 2022; Yu et al., 2020). The main biomarkers of systemic inflammation are NLR, fibrinogen, albumin, fibrinogen,

platelet count and CRP (Chargi et al., 2022; Tsai et al., 2018; Handschel et al., 2013). The main markers of thrombosis risk are platelet count, fibrinogen and vWF:Ag (Tarabishy et al., 2020; Vanags et al, 2020; Drizlionoka et al., 2019; Du et al., 2015; Handschel et al., 2013). There was only one previous study that evaluated cytokines such as IL-6, IL-8 and macrophage stimulating factor from flap vein blood samples for the early prediction of flap loss (Finke et al., 2015).

Our narrative literature review also addressed intraoperative considerations and identified a clear need for further research to establish best practices for temperature, fluid management, and the use of vasopressors (Tapia et al., 2021; Moellhoff et al., 2021; Laitman et al., 2019; Ibrahim et al., 2014; Chen et al., 2010; Young et al., 2006). The identified preoperative and intraoperative risk factors for flap complications are summarised in Figure 5.2.

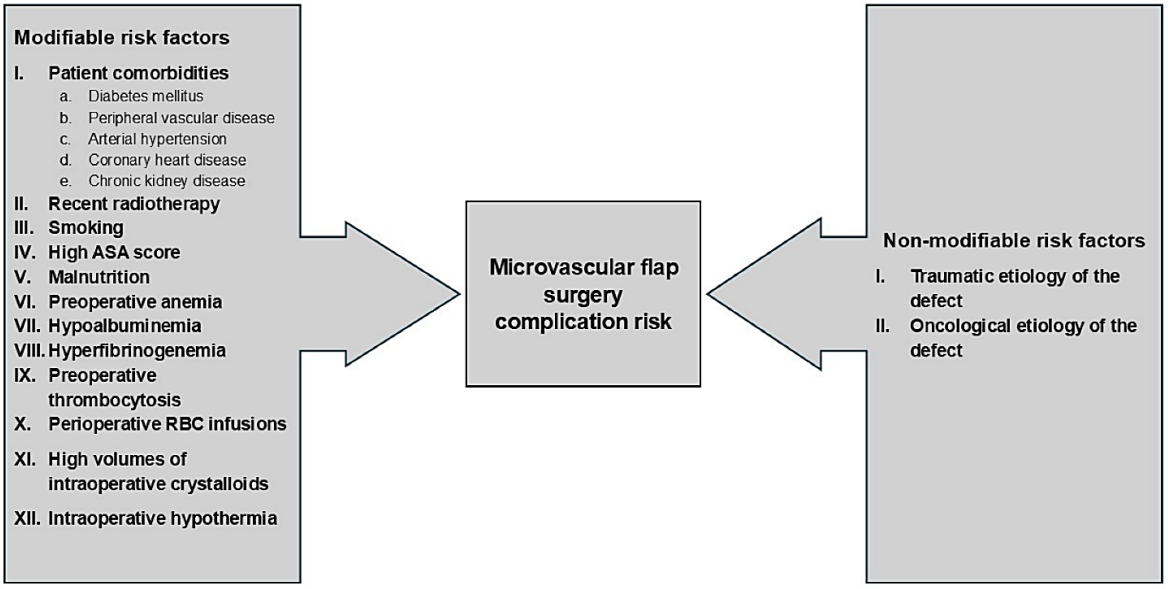

Figure 5.2. **Preoperative and intraoperative risk factors for postoperative complications in microvascular flap surgery**

ASA – American Society of Anesthesiologists; RBC – red blood cell.

Postoperatively, continuous instrumental monitoring of free flap perfusion is generally preferred, however the optimal strategy for antithrombotic prophylaxis remains unclear and warrants further study (Rothweiler et al., 2022; Lee et al., 2015; Bashir et al., 2014; Lighthall et al., 2013).

In general, the narrative literature review has provided valuable insights to guide future investigations into the perioperative management of patients undergoing microvascular flap surgery. Although the field has witnessed significant advancements, several critical knowledge gaps remain unresolved. It is increasingly evident that the determinants of complications and successful outcomes in free flap microvascular reconstruction are complex and multifactorial. While substantial evidence exists identifying variables associated with adverse outcomes in microvascular flap surgery, many previous studies are limited by their small sample sises and retrospective designs, which may introduce risks of selection and classification biases.

5.2 Prospective study evaluating CONUT score for prediction of microvascular flap complications in reconstructive surgery (First prospective study of the second part)

The first study of the prospective part that an elevated preoperative CONUT index is a reliable predictor of flap complications, with a score >2 being the optimal cut-off. Flap complications were associated with lymphocytopenia, monocytopenia, low haematocrit, and obesity.

Systemic factors such as surgical stress response, tissue healing, and nutritional status are key contributors to surgical outcomes in microvascular flap surgery (Košec et al., 2022). Malnourished patients are more likely to suffer from surgical complications, extended hospitalisations, and delayed recovery in both general surgery and microvascular flap reconstruction (Kutnik et al., 2023; Venianaki et al., 2021; Yu et al., 2020; Ho et al., 2015). Yu et al. demonstrated the utility of the PNI, which shares components with CONUT, in predicting flap failure (Yu et al., 2020). Our findings show that a higher CONUT score is positively linked to increased flap complication risk. To our knowledge, this is the first study highlighting the predictive value of CONUT in microvascular flap surgery, aligning with results from other surgical populations (Cozzani et al., 2024; Cai et al., 2023; Hara et al., 2020).

The CONUT >2 threshold aligns with cut-offs reported in other surgical studies, though it demonstrated low sensitivity (21.1 %) and high specificity (95.6 %), making it more suitable for ruling out low-risk patients (Quian et al, 2021, Tokoyawa et al., 2016; Iseki et al., 2015).

While we found that CONUT predicted minor flap complications, it was not a reliable predictor of true flap loss, which may result from different mechanisms, such as compromised microvascular anastomosis (Lee et al., 2010). Minor complications like wound dehiscence, infection, and fistulae are more likely to stem from impaired tissue healing, often linked to malnutrition (Shum et al., 2014; Pohlenz et al., 2012; Felekis et al., 2010). These complications, although less severe, can still necessitate re-exploration or repeat surgery (Pohlenz et al., 2013). Importantly, patients undergoing microvascular flap surgery are particularly vulnerable to delayed healing at both donor and recipient sites (Pohlenz et al., 2013).

Lymphocyte count, a CONUT component, likely plays a key role in flap healing. Preoperative lymphocytopenia has been linked to increased complication rates (Chiarelli et al., 2019; Lee et al., 2012). Our study found that monocytopenia was also linked to flap complications. Increased monocyte counts after major surgery are linked to surgical site infection, although there are no studies on preoperative monocytopenia and surgical outcomes (Snow et al., 2024). Interestingly, we observed a link between monocytopenia and flap complications, which contrasts with the findings of Košec and co-authors who reported no such association (Košec et al., 2022).

While the role of obesity is debated, some studies support its association with increased perioperative risks in microvascular flap surgery (Sinha et al., 2016; Fischer et al., 2013). Other studies found no such link in microvascular flap surgery (Crippen et al., 2018; Chang et al., 2018; de la Garza et al., 2016). Importantly, obesity and malnutrition can occur together, and this "double burden" malnutrition can lead to poor health outcomes (Wells et al., 2020; Barazzoni et al., 2020). BMI alone is not a reliable indicator of acute malnutrition, as shown by Ignacio de Ulíbarri and co-authors (de Ulibarri et al., 2005). Our results support that both obesity and nutritional risk independently raise the likelihood of complications and should be addressed preoperatively.

Although there is evidence for identifying patients at risk of malnutrition, data on the impact of nutrition in this setting remains limited. At the time of writing, only one clinical trial by Hwang et al. has shown that intraoperative enteral nutrition improves wound healing during flap reconstruction in head and neck surgery (Hwang et al., 2023).

This study had several limitations. Many patients had cancer as a primary diagnosis, which introduces additional risk factors for flap complications (Katna et al., 2023; Shankhdar et al., 2022; Chen et al., 2021). Furthermore, the presence of preoperative radiotherapy, another potential confounder, was not evaluated (Lee et al., 2015). Additionally, while serum albumin is included in both CONUT and PNI scores, it is not part of current malnutrition definitions, and the CONUT score should therefore be seen as a marker of nutritional risk rather than nutritional status (Evans et al., 2021). Further research is needed to validate malnutrition risk assessment tools and clarify their role in guiding interventions for patients undergoing microvascular flap surgery.

5.3 Prospective study evaluating FAR for predicting microvascular flap complications in reconstructive surgery (Second prospective study of the second part)

The second study of the prospective part found that both low and high preoperative FAR were associated with flap complications, suggesting a U-shaped relationship. Specifically, low FAR was linked to flap haematoma or flap loss, while high FAR was associated with minor complications. FAR was positively linked to length of hospital stay. Additional factors associated with the presence of flap complications included low lymphocyte count, high intraoperative haematocrit and obesity. The overall complication rate in this cohort was 17.7 %, with true flap loss occurring in 5.4 %. Patients with flap complications experienced significantly longer hospitalisations.

Elevated fibrinogen and low albumin levels in patients with complications support the role of inflammatory and coagulation markers in the pathophysiology of surgical

complications (Shang et al., 2023; La Vaccara et al., 2022). Albumin has been described as a key predictor of flap complications, with low levels associated with impaired healing, poor inflammatory response, and delayed or failed flap closure (La Vaccara et al., 2022; Utariani et al., 2020). As a marker of malnutrition and regulator of oncotic pressure, low albumin may reduce flap perfusion and oxygenation, increasing the risk of true flap loss (Xu et al., 2021).

In our cohort, patients with minor complications had higher fibrinogen levels, while patients with flap haematoma or flap loss had lower levels. Both hypofibrinogenemia and hyperfibrinogenaemia have been associated with flap complications (Drizlionoka et al., 2019; Kolbenschlag et al., 2016). Low fibrinogen can impair clot formation, leading to bleeding and flap failure (Vanags et al., 2020). Handschel et al. reported that high preoperative fibrinogen levels were linked to flap loss (Handschel et al., 2013). Drizlionoka et al. found a genetic variant associated with higher fibrinogen, which leads to increased flap thrombosis risk (Drizlionoka et al., 2019).

Interestingly, we observed a U-shaped relationship between FAR and flap complications. Patients with FAR greater than 0.13 had higher odds of flap complications, with further analysis showing that elevated FAR was particularly associated with minor flap complications. Several previous studies have similarly reported associations between increased FAR and the incidence of complications across various surgical populations (Xu et al., 2022; La Vaccara et al., 2022). This pattern, characterised by increased fibrinogen and decreased albumin, is consistent with the presence of chronic inflammation which is detrimental for flap success (Ma et al., 2024; Tarle et al., 2023; Ding et al., 2022; Du et al., 2015).

All previous articles on FAR in other surgical populations point to poor outcomes in high FAR patients (Xu et al., 2022; La Vaccara et al., 2022). Unexpectedly, FAR < 0.05 also predicted higher complication rates, specifically flap haematoma or flap loss. This suggests distinct mechanisms underlying major and minor complications in microvascular flap surgery. Low FAR may reflect coagulopathy, as low fibrinogen increases bleeding risk in microvascular flap surgery (Kolbenschlag et al., 2016). Additionally, increased albumin may exert anticoagulant effects, as albumin-induced coagulopathy can be reversed with fibrinogen supplementation (Paar et a., 2017; Winstedt et al., 2013). This may further explain the observed association between low FAR and bleeding.

The U-shaped relationship may reflect distinct underlying mechanisms: elevated FAR likely reflects systemic inflammation and impaired wound healing, while low FAR may indicate hypocoagulability (Lipa et al., 2025). These diverging states may both lead to flap complications through different pathophysiological pathways.

Overall, FAR may represent an easily accessible preoperative biomarker to stratify

surgical risk in microvascular flap patients. Identifying patients with FAR extremes could allow for early nutritional optimisation, inflammatory modulation, or perioperative coagulation management. Future investigations may elucidate whether perioperative interventions that normalise FAR such as targeted fibrinogen replacement, nutritional supplementation or reduction of chronic inflammation can reduce complication rates.

A limitation of this study is that the regression models did not include the presence of malignancy as primary indication for surgery, which is associated with elevated FAR and worse outcomes (Tomita et al., 2020). Furthermore, the identified cutoffs (< 0.06 and > 0.10) were based on regression analyses within our single centre cohort and warrant external validation. Whether these thresholds are generalizable across different surgical populations remains to be determined. Future research may validate these findings through multicentre studies, explore subgroup differences, and use experimental approaches to better define the role of FAR in different clinical contexts.

5.4 vWF:Ag, biomarkers of inflammation, and true flap loss in reconstructive surgery (Third prospective study of the second part)

The third prospective cohort study identified key associations between inflammatory biomarkers, preoperative vWF:Ag concentration, and postoperative complications in microvascular flap surgery. A principal finding was that elevated preoperative vWF:Ag levels significantly increase the risk of true flap loss. Additional associations were observed between flap complications and decreased lymphocyte count, reduced plasma albumin, elevated platelet count, increased plasma fibrinogen, and a higher NLR. Moreover, increased levels of CRP, fibrinogen, IL-6, and NLR were positively linked with elevated preoperative vWF:Ag concentrations.

Recent investigations have shown that various inflammatory states are generally accompanied by increased vWF:Ag levels (Manz et al., 2020; Gragnano et al., 2017). vWF, a large multimeric glycoprotein synthesised by endothelial cells and megakaryocytes, plays a central role in haemostasis, particularly in facilitating platelet plug formation at sites of vascular injury (O'Donnell et al., 2023; Mojzisch et al., 2021). vWF mediates platelet adhesion by binding to collagen and platelet receptors (Tsyu et al., 2023). vWF is also highly shear-sensitive and is therefore known to promote platelet aggregation at post-stenotic sites that have a negative shear rate gradient (Hoefer et al., 2021). Studies on atherosclerotic plaques reveal that interactions between the plaque geometries, local endothelial vWF release, and plasma levels of vWF:Ag can lead to thrombosis (Westein et al., 2013). The concept of vWF elongation and subsequent contribution to platelet aggregation at post-stenotic sites may be applicable to the anastomosis site in microvascular flap surgery (Rothweiler et al., 2021; Du et al., 2015; Handschel et al., 2013).

Although the exact mechanisms of vWF-mediated thrombosis are complex, total plasma vWF:Ag levels have been identified as predictors of thrombotic events. In microvascular flap surgery, Handschel et al. reported higher vWF:Ag concentrations in patients experiencing venous thrombosis at the anastomosis site, which aligns with our results (Handschel et al., 2013).

Among factors influencing vWF:Ag levels, chronic inflammation appears particularly prevalent in the population undergoing microvascular reconstruction (Chargi et al., 2022; Yu et al., 2020). Oncology and trauma as indications for microvascular reconstruction are particularly linked to elevated systemic inflammation, increased thrombosis risk, and higher vWF:Ag concentrations (Colonne et al., 2022; Zeineddin et al., 2021; Lelas et al., 2021; Abdol Razak et al., 2018). In our cohort, trauma cases exhibited the highest vWF:Ag levels, followed by oncology. Patients undergoing reconstruction for uncomplicated defects displayed the lowest concentrations. However, mean values across all three groups remained within previously reported normal ranges (Möller et al., 2020).

Elevated vWF:Ag has previously been associated with markers of systemic inflammation, including increased IL-6, reduced albumin, elevated CRP, and higher platelet counts (Möller et al., 2020; Tarabishy et al., 2020; Kim et al., 1999). In this study, all inflammatory biomarkers, except CRP and IL-6, were associated with flap complications. Specifically, increased NLR and decreased albumin were linked to secondary complications, while elevated fibrinogen was associated with true flap loss. These distinctions support our hypothesis that true flap loss and secondary complications arise from differing pathophysiological processes. Our findings suggest that inflammation also elevates fibrinogen and vWF:Ag levels, both of which have been shown to increase the likelihood of true flap loss (Vanags et al. 2020; Drizlionoka et al., 2019; Handschel et al., 2013).

These findings are consistent with those of Handschel et al., who observed elevated vWF:Ag in patients with flap thrombosis. However, our results further delineate the specific inflammatory markers associated with different types of complications, a distinction not emphasised in prior studies (Rothweiler et al., 2021; Handschel et al., 2013). Preoperative measurement of vWF:Ag, particularly when combined with fibrinogen or NLR, may offer clinicians a tool for early risk stratification. This may inform postoperative monitoring intensity, or prophylactic anticoagulation strategies (Stevens et al., 2024; Lacey et al., 2023).

This research part had several limitations. The analysis did not distinguish between arterial and venous thromboses at the anastomosis, despite existing literature addressing these differences (Rothweiler et al., 2021; Handschel et al., 2013). This study focuses solely on preoperative vWF:Ag concentrations, without accounting for the dynamic fluctuations in vWF:Ag levels intraoperatively and postoperatively. Continuous biomarker monitoring

throughout the perioperative period may offer deeper insight into mechanisms underlying flap complications. Our study design did not allow assessment of the cost-effectiveness of vWF:Ag relative to other biomarkers. Large-scale, multi-centre studies are needed to validate the use of vWF:Ag concentrations in clinical practice.

5.5 Early postoperative increase in TGF-β1 predicts microvascular flap loss in reconstructive surgery (Fourth prospective study of the second part)

The central finding of this study is that a postoperative increase in TGF-β1 is linked to true flap loss and, to a smaller extent, minor flap complications. Increased postoperative changes in TGF-β1 are linked to increased CRP and fibrinogen. Increased postoperative TGF-β1 concentrations are linked to increased preoperative haematocrit and haemoglobin.

While TGF-β is secreted by many cell types, platelets contribute approximately 45 % of the total TGF-β present in plasma, and TGF-β1 is its main isoform, accounting for 95 % of the total TGF-β (Deng et al., 2024; Wang et al., 2024; Karolczak et al., 2021; Meyer et al., 2012). Exposure to shear stress has been shown to stimulate TGF-β1 release from platelets both in vitro and in vivo (Wang et al., 2014; Ahamed et al., 2008). We propose that increased shear forces at a dysfunctional vascular anastomosis may contribute to the postoperative rise in TGF-β1. Prior research indicates that shear stress can activate latent TGF-β1 present in platelets and the extracellular matrix, highlighting the intricacies of flap survival under dynamic vascular settings (Wang et al., 2014). In thrombosed arteries during flap surgery, elevated shear stress may significantly amplify TGF-β1 release (Walshe et al., 2013). Co-secreted proteins such as thrombospondin-1 can further enhance TGF-β1 activation, and inflammatory mediators like matrix metalloproteinases may perpetuate a feedback loop of platelet activation and inflammation (Murphy-Ullrich et al., 2018; Murphy-Ullrich et al., 2000). Notably, TGF-β1 may itself increase shear stress on circulating platelets, and hypercholesterolemia has been shown to promote TGF-β1 release from platelets, though this was not evident in our findings (Wang et al., 2014). It must be noted that the shear stress interaction between the vessel wall and TGF-β1 has been previously studied only in the context of chronic vessel wall changes (Gómez-Bernal et al., 2023; Wang et al., 2014).

In addition to shear-induced release, thrombin may activate platelets via PAR1/4 receptors during thrombosis, promoting further TGF-β1 secretion (Boknäs et al., 2014). This process enhances monocyte tissue factor expression, increasing thrombin generation and contributing to inflammation and coagulation (Pihusch et al., 2005).

TGF-β1 also plays a role in the regulation of thrombosis (Jiang et al., 2022). Zhang et al. showed that TGF-β1 promotes neutrophil and monocyte recruitment to thrombi and facilitates neutrophil extracellular trap formation, supporting its role in venous thrombus development, as seen in Figure 5.3 (Zhang et al., 2024).

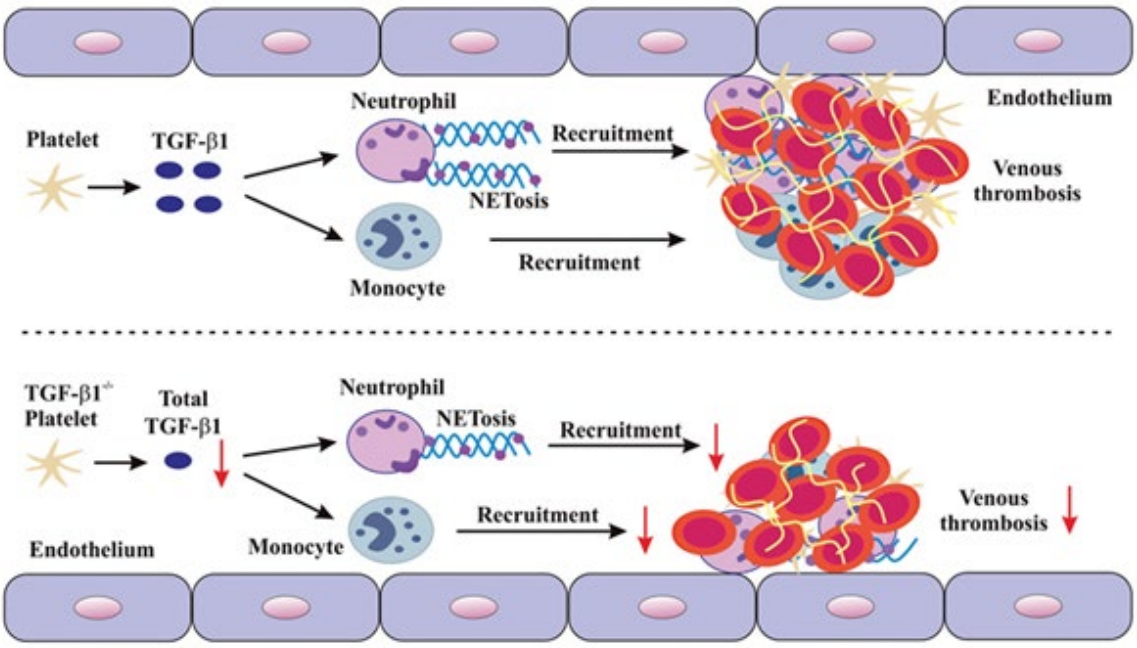

Figure 5.3. **Platelet-derived TGF-β1 promotes deep vein thrombosis via enhancing the recruitment of neutrophils and monocytes to venous thrombi as well as the formation of neutrophil extracellular traps**

Adapted from: Zhang, S., Li, Y., Zhang, J., Sun, Y., Chu, X., Gui, X., Tong, H., Ding, Y., Ju, W., Xu, M., Li, Z., Zeng, L., Xu, K., & Qiao, J. (2024). Platelet-Derived TGF-β1 Promotes Deep Vein Thrombosis. Thrombosis and haemostasis, 124(7), 641–648.

A study by Jiang et al. revealed that elevated platelet-derived TGF-β1 levels in portal venous thrombosis are associated with hypercoagulability and endothelial dysfunction (Jiang et al., 2022). However, the absence of TGF-β1 did not affect arterial thrombosis in mice (Zhang et al., 2024). While our results showed that a postoperative increase in TGF-β1 was linked to true flap loss, there was no link between increased preoperative TGF-β1 and the risk of true flap loss. Our findings corroborate recent evidence that platelet-derived TGF-β1 promotes the progression of venous thrombus development, rather than its initiation (Davis et al., 2025; Zhang et al., 2024; Jiang et al., 2022). This may imply that the promotion of coagulation is a product of local TGF-β1 release at the site of thrombus formation, rather than its cause.

Venous thrombosis due to pedicle kinking is a more frequent cause of flap failure than anastomotic failure and generally occurs under low shear stress (Williams et al., 2004). In such cases, blood stasis is the primary prothrombotic cause (Undas et al., 2011). The postoperative rise in TGF-β1 may indicate pedicle kinking, progressive anastomotic thrombosis and impending flap failure, as thrombus formation increases local TGF-β1 levels (Davis et al., 2025; Zhang et al., 2024; Smeda et al., 2024; Jiang et al., 2022). This is further supported by our observation of a positive correlation between postoperative TGF-β1 and preoperative plasma fibrinogen, which is a known risk factor for flap thrombosis (Handschel et al., 2013).

Postoperative TGF-β1 was also associated with preoperative haemoglobin and haematocrit. Though TGF-β1 correlates with haemoglobin in systemic lupus erythematosus, we did not observe an association between preoperative TGF-β1 and haematocrit in our cohort (Gómez-Bernal et al., 2022). Haematocrit may influence TGF-β1 through its effect on blood viscosity, which increases shear stress (Leo et al., 2020; Ameenuddin et al., 2019). Since platelet-derived TGF-β1 release is stimulated by shear stress, higher viscosity may increase TGF-β1 secretion at stenotic sites, including microvascular anastomoses (Wang et al., 2014). However, considering our study methodology, elevated viscosity may have also promoted TGF-β1 release during sample collection (Karolczak et al., 2021).

The observed postoperative rise in TGF-β1 could serve as an early biomarker of impending flap failure. In clinical settings where perfusion monitoring is limited, serial biomarker measurements may inform timely decisions regarding re-exploration (Wu et al., 2024). Additionally, future integration of TGF-β1 into postoperative monitoring protocols could stratify patients by thrombotic risk and guide intensity of clinical surveillance. To our knowledge, it is the first to systematically evaluate postoperative TGF-β1 changes in relation to flap outcomes, integrating both inflammatory and hematologic markers. The prospective design and use of serial postoperative samples enabled time-based association between TGF-β1 dynamics and clinical events.

This study has several limitations. First, TGF-β1 levels vary considerably across physiological and pathological conditions, many of which were not addressed by our exclusion criteria (Karolczak et al., 2021). Tracking TGF-β in plasma may be difficult due to rapid binding to target cells (Karolczak et al., 2021). Therefore, verification with immunohistochemical analysis of SMAD protein expression in target cells would further improve the reliability of the results (Karolczak et al., 2021). We did not differentiate arterial from venous thrombosis at the anastomotic site, a potentially important distinction given the role of TGF-β1 in venous thrombus progression (Deng et al., 2024). Given the use of multiple different flap types, the anastomosis site and subsequent vessel curvature were not individually evaluated, although they may influence blood flow and affect postoperative TGF-β1 levels (Wang et al., 2014). Finally, postoperative TGF-β1 increases may also be involved in minor flap complications, including fibrosis and impaired wound healing, even in successful flaps (Finnson et al., 2013; Tredget et al., 2005). Extended postoperative monitoring may clarify the predictive potential of TGF-β1 in microvascular flap surgery.

Conclusions

1. Perioperative care strategies for anaemia correction, analgesia, and PNB use are well-supported by current evidence in microvascular flap surgery. However, other aspects such as malnutrition assessment, inflammation, hypercoagulability, fluid and temperature management, and antithrombotic treatment strategies remain less clearly defined. The use of biomarkers to predict true flap loss represents a promising direction for future research.
2. Preoperative CONUT score > 2 is a reliable marker of malnutrition risk and predictor of minor flap complications. Patients with lymphocytopenia, monocytopenia, low haematocrit, or obesity, are at increased risk of minor flap complications, highlighting the need for routine malnutrition risk screening and targeted interventions in microvascular flap surgery.
3. Preoperative FAR shows a U-shaped association with flap complications, where low FAR below 0.06 predicts flap haematoma or true flap loss, high FAR above 0.10 predicts minor flap complications, and both are linked to longer hospital stays, supporting its utility as a preoperative risk stratification tool in microvascular flap surgery.
4. Elevated preoperative vWF:Ag levels above the cut-off of 163.73 IU/dL are linked to increased risk of true flap loss. Preoperative vWF:Ag concentration is positively associated with inflammatory biomarkers including increased fibrinogen, NLR, IL-6, CRP, and decreased plasma albumin, supporting the role of vWF:Ag as a predictive marker for true flap loss.
5. Postoperative increase in TGF-β1 greater than 1.00 ng/mL, driven by both shear stress induced platelet activation at sites of anastomotic dysfunction and active thrombus formation, is an effective biomarker for early detection of impending true flap loss.
6. Novel biomarker models integrating nutritional, inflammatory, coagulation, and immunological markers are useful for predicting complications in microvascular flap surgery. The newly identified perioperative biomarkers can help identify high-risk patients, guide perioperative care, and enable early detection of impending true flap loss. These insights support a more personalised, data-driven approach to improving outcomes in microvascular flap surgery.

Publications and reports on topics of Doctoral Thesis

1. **Rocans, R. P.,** Zarins, J., Bine, E., Deksnis, R., Citovica, M., Donina, S., Mamaja, B. (2023). The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery. *Journal of Clinical Medicine*, 12(14), 4794. https://doi.org/10.3390/jcm12144794
2. **Rocans, R. P.,** Zarins, J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Vanags, I., Gravelsina, S., Vilmane, A., Rasa-Dzelzkaleja, S., Mamaja, B. (2024). Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery. *Journal of Clinical Medicine*, 13(18), 5411. https://doi.org/10.3390/jcm13185411
3. **Ojuva, A. M., Rocans, R. P.,** Zarins, J., Bine, E., Mahauri, I., Donina, S., Mamaja, B., Vanags, I. (2024). Novel Challenges and Opportunities for Anaesthesia and Perioperative Care in Microvascular Flap Surgery: A Narrative Review. Clinics and Practice, 14(5), 2187-2201. https://doi.org/10.3390/clinpract14050172
4. **Rocans, R. P.,** Zarins, J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Vanags, I., & Mamaja, B. (2025). Fibrinogen-to-albumin ratio (FAR) for predicting microvascular flap complications in reconstructive surgery. JPRAS open, 44, 414–423. https://doi.org/10.1016/j.jpra.2025.03.022.
5. **Rocans, R. P.,** Zarins, J., Bine, E., Mahauri, I., Deksnis, R., Citovica, M., Donina, S., Gravelsina, S., Vilmane, A., Rasa-Dzelzkaleja, S., Sabelnikovs, O., Mamaja, B. (2025). Early Postoperative Increase in Transforming Growth Factor Beta-1 Predicts Microvascular Flap Loss in Reconstructive Surgery: A Prospective Cohort Study. Medicina (Kaunas, Lithuania), 61(5), 863. https://doi.org/10.3390/medicina61050863

Reports and theses at international congresses and conferences

1. **Rocans R.P.,** Mamaja B, Doniņa S43 General or regional anaesthesia for microvascular flap surgery: comparison of surgical complication rate and duration of hospitalization. Regional Anaesthesia & Pain Medicine 2021;70:A25. European Society of Regional Anaesthesia Hybrid Conference, 2021 (Poster presentation)
2. **Rocans R. P.,** Mamaja B, Doniņa (2022) General or regional anaesthesia for microvascular flap surgery: comparison of surgical site infection rate. Abstract from Euroanaesthesia 2022, AS-ESAIC-2022-00339. European Society of Anaesthesia and Intensive care, Milan, Italy, 2022 (Oral presentation)
3. **Rocans, R. P.,** Zarins, J., M., Donina, S., Mamaja, B. Full Blood Count Biomarkers for Prediction of Flap Complications in Microvascular Flap Surgery. RSU International Research Conference 2023: Knowledge for Use in Practice. Rīga Stradiņš University, Riga, Latvia, 2023 (Poster presentation)
4. **Rocans R. P.,** Zarins, J., Mamaja B, Doniņa (2023) Biomarkers for the preoperative prediction of flap loss in microvascular flap surgery: a single-center prospective analysis. Abstract from Euroanaesthesia 2023, AS-ESAIC-2022-00339. European Society of Anaesthesia and Intensive care, Glasgow, Scotland, UK, 2023 (Oral presentation)
5. **Rocans R. P.,** Zarins J, Deksnis R, Citovica M, Bine M, Mamaja B, Doniņa S. (2024) The Use of Peripheral Nerve Blocks for Extremity Microvascular Flap Surgery: Comparison of Different Surgical Complications. Baltic Society of Regional Anaesthesia 9th International Conference of Baltic Society of Regional Anaesthesia, Kuresaare, Estonia, 2024 (Oral presentation)

References

1. Abdol Razak, N. B., Jones, G., Bhandari, M., Berndt, M. C., & Metharom, P. (2018). Cancer-Associated Thrombosis: An Overview of Mechanisms, Risk Factors, and Treatment. *Cancers*, 10(10), 380. https://doi.org/10.3390/cancers10100380
2. Ahamed, J., Burg, N., Yoshinaga, K., Janczak, C. A., Rifkin, D. B., & Coller, B. S. (2008). In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor-beta1. *Blood*, 112(9), 3650–3660. https://doi.org/10.1182/blood-2008-04-151753
3. Altın, E. D. S., Canbolat, N., Buget, M. I., Altın, Y. F., Bayram, S., & Koltka, K. (2025). Correlation of preoperative fibrinogen/albumin ratio with morbidity following advanced-age hip fractures: an observational study. *Journal of Anaesthesia*, 39(2), 205–214. https://doi.org/10.1007/s00540-024-03444-z
4. Ameenuddin, Mohammed & Anand, Mohan & Massoudi, Mehrdad. (2019). Effects of shear-dependent viscosity and hematocrit on blood flow. *Applied Mathematics and Computation*. 356. 299-311. 10.1016/j.amc.2019.03.028
5. Barazzoni, R., & Gortan Cappellari, G. (2020). Double burden of malnutrition in persons with obesity. *Reviews in Endocrine & Metabolic Disorders*, 21(3), 307–313. https://doi.org/10.1007/s11154-020-09578-1
6. Bashir, M. M., Yousaf, N., & Khan, F. A. (2014). The outcome of microvascular free flap surgery with or without the use of postoperative heparin. *Journal of the College of Physicians and Surgeons – Pakistan* : JCPSP, 24(6), 412–415.
7. Boknäs, N., Faxälv, L., Sanchez Centellas, D., Wallstedt, M., Ramström, S., Grenegård, M., & Lindahl, T. L. (2014). Thrombin-induced platelet activation via PAR4: pivotal role for exosite II. *Thrombosis and Haemostasis*, 112(3), 558–565. https://doi.org/10.1160/TH13-12-1013
8. Cai, Y. S., Li, X. Y., Ye, X., Li, X., Fu, Y. L., Hu, B., Li, H., & Miao, J. B. (2023). Preoperative controlling nutritional status score (CONUT) predicts postoperative complications of patients with bronchiectasis after lung resections. *Frontiers in Nutrition*, 10, 1000046. https://doi.org/10.3389/fnut.2023.1000046
9. Chang, E. I., & Liu, J. (2018). Prospective Evaluation of Obese Patients Undergoing Autologous Abdominal Free Flap Breast Reconstruction. *Plastic and Reconstructive Surgery*, 142(2), 120e–125e. https://doi.org/10.1097/PRS.0000000000004550
10. Chargi, N., Breik, O., Forouzanfar, T., Martin, T., Praveen, P., Idle, M., Parmar, S., & de Bree, R. (2022). Association of low skeletal muscle mass and systemic inflammation with surgical complications and survival after microvascular flap reconstruction in patients with head and neck cancer. *Head & Neck*, 44(10), 2077–2094. https://doi.org/10.1002/hed.27113
11. Chen, C., Nguyen, M. D., Bar-Meir, E., Hess, P. A., Lin, S., Tobias, A. M., Upton, J., 3rd, & Lee, B. T. (2010). Effects of vasopressor administration on the outcomes of microsurgical breast reconstruction. *Annals of Plastic Surgery*, 65(1), 28–31. https://doi.org/10.1097/SAP.0b013e3181bda312
12. Chen, J., Dennis, S. K., & Abouyared, M. (2021). Sarcopenia and microvascular free flap reconstruction. *Current Opinion in Otolaryngology & Head and Neck Surgery*, 29(5), 419–423. https://doi.org/10.1097/MOO.0000000000000756
13. Chiarelli, M., Achilli, P., Tagliabue, F., Brivio, A., Airoldi, A., Guttadauro, A., Porro, F., & Fumagalli, L. (2019). Perioperative lymphocytopenia predicts mortality and severe complications after intestinal surgery. *Annals of Translational Medicine*, 7(14), 311. https://doi.org/10.21037/atm.2019.06.46
14. Colonne, C. K., Favaloro, E. J., & Pasalic, L. (2022). The Intriguing Connections between von Willebrand Factor, ADAMTS13 and Cancer. *Healthcare (Basel, Switzerland)*, 10(3), 557. https://doi.org/10.3390/healthcare10030557

15. Cozzani, F., Ricchiuto, M., Virgilio, E., Viani, L., Rossini, M., Pedrazzi, G., Del Rio, P. (2024). The Controlling Nutritional Status (CONUT) Score Predicts Post-operatory Risks and Prognosis in Patients With Surgically Treated Colon Cancer: A Retrospective Study. *Anticancer Research*, 44(9), 3955–3964. https://doi.org/10.21873/anticanres.17224

16. Crippen, M. M., Brady, J. S., Mozeika, A. M., Eloy, J. A., Baredes, S., & Park, R. C. W. (2018). Impact of Body Mass Index on Operative Outcomes in Head and Neck Free Flap Surgery. Otolaryngology – head and neck surgery*: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 159(5), 817–823. https://doi.org/10.1177/0194599818777240

17. Davis, J., Maranto, M., Kennedy, J., Wang, X., Azhar, M., Jain, A., & Evans, C. E. (2025). Transforming Growth Factors in Venous Thrombus Formation and Resolution. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 45(5), 643–653. https://doi.org/10.1161/ATVBAHA.124.322395

18. de la Garza, G., Militsakh, O., Panwar, A., Galloway, T. L., Jorgensen, J. B., Ledgerwood, L. G., Kaiser, K., Kitzerow, C., Shnayder, Y., Neumann, C. A., Khariwala, S. S., Chad Spanos, W., & Pagedar, N. A. (2016). Obesity and perioperative complications in head and neck free tissue reconstruction. *Head & Neck*, 38 Suppl 1(Suppl 1), E1188–E1191. https://doi.org/10.1002/hed.24189

19. Deng, Z., Fan, T., Xiao, C., Tian, H., Zheng, Y., Li, C., & He, J. (2024). TGF-β signaling in health, disease, and therapeutics. *Signal Transduction and Targeted Therapy*, 9(1), 61. https://doi.org/10.1038/s41392-024-01764-w

20. Dettori, J. R., Norvell, D. C., Chapman, J. R. (2019). Grab Control! Choosing the Right Comparison Group in an Observational Study. *Global Spine Journal*, 9(4), 456–458. https://doi.org/10.1177/2192568219847206

21. Ding, Y., & Xue, L. (2022). The potential value of fibrinogen to albumin ratio (FAR) in the assessment of inflammation in spondyloarthritis. *BMC Musculoskeletal Disorders*, 23(1), 864. https://doi.org/10.1186/s12891-022-05797-6

22. Dolan, R. T., Butler, J. S., Murphy, S. M., & Cronin, K. J. (2012). Health-related quality of life, surgical and aesthetic outcomes following microvascular free flap reconstructions: an 8-year institutional review. *Annals of the Royal College of Surgeons of England*, 94(1), 43–51. https://doi.org/10.1308/003588412X13171221498749

23. Drizlionoka, K., Zariņš, J., Ozoliņa, A., Ņikitina-Zaķe, 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* (Kaunas, Lithuania), 55(9), 563. https://doi.org/10.3390/medicina55090563

24. Du, W., Wu, P. F., Qing, L. M., Wang, C. Y., Liang, J. Y., Yu, F., & Tang, J. Y. (2015). Systemic and flap inflammatory response associates with thrombosis in flap venous crisis. *Inflammation*, 38(1), 298–304. https://doi.org/10.1007/s10753-014-0033-9

25. Eckart, A., Struja, T., Kutz, A., Baumgartner, A., Baumgartner, T., Zurfluh, S., Neeser, O., Huber, A., Stanga, Z., Mueller, B., & Schuetz, P. (2020). Relationship of Nutritional Status, Inflammation, and Serum Albumin Levels During Acute Illness: A Prospective Study. *The American Journal of Medicine*, 133(6), 713–722.e7. https://doi.org/10.1016/j.amjmed.2019.10.031

26. Evans, D. C., Corkins, M. R., Malone, A., Miller, S., Mogensen, K. M., Guenter, P., Jensen, G. L., & ASPEN Malnutrition Committee (2021). The Use of Visceral Proteins as Nutrition Markers: An ASPEN Position Paper. Nutrition in Clinical Practice : *Official Publication of the American Society for Parenteral and Enteral Nutrition*, 36(1), 22–28. https://doi.org/10.1002/ncp.10588

27. Felekis, D., Eleftheriadou, A., Papadakos, G., Bosinakou, I., Ferekidou, E., Kandiloros, D., Katsaragakis, S., Charalabopoulos, K., & Manolopoulos, L. (2010). Effect of perioperative immuno-enhanced enteral nutrition on inflammatory response, nutritional status, and outcomes in head and neck cancer patients undergoing major surgery. *Nutrition and Cancer*, 62(8), 1105–1112. https://doi.org/10.1080/01635581.2010.494336

28. Finke, J. C., Yang, J., Bredell, M., Fritschen, U. von, & Glocker, M. O. (2018). Plasma Cytokine and Growth Factor Profiling during Free Flap Transplantation. *InTech*. doi: 10.5772/intechopen.70054

29. Finnson, K. W., McLean, S., Di Guglielmo, G. M., & Philip, A. (2013). Dynamics of Transforming Growth Factor Beta Signaling in Wound Healing and Scarring. *Advances in Wound Care*, 2(5), 195–214. https://doi.org/10.1089/wound.2013.0429

30. Fischer, J. P., Nelson, J. A., Sieber, B., Cleveland, E., Kovach, S. J., Wu, L. C., Serletti, J. M., & Kanchwala, S. (2013). Free tissue transfer in the obese patient: an outcome and cost analysis in 1258 consecutive abdominally based reconstructions. *Plastic and Reconstructive Surgery*, 131(5), 681e–692e. https://doi.org/10.1097/PRS.0b013e31828e2159

31. Galviz Tabares, B., Ruiz Geithner, C. M., Pierpoline, J., & Mosquera, C. (2025). Long-Term Functional Outcomes of Free Flaps Versus Locoregional Flaps in Soft Tissue Reconstruction for Oral Cavity Cancer: A Systematic Review. *The Journal of Craniofacial Surgery*, 36(4), 1278–1285. https://doi.org/10.1097/SCS.0000000000011158

32. Go, B. C., Go, C. C., Chorath, K., Moreira, A., & Rajasekaran, K. (2022). Multimodal Analgesia in Head and Neck Free Flap Reconstruction: A Systematic Review. Otolaryngology – head and neck surgery : *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 166(5), 820–831. https://doi.org/10.1177/01945998211032910

33. Gómez-Bernal, F., Quevedo-Abeledo, J. C., García-González, M., Fernández-Cladera, Y., González-Rivero, A. F., de Vera-González, A., Martín-González, C., González-Gay, M. Á., & Ferraz-Amaro, I. (2022). Serum Levels of Transforming Growth Factor Beta 1 in Systemic Lupus Erythematosus Patients. *Biomolecules*, 13(1), 73. https://doi.org/10.3390/biom13010073

34. Gómez-Bernal, F., Quevedo-Abeledo, J. C., García-González, M., Fernández-Cladera, Y., González-Rivero, A. F., Martín-González, C., González-Gay, M. Á., & Ferraz-Amaro, I. (2023). Transforming growth factor beta 1 is associated with subclinical carotid atherosclerosis in patients with systemic lupus erythematosus. *Arthritis Research & Therapy*, 25(1), 64. https://doi.org/10.1186/s13075-023-03046-2

35. Gragnano, F., Sperlongano, S., Golia, E., Natale, F., Bianchi, R., Crisci, M., Fimiani, F., Pariggiano, I., Diana, V., Carbone, A., Cesaro, A., Concilio, C., Limongelli, G., Russo, M., & Calabrò, P. (2017). The Role of von Willebrand Factor in Vascular Inflammation: From Pathogenesis to Targeted Therapy. *Mediators of Inflammation*, 2017, 5620314. https://doi.org/10.1155/2017/5620314

36. Hanasono, M. M., & Butler, C. E. (2008). Prevention and treatment of thrombosis in microvascular surgery. *Journal of Reconstructive Microsurgery*, 24(5), 305–314. https://doi.org/10.1055/s-2008-1080530

37. Handschel, J., Burghardt, S., Naujoks, C., Kübler, N. R., & Giers, G. (2013). Parameters predicting complications in flap surgery. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 115(5), 589–594. https://doi.org/10.1016/j.oooo.2012.09.007

38. Hara, M., Fujii, T., Masuhara, H., Kawasaki, M., Tokuhiro, K., & Watanabe, Y. (2020). The prognostic impact of the controlling nutritional status (CONUT) score in patients undergoing cardiovascular surgery. *General Thoracic and Cardiovascular Surgery*, 68(10), 1142–1147. https://doi.org/10.1007/s11748-020-01346-x

39. Hassanzad, M., Hajian-Tilaki, K. (2024). Methods of determining optimal cut-point of diagnostic biomarkers with application of clinical data in ROC analysis: an update review. *BMC Medical Research Methodology*, 24(1), 84. https://doi.org/10.1186/s12874-024-02198-2

40. Hill, J. B., Patel, A., Del Corral, G. A., Sexton, K. W., Ehrenfeld, J. M., Guillamondegui, O. D., & Shack, R. B. (2012). Preoperative anemia predicts thrombosis and free flap failure in microvascular reconstruction. *Annals of Plastic Surgery*, 69(4), 364–367. https://doi.org/10.1097/SAP.0b013e31823ed606

41. Ho, J. W., Wu, A. H., Lee, M. W., Lau, S. Y., Lam, P. S., Lau, W. S., Kwok, S. S., Kwan, R. Y., Lam, C. F., Tam, C. K., & Lee, S. O. (2015). Malnutrition risk predicts surgical outcomes in patients undergoing gastrointestinal operations: Results of a prospective study. *Clinical Nutrition* (Edinburgh, Scotland), 34(4), 679–684. https://doi.org/10.1016/j.clnu.2014.07.012

42. Hoefer, T., Rana, A., Niego, B., Jagdale, S., Albers, H. J., Gardiner, E. E., Andrews, R. K., Van der Meer, A. D., Hagemeyer, C. E., & Westein, E. (2021). Targeting shear gradient activated von Willebrand factor by the novel single-chain antibody A1 reduces occlusive thrombus formation in vitro. *Haematologica*, 106(11), 2874–2884. https://doi.org/10.3324/haematol.2020.250761

43. Hwang, T. Z., Wang, Y. M., Jeng, S. F., Lee, Y. C., Chen, T. S., Su, S. Y., Huang, C. C., & Lam, C. F. (2023). Intraoperative Enteral Nutrition Feeding in Free-Flap Healing after Reconstruction Surgery for Head and Neck Cancers. Otolaryngology – head and neck surgery : *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 169(4), 843–851. https://doi.org/10.1002/ohn.335

44. Ibrahim, A. M., Kim, P. S., Rabie, A. N., Lee, B. T., & Lin, S. J. (2014). Vasopressors and reconstructive flap perfusion: a review of the literature comparing the effects of various pharmacologic agents. *Annals of Plastic Surgery*, 73(2), 245–248. https://doi.org/10.1097/SAP.0b013e31828d70b3

45. Ignacio de Ulíbarri, J., González-Madroño, A., de Villar, N. G., González, P., González, B., Mancha, A., Rodríguez, F., & Fernández, G. (2005). CONUT: a tool for controlling nutritional status. First validation in a hospital population. *Nutricion Hospitalaria*, 20(1), 38–45.

46. Iseki, Y., Shibutani, M., Maeda, K., Nagahara, H., Ohtani, H., Sugano, K., Ikeya, T., Muguruma, K., Tanaka, H., Toyokawa, T., Sakurai, K., & Hirakawa, K. (2015). Impact of the Preoperative Controlling Nutritional Status (CONUT) Score on the Survival after Curative Surgery for Colorectal Cancer. *PloS One*, 10(7), e0132488. https://doi.org/10.1371/journal.pone.0132488

47. Jaxa-Kwiatkowski, A., Jaxa-Kwiatkowski, M., Jaxa-Kwiatkowska, K., Gerber, H., Kubiak, M., & Łysenko, L. (2025). Perioperative Nutritional and Metabolic Factors Affecting Surgical Outcomes in Head and Neck Cancer Free Flap Reconstruction: A Comprehensive Review. *Journal of Clinical Medicine*, 14(11), 3679. https://doi.org/10.3390/jcm14113679

48. Jiang, S., Ai, Y., Ni, L., Wu, L., Huang, X., & Chen, S. (2022). Platelet-derived TGF-β1 is related to portal vein thrombosis in cirrhosis by promoting hypercoagulability and endothelial dysfunction. *Frontiers in Cardiovascular Medicine*, 9, 938397. https://doi.org/10.3389/fcvm.2022.938397

49. Karolczak, K., & Watala, C. (2021). Blood Platelets as an Important but Underrated Circulating Source of TGFβ. *International Journal of Molecular Sciences*, 22(9), 4492. https://doi.org/10.3390/ijms22094492

50. Katna, R., Naik, G., Girkar, F., Deshpande, A., Chalke, S., Bhosale, B., & Kalyani, N. (2023). Clinical outcomes for microvascular reconstruction in oral cancers: experience from a single surgical centre. *Annals of the Royal College of Surgeons of England*, 105(3), 247–251. https://doi.org/10.1308/rcsann.2021.0295

51. Keller U. Nutritional Laboratory Markers in Malnutrition. *Journal of Clinical Medicine*. 2019; 8(6):775. https://doi.org/10.3390/jcm8060775

52. Kim, S. B., Chi, H. S., Park, J. S., Hong, C. D., & Yang, W. S. (1999). Effect of increasing serum albumin on plasma D-dimer, von Willebrand factor, and platelet aggregation in CAPD patients. *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation*, 33(2), 312–317. https://doi.org/10.1016/s0272-6386(99)70306-9

53. Kolbenschlag, J., Diehm, Y., Daigeler, A., Kampa, D., Fischer, S., Kapalschinski, N., Goertz, O., & Lehnhardt, M. (2016). Insufficient fibrinogen response following free flap surgery is associated with bleeding complications. *GMS Interdisciplinary Plastic and Reconstructive Surgery DGPW*, 5, Doc22. https://doi.org/10.3205/iprs000101

54. Košec, A., Solter, D., Ribić, A., Knežević, M., Vagić, D., & Pegan, A. (2022). Systemic Inflammatory Markers as Predictors of Postoperative Complications and Survival in Patients With Advanced Head and Neck Squamous Cell Carcinoma Undergoing Free-Flap Reconstruction. *Journal of Oral and Maxillofacial Surgery : Official Journal of the American Association of Oral and Maxillofacial Surgeons*, 80(4), 744–755. https://doi.org/10.1016/j.joms.2021.12.011

55. Koster, I. T., den Os, M. M., Rutten, M. V., van den Dungen, T. R., de Jong, T., Winters, H. A., & Driessen, C. (2024). The Effect of Regional Anaesthesia on Free Flap Survival in Lower Extremity Reconstructions. *Strategies in Trauma and Limb Reconstruction*, 19(1), 15–20. https://doi.org/10.5005/jp-journals-10080-1612

56. Kutnik, P., Wichowska, O., Sysiak-Sławecka, J., Szczukocka, M., Rypulak, E., Piwowarczyk, P., Borys, M., & Czuczwar, M. (2023). Malnutrition risk in elective surgery patients and effectiveness of preoperative nutritional interventions at a pre-anaesthetic clinic: a 4-year apart, single-centre, observational study. *Anaesthesiology Intensive Therapy*, 55(3), 179–185. https://doi.org/10.5114/ait.2023.130632

57. La Vaccara, V., Cammarata, R., Coppola, A., Farolfi, T., Cascone, C., Angeletti, S., Maltese, G., Coppola, R., & Caputo, D. (2022). Data of postoperative complications related to fibrinogen-to-albumin ratio in pancreatic resections. *Data in Brief*, 42, 108064. https://doi.org/10.1016/j.dib.2022.108064

58. Lacey, H., Kanakopoulos, D., Hussein, S., Moyasser, O., Ward, J., & King, I. C. C. (2023). Adjunctive technologies in postoperative free-flap monitoring: a systematic review. *Journal of Plastic, Reconstructive & Aesthetic Surgery: JPRAS*, 87, 147–155. https://doi.org/10.1016/j.bjps.2023.09.015

59. Laitman, B. M., Ma, Y., Hill, B., Teng, M., Genden, E., DeMaria, S., & Miles, B. A. (2019). Mild hypothermia is associated with improved outcomes in patients undergoing microvascular head and neck reconstruction. *American Journal of Otolaryngology*, 40(3), 418–422. https://doi.org/10.1016/j.amjoto.2019.03.010

60. Le, B., Flier, S., Madill, J., Joyes, C., Dawson, E., Wellington, C., Adekunte, S., Cheng, D., & John-Baptiste, A. (2023). Malnutrition risk, outcomes, and costs among older adults undergoing elective surgical procedures: A retrospective cohort study. *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition*, 38(5), 1045–1062. https://doi.org/10.1002/ncp.11043

61. Lee, K. T., & Mun, G. H. (2015). The efficacy of postoperative antithrombotics in free flap surgery: a systematic review and meta-analysis. *Plastic and Reconstructive Surgery*, 135(4), 1124–1139. https://doi.org/10.1097/PRS.0000000000001100

62. Lee, M., Chin, R. Y., Eslick, G. D., Sritharan, N., & Paramaesvaran, S. (2015). Outcomes of microvascular free flap reconstruction for mandibular osteoradionecrosis: A systematic review. *Journal of Cranio-Maxillo-Facial Surgery : Official Publication of the European Association for Cranio-Maxillo-Facial Surgery*, 43(10), 2026–2033. https://doi.org/10.1016/j.jcms.2015.03.006

63. Lee, S., & Thiele, C. (2010). Factors associated with free flap complications after head and neck reconstruction and the molecular basis of fibrotic tissue rearrangement in preirradiated soft tissue. *Journal of Oral And Maxillofacial Surgery : Official Journal of the American Association of Oral And Maxillofacial Surgeons*, 68(9), 2169–2178. https://doi.org/10.1016/j.joms.2009.08.026

64. Lee, Y. Y., Choi, C. H., Sung, C. O., Do, I. G., Hub, S. J., Kim, H. J., Kim, T. J., Lee, J. W., Bae, D. S., & Kim, B. G. (2012). Clinical significance of changes in peripheral lymphocyte count after surgery in early cervical cancer. *Gynecologic Oncology*, 127(1), 107–113. https://doi.org/10.1016/j.ygyno.2012.05.039

65. Lelas, A., Greinix, H. T., Wolff, D., Eissner, G., Pavletic, S. Z., & Pulanic, D. (2021). Von Willebrand Factor, Factor VIII, and Other Acute Phase Reactants as Biomarkers of Inflammation and Endothelial Dysfunction in Chronic Graft-Versus-Host Disease. *Frontiers in Immunology*, 12, 676756. https://doi.org/10.3389/fimmu.2021.676756

66. Leo, J. A., Simmonds, M. J., & Sabapathy, S. (2020). Shear-thinning behaviour of blood in response to active hyperaemia: Implications for the assessment of arterial shear stress-mediated dilatation. *Experimental Physiology*, 105(2), 244–257. https://doi.org/10.1113/EP088226

67. Lese, I., Biedermann, R., Constantinescu, M., Grobbelaar, A. O., & Olariu, R. (2021). Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *Journal of Plastic, Reconstructive & Aesthetic Surgery JPRAS*, 74(3), 512–522. https://doi.org/10.1016/j.bjps.2020.08.126

68. Lighthall, J. G., Cain, R., Ghanem, T. A., & Wax, M. K. (2013). Effect of postoperative aspirin on outcomes in microvascular free tissue transfer surgery. Otolaryngology – head and neck surgery : *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 148(1), 40–46. https://doi.org/10.1177/0194599812463320

69. Lipa, A. J., Andreikovits, P., Stoeckl, M., Domanovits, H., Schoergenhofer, C., Schwameis, M., & Grafeneder, J. (2025). Fibrinogen-to-Albumin Ratio as Predictor of Mortality in Acute Aortic Syndromes. *Journal of Clinical Medicine*, 14(5), 1669. https://doi.org/10.3390/jcm14051669

70. Lo Buglio, A., Bellanti, F., Carmignano, D. F. P., Serviddio, G., & Vendemiale, G. (2024). Association between Controlling Nutritional Status (CONUT) Score and Body Composition, Inflammation and Frailty in Hospitalized Elderly Patients. *Nutrients*, 16(5), 576. https://doi.org/10.3390/nu16050576

71. Lo, S. L., Yen, Y. H., Lee, P. J., Liu, C. C., & Pu, C. M. (2017). Factors Influencing Postoperative Complications in Reconstructive Microsurgery for Head and Neck Cancer. *Journal of Oral and Maxillofacial Surgery : Official Journal of the American Association of Oral and Maxillofacial Surgeons*, 75(4), 867–873. https://doi.org/10.1016/j.joms.2016.09.025

72. Luyendyk, J. P., Schoenecker, J. G., & Flick, M. J. (2019). The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood,* 133(6), 511–520. https://doi.org/10.1182/blood-2018-07-818211

73. Ma, S., & Wang, L. (2024). Fibrinogen-to-albumin ratio (FAR) is the best biomarker for the overall survival of patients with non-small-cell lung cancer. *Frontiers in Oncology*, 14, 1396843. https://doi.org/10.3389/fonc.2024.1396843

74. Mann, C.J. (2012). Observational research methods – Cohort studies, cross sectional studies, and case–control studies. *African Journal of Emergency Medicine*. 2. 38–46. 10.1016/j.afjem. 2011.12.004.

75. Manz, X. D., Bogaard, H. J., & Aman, J. (2022). Regulation of VWF (Von Willebrand Factor) in Inflammatory Thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 42(11), 1307–1320. https://doi.org/10.1161/ATVBAHA.122.318179

76. Meyer, A., Wang, W., Qu, J., Croft, L., Degen, J. L., Coller, B. S., & Ahamed, J. (2012). Platelet TGF-β1 contributions to plasma TGF-β1, cardiac fibrosis, and systolic dysfunction in a mouse model of pressure overload. *Blood,* 119(4), 1064–1074. https://doi.org/10.1182/blood-2011-09-377648

77. Min, K., Hong, J. P., & Suh, H. P. (2022). Risk Factors for Partial Flap Loss in a Free Flap: A 12-Year Retrospective Study of Anterolateral Thigh Free Flaps in 303 Lower Extremity Cases. *Plastic and Reconstructive Surgery*, 150(5), 1071e–1081e. https://doi.org/10.1097/PRS.0000000000009646

78. 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. https://doi.org/10.1055/s-0040-1715880

79. Mojzisch, A., & Brehm, M. A. (2021). The Manifold Cellular Functions of von Willebrand Factor. *Cells*, 10(9), 2351. https://doi.org/10.3390/cells10092351

80. Möller, C., Schutte, A. E., Smith, W., & Botha-Le Roux, S. (2020). Von Willebrand factor, its cleaving protease (ADAMTS13), and inflammation in young adults: The African-PREDICT study. *Cytokine*, 136, 155265. https://doi.org/10.1016/j.cyto.2020.155265

81. Murphy-Ullrich, J. E., & Poczatek, M. (2000). Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. *Cytokine & Growth Factor Reviews*, 11(1-2), 59–69. https://doi.org/10.1016/s1359-6101(99)00029-5

82. Murphy-Ullrich, J. E., & Suto, M. J. (2018). Thrombospondin-1 regulation of latent TGF-β activation: A therapeutic target for fibrotic disease. *Matrix Biology : Journal of the International Society for Matrix Biology*, 68-69, 28–43. https://doi.org/10.1016/j.matbio.2017.12.009

83. O'Donnell, J. S., Byrne, C., & Preston, R. J. S. (2023). Von Willebrand factor-inflammation crosstalk in deep vein thrombosis. *Journal of Thrombosis and Haemostasis : JTH*, 21(3), 453–455. https://doi.org/10.1016/j.jtha.2022.11.038

84. Olivier, J., Johnson, W. D., & Marshall, G. D. (2008). The logarithmic transformation and the geometric mean in reporting experimental IgE results: what are they and when and why to use them?. *Annals of Allergy, Asthma & Immunology : Official Publication of the American College of Allergy, Asthma, & Immunology*, 100(4), 333–337. https://doi.org/10.1016/S1081-1206(10)60595-9

85. Paar, M., Rossmann, C., Nusshold, C., Wagner, T., Schlagenhauf, A., Leschnik, B., Oettl, K., Koestenberger, M., Cvirn, G., & Hallström, S. (2017). Anticoagulant action of low, physiologic, and high albumin levels in whole blood. *PloS One*, 12(8), e0182997. https://doi.org/10.1371/journal.pone.0182997

86. Park, S., Nam, K., & Kim, T. K. (2022). Association Between Preoperative Fibrinogen-to-Albumin Ratio and All-Cause Mortality After Off-Pump Coronary Artery Bypass Grafting: A Retrospective Observational Study. *Anaesthesia and Analgesia*, 134(5), 1021–1027. https://doi.org/10.1213/ANE.0000000000005948

87. Pihusch, V., Pihusch, M., Penovici, M., Kolb, H. J., Hiller, E., & Pihusch, R. (2005). Transforming growth factor beta-1 released from platelets contributes to hypercoagulability in veno-occlusive disease following hematopoetic stem cell transplantation. *Thrombosis Research*, 116(3), 233–240. https://doi.org/10.1016/j.thromres.2004.12.010

88. Pohlenz, P., Klatt, J., Schön, G., Blessmann, M., Li, L., & Schmelzle, R. (2012). Microvascular free flaps in head and neck surgery: complications and outcome of 1000 flaps. *International journal of Oral and Maxillofacial Surgery*, 41(6), 739–743. https://doi.org/10.1016/j.ijom.2012.02.012

89. Portuondo, J. I., Probstfeld, L., Massarweh, N. N., Le, L., Wei, Q., Chai, C. Y., Taylor, J., Awad, S. S., & Tran Cao, H. S. (2020). Malnutrition in elective surgery: How traditional markers might be failing surgeons and patients. *Surgery*, 168(6), 1144–1151. https://doi.org/10.1016/j.surg.2020.08.012

90. Qian, Y., Liu, H., Pan, J., Yu, W., Lv, J., Yan, J., Gao, J., Wang, X., Ge, X., & Zhou, W. (2021). Preoperative Controlling Nutritional Status (CONUT) score predicts short-term outcomes of patients with gastric cancer after laparoscopy-assisted radical gastrectomy. *World Journal of Surgical Oncology*, 19(1), 25. https://doi.org/10.1186/s12957-021-02132-6

91. Reed, W. T., Jiang, R., Ohnuma, T., Kahmke, R. R., Pyati, S., Krishnamoorthy, V., Raghunathan, K., & Osazuwa-Peters, N. (2024). Malnutrition and Adverse Outcomes After Surgery for Head and Neck Cancer. *JAMA Otolaryngology – Head & Neck Surgery*, 150(1), 14–21. https://doi.org/10.1001/jamaoto.2023.3486

92. Ren, J. Y., Wang, D., Zhu, L. H., Liu, S., Yu, M., & Cai, H. (2024). Combining systemic inflammatory response index and albumin fibrinogen ratio to predict early serious complications and prognosis after resectable gastric cancer. *World Journal of Gastrointestinal Oncology*, 16(3), 732–749. https://doi.org/10.4251/wjgo.v16.i3.732

93. Rothweiler, R. M., Metzger, M. C., Zieger, B., Huber-Schumacher, S., Schmelzeisen, R., & Kalbhenn, J. (2021). The Role of von Willebrand Factor in Microvascular Surgery in Severely Injured Patients. *Plastic and Reconstructive Surgery. Global open*, 9(10), e3836. https://doi.org/10.1097/GOX.0000000000003836

94. Rothweiler, R., Gerlach, V., Voss, P., Poxleitner, P., Ermer, M., Gross, C., Schwer, C., Vach, K., Kalbhenn, J., & Metzger, M. (2022). Aspirin, heparin and ischemia time in microvascular free flap surgery - their influence and an optimal anticoagulation protocol. *Journal of Stomatology, Oral and Maxillofacial Surgery*, 123(5), e556–e562. https://doi.org/10.1016/j.jormas.2022.03.001

95. Salibian, A. A., Frey, J. D., Karp, N. S., & Choi, M. (2018). Abstract: Transversus Abdominis Plane Blocks in Microsurgical Breast Reconstruction: An Analysis of Pain, Narcotic Consumption, Length of Stay and Cost Implications. *Plastic and Reconstructive Surgery Global Open*, 6(9 Suppl), 174–175. https://doi.org/10.1097/01.GOX.0000547056.22407.b1

96. Schmidt, A., Bengtsson, A., Tylman, M., & Blomqvist, L. (2007). Pro-inflammatory cytokines in elective flap surgery. *The Journal of Surgical Research*, 137(1), 117–121. https://doi.org/10.1016/j.jss.2006.05.040

97. Schuderer, J. G., Dinh, H. T., Spoerl, S., Taxis, J., Fiedler, M., Gottsauner, J. M., Maurer, M., Reichert, T. E., Meier, J. K., Weber, F., & Ettl, T. (2023). Risk Factors for Flap Loss: Analysis of Donor and Recipient Vessel Morphology in Patients Undergoing Microvascular Head and Neck Reconstructions. *Journal of Clinical Medicine*, 12(16), 5206. https://doi.org/10.3390/jcm12165206

98. Shang, J., Jiang, S., Gong, J., Zhao, G., Su, D., & Wang, L. (2023). Low albumin-to-fibrinogen ratio predicts adverse clinical outcomes after primary total joint arthroplasty: A retrospective observational investigation. I*nternational Wound Journal*, 20(9), 3690–3698. https://doi.org/10.1111/iwj.14260

99. Shankhdhar, V. K., Mantri, M. R., Wagh, S., Thiagarajan, S., Chaukar, D., Jaiswal, D., & Mathews, S. (2022). Microvascular Flap Reconstruction for Head and Neck Cancers in Previously Operated and/or Radiated Neck: Is It Safe?. *Annals of Plastic Surgery*, 88(1), 63–67. https://doi.org/10.1097/SAP.0000000000002951

100. Shum, J., Markiewicz, M. R., Park, E., Bui, T., Lubek, J., Bell, R. B., & Dierks, E. J. (2014). Low prealbumin level is a risk factor for microvascular free flap failure. *Journal of Oral and Maxillofacial Surgery : Official Journal of the American Association of Oral and Maxillofacial Surgeons*, 72(1), 169–177. https://doi.org/10.1016/j.joms.2013.05.022

101. Sinha, S., Ruskin, O., D'Angelo, A., McCombe, D., Morrison, W. A., & Webb, A. (2016). Are overweight and obese patients who receive autologous free-flap breast reconstruction satisfied with their postoperative outcome? A single-centre study. *Journal of Plastic, Reconstructive & Aesthetic Surgery* : JPRAS, 69(1), 30–36. https://doi.org/10.1016/j.bjps.2015.08.036

102. Smeda, M., Maleki, E. H., & Jasztal, A. (2024). A possible role of plasmin-dependent activation of TGF-β in cancer-associated thrombosis: Implications for therapy. *Cancer Metastasis Reviews*, 44(1), 2. https://doi.org/10.1007/s10555-024-10222-6

103. Snow, T. A. C., Waller, A. V., Loye, R., Ryckaert, F., Cesar, A., Saleem, N., Roy, R., Whittle, J., Al-Hindawi, A., Das, A., Singer, M., Brealey, D., Arulkumaran, N., & University College London Hospitals Critical Care Research Team (2024). Early dynamic changes to monocytes following major surgery are associated with subsequent infections. *Frontiers in Immunology*, 15, 1352556. https://doi.org/10.3389/fimmu.2024.1352556

104. Stevens, M. N., Freeman, M. H., Shinn, J. R., Kloosterman, N., Carr, S., Mannion, K., & Rohde, S. L. (2023). Preoperative Predictors of Free Flap Failure. Otolaryngology – head and neck surgery : *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 168(2), 180–187. https://doi.org/10.1177/01945998221091908

105. Stevens, M. N., Prasad, K., Sharma, R. K., Gallant, J. N., Habib, D. R. S., Langerman, A., Mannion, K., Rosenthal, E., Topf, M. C., & Rohde, S. L. (2024). Comparative Outcomes for Microvascular Free Flap Monitoring Outside the Intensive Care Unit. Otolaryngology – head and neck surgery : *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 171(2), 381–386. https://doi.org/10.1002/ohn.780

106. Takagi, K., Domagala, P., Polak, W. G., Buettner, S., Wijnhoven, B. P. L., & Ijzermans, J. N. M. (2019). Prognostic significance of the controlling nutritional status (CONUT) score in patients undergoing gastrectomy for gastric cancer: a systematic review and meta-analysis. *BMC Surgery*, 19(1), 129. https://doi.org/10.1186/s12893-019-0593-6

107. Tapia, B., Garrido, E., Cebrian, J. L., Del Castillo, J. L., Gonzalez, J., Losantos, I., & Gilsanz, F. (2021). Impact of Goal Directed Therapy in Head and Neck Oncological Surgery with Microsurgical Reconstruction: Free Flap Viability and Complications. *Cancers*, 13(7), 1545. https://doi.org/10.3390/cancers13071545

108. Tarabishy, S. P., Inglesby, D., Tapp, M., Corral, G. D., & Herrera, F. A. (2020). Thrombocytosis is associated with complications after microvascular surgery: An NSQIP data analysis. *Microsurgery*, 40(3), 288–297. https://doi.org/10.1002/micr.30530

109. Tarle, M., Čvrljević, I., Raguž, M., & Lukšić, I. (2023). Hemoglobin-Albumin-Lymphocyte-Platelet (HALP) Score as a Predictive Model for the Success of Reconstruction of Head and Neck Defects with Free Microvascular Flaps. *Journal of Clinical Medicine*, 12(16), 5314. https://doi.org/10.3390/jcm12165314

110. Tobert, C. M., Hamilton-Reeves, J. M., Norian, L. A., Hung, C., Brooks, N. A., Holzbeierlein, J. M., Downs, T. M., Robertson, D. P., Grossman, R., & Nepple, K. G. (2017). Emerging Impact of Malnutrition on Surgical Patients: Literature Review and Potential Implications for Cystectomy in Bladder Cancer. *The Journal of Urology*, 198(3), 511–519. https://doi.org/10.1016/j.juro.2017.01.087

111. Tomita, K., Ochiai, S., Gunji, T., Hikita, K., Kobayashi, T., Sano, T., Chiba, N., & Kawachi, S. (2020). Prognostic Significance of Plasma Fibrinogen/Serum Albumin Ratio in the Postoperative Outcome of Pancreatic Ductal Adenocarcinoma. *Anticancer Research*, 40(12), 7017–7023. https://doi.org/10.21873/anticanres.14727

112. Toyokawa, T., Kubo, N., Tamura, T., Sakurai, K., Amano, R., Tanaka, H., Muguruma, K., Yashiro, M., Hirakawa, K., & Ohira, M. (2016). The pretreatment Controlling Nutritional Status (CONUT) score is an independent prognostic factor in patients with resectable thoracic esophageal squamous cell carcinoma: results from a retrospective study. *BMC Cancer*, 16(1), 722. https://doi.org/10.1186/s12885-016-2696-0

113. Tredget, E. B., Demare, J., Chandran, G., Tredget, E. E., Yang, L., & Ghahary, A. (2005). Transforming growth factor-beta and its effect on reepithelialization of partial-thickness ear wounds in transgenic mice. Wound repair and regeneration : *Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, 13(1), 61–67. https://doi.org/10.1111/j.1067-1927.2005.130108.x

114. Tsai, M. H., Chuang, H. C., Lin, Y. T., Lu, H., Chen, W. C., Fang, F. M., & Chien, C. Y. (2018). Clinical impact of albumin in advanced head and neck cancer patients with free flap reconstruction-a retrospective study. *PeerJ*, 6, e4490. https://doi.org/10.7717/peerj.4490

115. Tsyu, N. G., & Belyaev, A. V. (2023). Coarse-grained simulations of von Willebrand factor adsorption to collagen with consequent platelet recruitment. *International Journal for Numerical Methods in Biomedical Engineering*, 39(11), e3747. https://doi.org/10.1002/cnm.3747

116. Unal I. (2017). Defining an Optimal Cut-Point Value in ROC Analysis: An Alternative Approach. *Computational and Mathematical Methods in Medicine*, 2017, 3762651. https://doi.org/10.1155/2017/3762651

117. Undas, A., & Ariëns, R. A. (2011). Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31(12), e88–e99. https://doi.org/10.1161/ATVBAHA.111.230631

118. Utariani, A., Rahardjo, E., & Perdanakusuma, D. S. (2020). Effects of Albumin Infusion on Serum Levels of Albumin, Proinflammatory Cytokines (TNF-α, IL-1, and IL-6), CRP, and MMP-8; Tissue Expression of EGRF, ERK1, ERK2, TGF-β, Collagen, and MMP-8; and Wound Healing in Sprague Dawley Rats. *International Journal of Inflammation*, 2020, 3254017. https://doi.org/10.1155/2020/3254017

119. 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, 289. https://doi.org/10.3389/fmed.2020.00289

120. Venianaki, M., Andreou, A., Nikolouzakis, T. K., Chrysos, E., Chalkiadakis, G., & Lasithiotakis, K. (2021). Factors Associated with Malnutrition and Its Impact on Postoperative Outcomes in Older Patients. *Journal of Clinical Medicine*, 10(12), 2550. https://doi.org/10.3390/jcm10122550

121. Vincent, A., Sawhney, R., & Ducic, Y. (2019). Perioperative Care of Free Flap Patients. *Seminars in Plastic Surgery*, 33(1), 5–12. https://doi.org/10.1055/s-0038-1676824

122. Walshe, T. E., dela Paz, N. G., & D'Amore, P. A. (2013). The role of shear-induced transforming growth factor-β signaling in the endothelium. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 33(11), 2608–2617. https://doi.org/10.1161/ATVBAHA.113.302161

123. Wang, L., Wang, H., Zhu, M., Ni, X., Sun, L., Wang, W., Xie, J., Li, Y., Xu, Y., Wang, R., Han, S., Zhang, P., Peng, J., Hou, M., & Hou, Y. (2024). Platelet-derived TGF-β1 induces functional reprogramming of myeloid-derived suppressor cells in immune thrombocytopenia. *Blood,* 144(1), 99–112. https://doi.org/10.1182/blood.2023022738

124. Wang, W., Vootukuri, S., Meyer, A., Ahamed, J., & Coller, B. S. (2014). Association between shear stress and platelet-derived transforming growth factor-β1 release and activation in animal models of aortic valve stenosis. A*rteriosclerosis, Thrombosis, and Vascular Biology*, 34(9), 1924–1932. https://doi.org/10.1161/ATVBAHA.114.303852

125. Wells, J. C., Sawaya, A. L., Wibaek, R., Mwangome, M., Poullas, M. S., Yajnik, C. S., & Demaio, A. (2020). The double burden of malnutrition: aetiological pathways and consequences for health. *Lancet (London, England)*, 395(10217), 75–88. https://doi.org/10.1016/S0140-6736(19)32472-9

126. Westein, E., van der Meer, A. D., Kuijpers, M. J., Frimat, J. P., van den Berg, A., & Heemskerk, J. W. (2013). Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), 1357–1362. https://doi.org/10.1073/pnas.1209905110

127. Williams, J. G., French, R. J., & Lalonde, D. H. (2004). Why do free flap vessels thrombose? Lessons learned from implantable Doppler monitoring. *The Canadian Journal of Plastic Surgery = Journal Canadien de Chirurgie Plastique*, 12(1), 23–26. https://doi.org/10.1177/22925503 0401200112

128. Winstedt, D., Hanna, J., & Schött, U. (2013). Albumin-induced coagulopathy is less severe and more effectively reversed with fibrinogen concentrate than is synthetic colloid-induced coagulopathy. *Scandinavian Journal of Clinical and Laboratory Investigation*, 73(2), 161–169. https://doi.org/10.3109/00365513.2012.762114

129. Wu, Z. Y., Zhou, Y., Ma, S. R., Yu, Z. L., & Jia, J. (2024). Multi-factor early monitoring method based on D-dimer for iliac crest flap loss. *BMC Oral Health*, 24(1), 963. https://doi.org/10.1186/s 12903-024-04712-w

130. Xiao, Q., Li, X., Duan, B., Li, X., Liu, S., Xu, B., Shi, S., Zhang, J., Qin, H., Duan, X., & Pu, Y. (2022). Clinical significance of controlling nutritional status score (CONUT) in evaluating outcome of postoperative patients with gastric cancer. *Scientific Reports*, 12(1), 93. https://doi.org/ 10.1038/s41598-021-04128-4

131. Xu, H., Han, Z., Ma, W., Zhu, X., Shi, J., & Lin, D. (2021). Perioperative Albumin Supplementation is Associated With Decreased Risk of Complications Following Microvascular Head and Neck Reconstruction. *Journal of Oral and Maxillofacial Surgery : Official Journal of the American Association of Oral and Maxillofacial Surgeons*, 79(10), 2155–2161. https://doi.org/ 10.1016/j.joms.2021.04.030

132. Xu, J., Li, S., Feng, Y., Zhang, J., Peng, Y., Wang, X., & Wang, H. (2022). The Fibrinogen/Albumin Ratio Index as an Independent Prognostic Biomarker for Patients with Combined Hepatocellular Cholangiocarcinoma After Surgery. *Cancer Management and Research*, 14, 1795–1806. https://doi.org/10.2147/CMAR.S361462

133. Young, V. L., & Watson, M. E. (2006). Prevention of perioperative hypothermia in plastic surgery. *Aesthetic Surgery Journal*, 26(5), 551–571. https://doi.org/10.1016/j.asj.2006.08.009

134. Yu, J., Hong, J. P., Suh, H. P., Park, J. Y., Kim, D. H., Ha, S., Lee, J., Hwang, J. H., & Kim, Y. K. (2020). Prognostic Nutritional Index is a Predictor of Free Flap Failure in Extremity Reconstruction. *Nutrients*, 12(2), 562. https://doi.org/10.3390/nu12020562

135. Zeineddin, A., Dong, J. F., Wu, F., Terse, P., & Kozar, R. A. (2021). Role of Von Willebrand Factor after Injury: It May Do More Than We Think. *Shock* (Augusta, Ga.), 55(6), 717–722. https://doi.org/10.1097/SHK.0000000000001690

136. Zhang, F., Hu, E. C., Topp, S., Lei, M., Chen, W., & Lineaweaver, W. C. (2006). Proinflammatory cytokines gene expression in skin flaps with arterial and venous ischemia in rats. *Journal of Reconstructive Microsurgery*, 22(8), 641–647. https://doi.org/10.1055/s-2006-956238

137. Zhang, L. P., Ren, H., Du, Y. X., Zheng, X. H., Zhang, Z. M., & Wang, C. F. (2021). Combination of preoperative fibrinogen and D-dimer as a prognostic indicator in pancreatic ductal adenocarcinoma patients undergoing R0 resection. *World Journal of Gastrointestinal Surgery*, 13(3), 279–302. https://doi.org/10.4240/wjgs.v13.i3.279

138. Zhang, S., Li, Y., Zhang, J., Sun, Y., Chu, X., Gui, X., Tong, H., Ding, Y., Ju, W., Xu, M., Li, Z., Zeng, L., Xu, K., & Qiao, J. (2024). Platelet-Derived TGF-β1 Promotes Deep Vein Thrombosis. *Thrombosis and Haemostasis*, 124(7), 641–648. https://doi.org/10.1055/a-2235-7485

Acknowledgments

First and foremost, I would like to sincerely thank my research supervisors, Emeritus Professor Biruta Mamaja and Associate Professor Simona Doniņa, for their invaluable support throughout my research and the writing of this Thesis. I am especially grateful to Emeritus Professor Biruta Mamaja for her dedication, guidance, and encouragement, which have meant a great deal to me. Her insightful advice and unwavering support helped shape this work and contributed greatly to my development as a researcher. I truly appreciate the time she invested in my progress and the belief she had in me.

I am also deeply thankful to Associate Professor Simona Doniņa for her trust, supervision, and thoughtful recommendations during my PhD journey. Her mentorship has been a key influence in refining my research skills.

My heartfelt thanks go to my good colleague, Dr. med. Jānis Zariņš, whose expertise, energy, and generous help with the surgical aspects of this project were crucial. His insight greatly enriched the quality of this research. I am grateful to my friend and colleague Evita Bine for her unwavering support and assistance throughout the research process.

I would also like to thank Margarita Citoviča and Renārs Deksnis for their help in planning the research methodology and handling the samples. My appreciation extends to Sabīne Grāvelsiņa, Anda Vīlmane, and Santa Rasa-Dzelzkalēja for their dedicated support with the laboratory work. Each of them has played an important part in making this research possible.

Lastly, and most importantly, I want to thank my family for their unwavering love, patience, and encouragement. Their support has given me the strength to keep going, even during the most challenging times. I am especially grateful to my wife, Kristiāna, for her endless care and belief in me, and to our children – Alise, Nora, and Aleksandrs – for bringing joy to my life.

Annexes

First Publication

clinics and practice

MDPI

Review

Novel Challenges and Opportunities for Anesthesia and Perioperative Care in Microvascular Flap Surgery: A Narrative Review

Aleksi Matias Ojuva [1,2,*], Rihards Peteris Rocans [1,3], Janis Zarins [4,5], Evita Bine [3], Insana Mahauri [1], Simona Donina [6,7], Biruta Mamaja [1] and Indulis Vanags [1]

[1] Department of Anaesthesia and Intensive Care, Riga Stradiņš University, Dzirciema Street 16, LV-1007 Riga, Latvia; rihardspeteris.rocans@rsu.lv (R.P.R.); insana.mahauri@rsu.lv (I.M.); biruta.mamaja@aslimnica.lv (B.M.); indulis.vanags@rsu.lv (I.V.)
[2] Department of Internal Diseases, South Karelia Central Hospital, Valto Kakelan Street 1, 53130 Lappeenranta, Finland
[3] Intensive Care Clinic, Riga East Clinical University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; evitabine@rsu.edu.lv
[4] Department of Hand and Plastic Surgery, Microsurgery Centre of Latvia, Brivibas Street 410, LV-1024 Riga, Latvia; janis.zarins@mcl.lv
[5] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Pulka Street 3, LV-1007 Riga, Latvia
[6] Institute of Microbiology and Virology, Riga Stradins University, Ratsupites Street 5, LV-1067 Riga, Latvia; donsimon@inbox.lv
[7] Outpatient Department, Riga East Clinical University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia
* Correspondence: aleksi.ojuva@ekhva.fi or aleksi.ojuva@outlook.com

Abstract: Complex microvascular techniques and in-depth knowledge of blood rheology and microanastomosis function are required for success in microvascular flap surgery. Substantial progress has been achieved in preventing complications, but the rate of flap loss is still significant and can have significant adverse effects on the patient. Flap thrombosis, flap hematoma, and flap loss are the most frequent and severe major surgical complications. Advances in understanding the pathophysiology of different flap complications, the use of preoperative risk assessment and new treatment concepts could improve the perioperative care of microvascular flap surgery patients. Our aim was to outline novel avenues for best practice and provide an outlook for further research of anesthesia and perioperative care concepts in microvascular flap surgery.

Keywords: anesthesia; perioperative care; microvascular flap complications; reconstructive surgery

Citation: Ojuva, A.M.; Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Donina, S.; Mamaja, B.; Vanags, I. Novel Challenges and Opportunities for Anesthesia and Perioperative Care in Microvascular Flap Surgery: A Narrative Review. *Clin. Pract.* **2024**, *14*, 2187–2201. https://doi.org/10.3390/clinpract14050172

Academic Editors: Athanassios A. Kyrgidis and Ioannis Tilaveridis

Received: 23 September 2024
Revised: 13 October 2024
Accepted: 17 October 2024
Published: 18 October 2024

1. Introduction

Microvascular flap surgery has secured its place in reconstructive surgery as an important technique to achieve the closure of various tissue defects [1–3]. The defect that requires correction can be caused by different etiologies, including trauma, oncology, chronic infection, or wounds of multiple etiologies [1]. The use of free flaps, in contrast to conventional surgical techniques, offers a wider range of donor sites, resulting in improved flap characteristics, such as size, function, tissue components, and form [2]. The use of free flaps also gives the benefit of earlier mobilization, reduced hospital stay and costs [2]. Despite good progress in surgical techniques and reduction of complication rates, flap loss remains a challenge in perioperative care for both surgeons and anesthesiologists. The main complication groups are true flap loss, minor flap complications, and flap hematoma [1,4]. The complication rate varies among studies, although overall, it is between 3 and 6% [3,5–7]. Venous thrombosis is the most common cause of true flap loss, while arterial thrombosis is the most common cause of early true flap loss [8]. Other more minor postoperative complications include infection, partial flap necrosis, postoperative bleeding, seroma, and

wound dehiscence [3]. Preoperatively, it is important to evaluate and ensure that the current comorbidities of the patient are controlled. Like in all major surgeries, the cardiac and respiratory condition should be optimal, including control of high blood pressure and high serum glucose level [1]. Multiple recent studies have described the pathophysiology of different flap complications and the use of biomarkers for preoperative risk assessment [8–10]. These biomarkers may open new avenues for the improvement of perioperative care. After preoperative preparations, high-quality intraoperative care is equally important and must employ good temperature, pain, and sympathetic control to prevent vasoconstriction [11–15]. Optimizing blood pressure and fluid management to maintain high cardiac output and low systemic vascular resistance is crucial to ensure the success of the surgery [1]. Postoperative antithrombotic treatment should be balanced to avoid both bleeding and thrombotic complications [16]. The issues presented are multifaceted; however, perioperative care in a multidisciplinary team-based approach could improve the results of microvascular flap surgery [17]. The aim of this narrative review is to outline novel avenues for best practice in microvascular flap surgery and provide an outlook for further research on preoperative risk assessment, anesthesia, and perioperative care. This narrative review separately addresses the three main groups of issues in preoperative, intraoperative, and postoperative care (Figure 1).

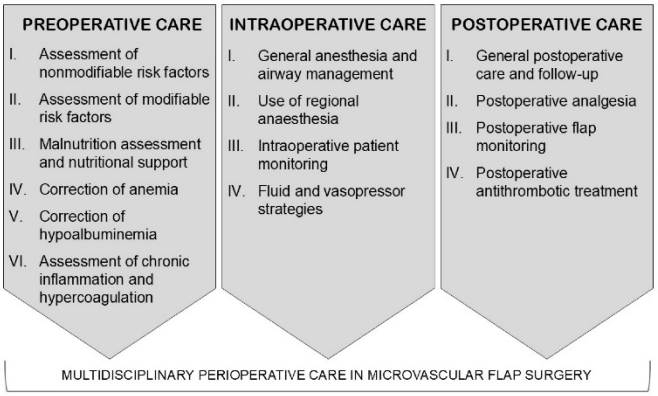

Figure 1. Suggested multidisciplinary team-based approach framework for perioperative care in microvascular flap surgery.

2. Materials and Methods

For this literature review, PubMed, Scopus, and Web of Science databases were used. The search was carried out using the following algorithm and key terms: ''preoperative risk", ''risk factors", ''biomarkers", ''fibrinogen", ''malnutrition", ''comorbidities", ''anemia", ''coagulation", ''coagulogy assessment", ''inflammation", ''regional anesthesia", ''general anesthesia", ''analgesia", ''crystalloid", ''vasopressor", ''fluid", ''monitoring", ''anticoagulants", ''antiaggregants", ''intraoperative care", ''preoperative care", ''postoperative care", "continuous instrumental free flap monitoring" combined with ''free flap failure", ''true flap loss", ''minor flap complications", ''flap complications", ''free flap thrombosis", ''free flap surgery", or ''microvascular flap surgery". Articles published from 1 January 2007 to 1 July 2024 were selected and analyzed. The articles were selected for the review based on relevance to daily clinical practice, with an emphasis on the studies reflecting new avenues for predicting risk, providing individualized patient care, and improving outcomes for clinical practice. Upon selection for review, the highest priority was placed on randomized clinical trials, larger observational studies, and the most recent articles.

The review uses the following definitions for complications: true flap loss is defined as the impairment of the flap blood flow due to venous or arterial anastomosis dysfunction or

thrombosis leading to congestion or ischemia and complete loss of the free transposed flap; flap hematoma is defined as the presence of a postoperative hematoma adjacent to the flap recipient site without interfering with the flap blood supply either due to surgical causes or insufficient coagulation function; minor flap complications are defined as the presence of flap recipient or donor site wound infection, slow or difficult flap wound healing, partial or marginal flap necrosis, or difficult healing at the donor site.

3. Results and Discussion

3.1. Preoperative Assessment of Non-Modifiable Risk Factors

To properly evaluate preoperative risk factors, it is important to assess the patient's non-modifiable risk factors [3,18]. Evidence on the gender of the patient in relation to the risk of flap failure remains inconclusive. A study by Sanati-Mehrizy et al. that included 1921 patients identified male gender as an independent risk factor for flap failure in all flap types included in the study [18]. However, some studies also found no statistically significant link between male gender and free flap failure [3], and on the other hand, female sex was found to be a risk factor in the case of head and neck free flap failure [10].

Age was not identified as a direct risk factor for flap failure on its own [3]. Despite the increased rate of vascular damage and atherosclerotic changes in older patients, the risk of flap thrombosis is not considered to be higher [3]. Therefore, age should not be a contraindication to microvascular flap surgery [3,18].

The etiology of the defect is non-modifiable at the time of surgery and might also influence the risk of free flap failure. Among the patients in the study conducted by Lese et al., it was found that in elective non-cancer patients, vascular compromise occurred in 3.8% of cases, the cancer group had a compromise rate of 6.9%, and in the trauma group, the rate was even higher at 8.9% [3]. Another study by Bui et al. also found that a history of trauma is linked to increased rates of flap complications [19].

3.2. Preoperative Assessment of Comorbidities and Modifiable Risk Factors

Patient comorbidities should be evaluated before surgery to predict and potentially improve outcomes. Comorbidities and factors that have been identified as a risk factor for flap failure include diabetes mellitus [3], peripheral vascular disease [3,8], arterial hypertension [3], coronary heart disease [3], renal failure [6], recent radiotherapy [6], and smoking [18]. However, not all of these were recognized in all studies as independent risk factors, and some of them remain inconclusive. The ASA class should be considered as one of the possible predictors of postoperative complications, with a higher ASA class being a risk factor for flap failure [3]. Smoking was identified as a risk factor for free flap failure in some studies [18], but there were others in which it was not considered an independent risk factor [3,8,20]. Smoking cessation 4–8 weeks before surgery is generally preferred to decrease perioperative morbidity and improve wound healing [20,21].

3.3. Preoperative Malnutrition Risk Assessment and Nutritional Support

Nutritional status is an important feature that influences surgical outcome, and malnutrition can be a major risk factor for complications in all surgical patients [22]. Malnutrition has been found to negatively impact wound healing [23], and the same applies to microvascular flap surgery [4,5]. A recent study by Yu et al. showed that a low prognostic nutritional index (PNI) was found to be a risk factor for free flap failure [5]. PNI is calculated using the serum albumin and total lymphocyte count ($10 \times$ serum albumin g/dL $+ 0.005 \times$ total lymphocyte count per microliter) [5]. The study used the cutoff of PNI < 40 to predict the risk of flap complications [5].

In another study, the Controlling Nutritional Status Score (CONUT) was used to indicate the risk of malnutrition [4]. An increased preoperative CONUT score was found to reliably predict flap complications with a score of more than 2 [4]. This score includes some of the same parameters as PNI, such as serum albumin and total lymphocyte count, but also includes total cholesterol [4].

Although evidence can be found for the assessment of the risk of malnutrition, direct evidence for nutritional support in microvascular flap surgery is limited. Upon the writing of this review, only a single clinical trial by Hwang et al. showed that intraoperative enteral feeding during microvascular flap reconstruction in head and neck surgery enhances wound regeneration [24].

Options for nutritional support include oral supplementation, enteral feeding (via a gastric or postpyloric tube), or parenteral feeding [25]. Enteral feeding is generally preferred to parenteral feeding if possible due to better safety, reduced complications, and cost [25]. Providing perioperative nutritional support could benefit patients who are malnourished but also lead to postponed surgery and the additional cost of artificial nutrition [26]. Additionally, it is worth noting that preoperative fasting should be minimized. Clear fluids should be allowed up to 2 h before the beginning of anesthesia, and solid foods up to 6 h prior [12].

In summary, patients with both mild and severe malnutrition may benefit from preoperative nutritional evaluation [4,5] and nutritional support [4], and intraoperative or early postoperative enteral nutrition [12,24] should be encouraged.

3.4. Correction of Preoperative Anemia

In microvascular surgery, it is generally accepted that hemoglobin will decrease during the surgery due to hemodilution. In fact, it is traditionally accepted that hemodilution and hyperdynamic circulation can reduce the risk of anastomotic failure [27]. However, both preoperative anemia and intraoperative hematocrit < 30% could be associated with flap failure [28,29]. Furthermore, preoperative anemia is linked to impaired wound healing [30]. A meta-analysis of 4984 patients indicated that preoperative anemia is a possible risk factor for free flap failure [29]. It also indicated that postoperative transfusions are associated with more complications [29]. Perioperatively, the amount of blood loss and blood transfusions should be limited, as they could increase the risk of flap failure and other complications like infection [31]. This, in turn, leads to the importance of identifying preoperative anemia to prevent intraoperative transfusions during surgery [30].

If the cause of anemia is iron deficiency, intravenous or oral iron administration should be considered if the Hb is below 120, depending on the time to the surgery [30,31]. It should be noted that when administering iron treatment, there should be enough time for the correction of the anemia to be effective [31]. For partial correction, two to four weeks, and for full correction, six to eight weeks should be sufficient [31,32]. Intravenous iron corrects iron-deficiency anemia more rapidly than oral iron, but it still requires weeks to improve the results of the complete blood count [32], which must be taken into account during surgical planning.

3.5. Correction of Hypoalbuminemia

Albumin is an important protein involved in the regulation of serum osmolality, tissue repair, and systemic inflammation [33]. It acts as the main extracellular scavenger in the interstitium and as an antioxidant, and it also provides amino acids for the synthesis of tissues [33]. In the case of inflammation, capillary permeability increases, which, in turn, leads to an expansion of the interstitial space and an increased distribution of albumin [33]. Furthermore, the half-life of albumin has been shown to decrease the albumin levels in the case of an inflammatory condition [33]. This leads to the conclusion that hypoalbuminemia correlates with inflammation and can be a predictive factor for a negative outcome of the surgery [33,34].

Min, Hong, and Suh found in their study that when the preoperative albumin level was increased by 1 g/dL, the probability of partial flap loss was reduced to less than half [1]. Another study found that preoperative hypoalbuminemia is a negative prognostic factor in patients who have had tumor excision and free flap reconstruction in an advanced stage of head and neck squamous cell carcinoma [9]. A study by da Silva et al. that included 35 patients found that hypoalbuminemia had no impact on the frequency of complications

in extremity free flap reconstruction but was associated with a prolonged hospital stay [35]. As mentioned in the previous section, low albumin levels as a marker of malnutrition risk have been associated with an increased risk of free flap complications [4,5,36]. If low albumin levels are measured preoperatively, the cause of hypoalbuminemia should be considered, whether it is due to malnutrition, inflammation, or other causes [33].

Intriguingly, there is evidence that perioperative albumin supplementation may reduce postoperative complications and shorten hospital stays. A study by Xu et al. included 315 patients who underwent oral and maxillofacial tumor resection and reconstruction with free flap. It found that administering 100 mL of 20% albumin intraoperatively and 50 mL every day for 2 postoperative days was associated with fewer local complications and shortened hospital stays. However, this had no effect on systemic complications [37].

3.6. Other Preoperative Biomarkers

Fibrinogen plays an important role in tissue inflammation, wound healing, and hemostasis [38]. It is produced mainly by the liver and is also considered an acute-phase protein, so it is also increased by inflammatory reactions [38]. A study by Handschel et al. found that increased preoperative plasma fibrinogen is associated with true flap loss [39]. A study by Drizlionoka et al. involving carriers of a single-nucleotide polymorphism in the gene coding for the gamma chain of fibrinogen showed that these individuals had higher plasma fibrinogen levels and, therefore, also had a higher rate of free flap thrombosis [10].

A recent study found that a high fibrinogen/albumin ratio was associated with decreased microvascular perfusion in patients with ST elevation myocardial infarction who underwent primary percutaneous coronary intervention [40]. A higher fibrinogen-to-albumin ratio has also been associated with poorer prognosis in cancer patients [41,42]. A high fibrinogen-to-albumin ratio could indicate both a state of chronic inflammation [43] and hypercoagulation [44], and both conditions could be hypothesized to be linked to predict flap complications in the future. A study by Vanags et al. showed that Rotational Thromboelastometry could be used in microvascular flap surgery to predict hypercoagulable states and true flap loss in late-surgery trauma cases [45]. When studying only head and neck reconstructions, Stevens et al. found preoperative thrombocytosis to be a strong predictor of flap failure. Whether or not to use antithrombotic treatment in order to prevent this still remains controversial [8].

The neutrophil-to-lymphocyte ratio (NLR) is another biomarker of both inflammation and malnutrition risk [46,47]. A study by Chargi et al. found both the NLR and low skeletal muscle mass are associated with flap complications and increased length of stay [48].

3.7. General Anesthesia (GA) and Airway Management

Choosing the appropriate anesthetic technique is dependent on a number of different clinical factors. Anesthesia decision-making is influenced by surgical requirements for the procedure, expected duration, patient comorbidities, postoperative analgesia, and individual anesthesia factors [49]. Generally, due to the multiple surgical sites involved and the positioning of the patient, most microvascular reconstructions are performed under general anesthesia [50]. Specifically, for head and neck reconstruction, airway management for GA can pose specific challenges [12]. In these cases, it is important to carefully examine the patient's airway before intubation to identify possible tumors, lymphedema, or fibrosis that might have occurred if the patient has received irradiation therapy for cancer [12,51]. Awake fiberoptic intubation is preferred if difficult intubation is expected [12]. Elective tracheostomy prior to microvascular flap surgery has been proposed in extremely difficult airway cases [52].

Regarding the anesthetic agent, there is some evidence supporting the use of Sevoflurane over Propofol. Sevoflurane has been shown to protect the endothelium from ischemia-reperfusion injury in animal models [53]. Sevoflurane creates a lower capillary filtration coefficient when compared to Propofol [11], which may be beneficial in microvascular flap surgery.

After the use of GA, nausea and vomiting prophylaxis must be considered. Specifically for head and neck surgery, postoperative vomiting can cause suture dehiscence, wound infection, and fistula formation [54]. For prophylaxis, patients with increased risk should be identified, and the administration of a combination of antiemetics and corticosteroids intraoperatively should be considered [55]. The choice of anesthetic should also be taken into consideration regarding postoperative nausea and vomiting and favors Propofol over Sevoflurane [12,56].

3.8. Use of Regional Anesthesia

The exclusive use of regional anesthesia (RA) instead of GA can help avoid several complications, such as postoperative nausea, airway injury, and respiratory insufficiency. RA could also reduce the number of patients admitted to the ICU postoperatively and improve pain management [57,58]. The use of combined spinal and epidural anesthesia is a considerable option for lower limb reconstruction [57]. Epidural anesthesia can be used exclusively in lower limb reconstruction [59] or be combined with GA as it improves postoperative pain and, according to recent studies, does not increase the risk of flap thrombosis or reduce flap blood flow [14,26]. However, there is also some conflicting evidence on the use of regional anesthesia in microvascular flap surgery [50]. A retrospective study by Jayaram et al. involving 165 patients found that spinal and epidural anesthesia was associated with a higher rate of failure in microvascular free flaps in patients with acute trauma [50]. It has been postulated that the sympathectomy that accompanies regional anesthesia can cause a "steal phenomenon" that diverts blood from the transferred flap to the intact tissue that still has innervation of the autonomic nervous system [50,60]. However, the concept of the ''steal phenomenon" is applicable to neuraxial anesthesia and may not be applicable to the use of peripheral nerve blocks (PNBs) [61]. The link between neuraxial anesthesia, hemodynamic parameters, and flap complications needs to be further elucidated [50].

Since hemodynamic parameters are generally much less affected when PNBs are used [62], it is reasonable to believe that they are safe and effective for analgesia in microvascular flap surgery [61]. In particular, patients with GA supplemented with peripheral nerve block have no change in the risk of flap complications but have a shorter length of hospital stay [61]. The use of PNBs for the flap donor site [63] as the surgical location receives benefits from RA, much like any other surgical wound [64]. The main PNBs studied in microvascular flap surgery are transversus abdominis plane block for abdominal surgical procedures [65] and superficial cervical plexus block for neck surgery [66], as well as femoral, popliteal, and sciatic nerve blocks for lower extremity surgery [61].

3.9. Intraoperative Monitoring and Surgical Aspects

As with all major surgical operations, monitoring the vital parameters of patients is imperative to ensuring successful outcomes and, specifically for microvascular flap surgeries, to prevent true flap loss [67]. Standard basic physiological monitors should be used, such as usual pulse oximetry, electrocardiography, and invasive or non-invasive blood pressure measurement [49,67]. In the case of general anesthesia, additional capnography, end-tidal inhalation anesthetic concentrations, electroencephalography, and, when indicated, neuromuscular blockade monitoring [49] may be used.

Monitoring the intravascular fluid status is also important, as a high volume of perioperative crystalloid infusions has been associated with flap complications [11,12]. Fluids should be administered in a manner that achieves euvolemia, avoiding both hypovolemia and hypervolemia [11,12]. Diuresis, while informative, is not a comprehensive marker of fluid status [68]. More accurate depictions of fluid status can be obtained through the use of a respiratory variation of an arterial line graph, esophageal Doppler technology, and echocardiography monitoring [12,68,69]. It should also be noted that, as with any major surgery, changes in cardiac output are influenced by changes in the depth of anesthesia and surgical simulation [70].

It is mandatory that the patient's temperature is monitored throughout this type of surgery to maintain normothermia [11–13]. Normothermia can be achieved by using warm air covers, warming mattresses, and warming intravenous fluids during the operation [11,12]. Hypothermia has been associated with increased perioperative complications, like postoperative infection rates [11–13] and true flap loss [71,72]. A study by Laitman et al. found that hyperthermia also increases the risk of flap complication, and a mean temperature of 36.5 °C is protective against flap complications [73]. The same study found that the temperature interval of 34.5–36.0 °C reduces the risk of flap complications [73]; therefore, the general evidence regarding the optimal intraoperative body temperature remains inconclusive.

Although various macro-hemodynamic parameters can be monitored, there are also multiple ways that the surgeon could check the microcirculatory parameters of the flap intraoperatively. This could also provide information to the anesthesiologist. The main methods of monitoring flap microcirculation include photopletismography [74], the Acland test [75], indocyanine green angiography [76], and implantable Doppler flowmetry [77]. In this regard, the importance of the technical aspects of microvascular flap harvest, flap insertion, and precise blood vessel anastomosis must be emphasized. A meticulous approach to performing the above-mentioned aspects improves free flap outcomes, as this prevents hematoma formation, kinking of the vascular pedicle, and flap loss [78]. Furthermore, diligent instrumental and continuous flap monitoring must be continued postoperatively to optimize microvascular flap survival rates [79].

3.10. Fluids, Vasopressors, and Red Blood Cell Transfusions

It is generally accepted that a balanced fluid administration based on the patient's fluid responsiveness would be preferable [11,14]. The ideal goal would be to maximize cardiac output and tissue oxygenation by aiming toward the peak of the Frank–Starling curve [80] while avoiding flap edema [81]. A study by Dooley et al. about patients with head and neck cancer who underwent free tissue transfer found that higher volumes of intraoperative fluid were associated with an increased rate of both surgical and flap complications [82]. This could be due to the increased susceptibility of the transferred flaps to edema, as they initially do not have lymphatic drainage, and the permeability is increased due to capillary damage [82]. Crystalloid infusion of more than 130 mL/kg per 24 h was suggested by one study to be associated with an increased rate of complications [83]. Another found that patients who received more than 7 L of intraoperative crystalloids had more flap-related complications [81]. The preferred fluid strategy has not yet been elucidated; however, a recent study by Tapia et al. recommended a specific goal-directed fluid management therapy that reduced flap complication rates compared to conventional fluid treatment strategies [69]. It should be noted that the amount of fluid administered is most likely related to the duration of surgery [6]. A duration greater than 18 h was notably influenced by the occurrence of flap failure [6]. Longer duration may cause a longer ischemic period and lead to greater amounts of fluids given to the patient during the procedure [6], which may further exacerbate flap edema alongside increased flap ischemia.

The use of vasoconstrictors in the management of hypotension remains controversial [84–86]. In some animal studies, the use of vasopressors has been shown to reduce flap blood flow [87]. Conversely, in multiple studies involving microvascular surgery in humans, the use of vasopressors has not been shown to cause flap complications [82,85,88]. Dobutamine remains an alternative to pure vasoconstrictors and has been shown to improve flap blood flow [7,12,89]. Furthermore, norepinephrine has been shown to improve free flap blood flow, indicating that both of these vasoconstrictors are safe for microvascular flap surgery [90,91]. Conversely, a study by Chang found that the use of vasopressors increased the rate of arterial flap complications and the need for reoperation but did not increase the rate of true flap loss [84].

The infusion of red blood cells has been associated with an increased rate of flap complications and general complications [11,12,92,93], and this may be due to the immunomodulatory

effect of the transfused blood product [12,94]. A link between red blood cell infusions and wound infections has also been demonstrated [95,96]. Therefore, some authors propose a restrictive use of intraoperative RBC transfusions [12,13]. On the contrary, a study by Kim et al. used multivariate analysis to show that the lowest perioperative Hb level and age were significant predictors of flap failure, and the presence of perioperative blood transfusion was not associated with the risk of flap complications [97]. The overall preoperative and intraoperative risk factors have been summarized in Figure 2.

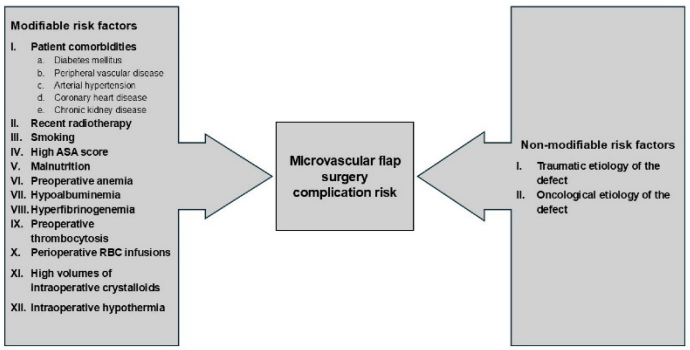

Figure 2. Preoperative and intraoperative risk factors for postoperative complications in microvascular flap surgery. Abbreviations: ASA—American Society of Anesthesiologists; RBC—red blood cell.

3.11. General Postoperative Care Principles and Postoperative Follow-Up

Optimal care for microvascular flap surgery patients does not end in the operating room. Postoperatively, the assignment of proper vitals monitoring and monitoring flap viability is of utmost importance. Regarding the location of postoperative care, the consensus within recent studies seems to point towards not routinely admitting patients to the ICU for postoperative care [12,98]. They have shown that there has been no difference in flaps lost due to complications between admissions to the specialist ward and admissions to the ICU [12,98,99]. There was also no difference in ICU readmissions, and general morbidity was not shown to increase in patients cared for in a specialist ward rather than in the ICU [12,98]. For example, a study by Yalamanchi et al. involving 338 patients did not show any differences in flap survival, reoperation, readmission, and complications postoperatively when comparing admission to the ICU and the non-ICU setting [99]. The findings of a meta-analysis by Mashrah et al. were also in line with this consensus [98].

If postoperative care is provided in the ICU, deep sedation and artificial ventilation should be avoided, as this could lead to a prolonged weaning time from mechanical ventilation and increase the risk of pneumonia [12,98]. Avoiding postoperative ICU admissions could also relieve ICU beds for other more critically ill patients and is likely more cost-effective [99,100].

Wherever the patient is admitted for postoperative care and monitoring, it should be performed by adequately trained nursing staff and in a controlled environment. As with all major surgeries, it should include at least vital signs such as heart rate, pulse oximetry, blood pressure measurement, core temperature, blood glucose, diuresis, drainage measurement, and fluid administration [101]. The monitoring should include a physical examination of the flap every hour. The color, capillary refill, tissue temperature, turgor, pinprick test, and Doppler signals should be examined [98]. In the case of flaps that are poorly accessible, there may be a higher benefit from an implantable Doppler [98] or other available instrumental tools.

3.12. Postoperative Pain Control

Many different analgesic medications have shown benefits in microvascular flap surgeries [12,102]. It is important for the patient's postoperative experience to effectively prevent pain for the patient during the postoperative period, and clinicians should depend on multimodal analgesia (MMA) by combining opioids, non-opioid analgesics, and RA, acting through multiple components of the pain pathway [12].

MMA combining non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and gabapentin with opioid analgesia has been shown to be safe and effective in postoperative analgesia in microvascular flap surgery patients [15,103]. Compared to standard opioid analgesia, the MMA group had a lower rate of partial flap loss [15]. Another study included ketamine in their multimodal analgesia protocol and found that with gabapentin it proved to be a viable option that could be considered to reduce opioid use [103]. Additionally, ketamine may have a more optimal side effect profile with respect to hemodynamics than opioids, which could affect flap viability [103]. There is also evidence that the use of antiemetic and analgesics could be reduced with a single dose of gabapentin before the surgery [11,104]. It has been suggested that ketorolac may reduce thrombotic complications in microvascular flaps of the lower extremities [105]. However, the general contraindications and side effects of NSAIDs must also be taken into account for all patients [106].

3.13. Postoperative Antithrombotic Treatment

The most common cause of flap failure is thrombosis at the anastomotic site; therefore, it is natural to consider using antithrombotic therapies to mitigate this risk. However, the use of anticoagulants also carries an increased risk of bleeding and the formation of hematoma [16]. Many patients undergoing microvascular flap surgery have cancer as an indication for reconstruction, which also increases the importance of good venous thromboembolism prophylaxis to prevent serious complications, such as deep vein thrombosis and pulmonary embolism [12,16]. However, it should be remembered that the pathogenesis of arterial thrombosis is related to endothelial damage, which leads to platelet aggregation, while venous thrombosis is due to fibrin clotting [16]. Furthermore, the risk of flap thrombosis and true flap loss is highest in the first 48 h after surgery and is significantly reduced after 72 h [16].

Commonly used anticoagulants and antiaggregants include unfractionated heparin (UFH), low molecular weight heparin (LMWH), aspirin, and dextran [7,11]. Dextran, a polysaccharide volume expander with antithrombotic properties, is no longer widely considered useful in microvascular flap surgery [107–109]. A meta-analysis by Dawoud et al. compiled eight studies on anticoagulation in head and neck reconstruction surgery and evaluated the use of UFH and LMWH [16]. The study found that UFH consistently increased the relative risk of flap hematoma and bleeding when compared to the control and LMWH [16]. LMWH can be used prophylactically, but additional therapeutic dose anticoagulation is not beneficial [16].

Another study that included 843 patients showed that the flap failure rate was not affected by postoperative antiplatelet treatment, intraoperative heparin bolus, or tPA [7]. Multiple studies have shown an increased risk of bleeding and hematoma formation and a lack of improvement in the risk of true flap loss with regard to UFH [110] and aspirin [111,112]. Conversely, a study by Rothweiler et al. that included 178 free flap surgeries found a decreased true flap loss risk using a combined anticoagulation regimen of aspirin 300 mg intraoperatively followed by aspirin 100 mg/day and an intraoperative bolus of UFH 20 IU/kg followed by UFH 500 IU/h with no APTT target value [113]. A study by Karimi et al. proposed a more delicate regimen of aspirin (100 mg/day) for the first 5 postoperative days and enoxaparin 40 mg/day subcutaneously for 3 days [114]. Given the heterogeneity of the evidence, it is clear that the optimal postoperative antithrombotic regimen has yet to be elucidated.

4. Areas of Future Research

Despite rapid improvements in this field, multiple knowledge gaps still persist. It has become clear that the factors affecting the complications and success of free flap microvascular reconstructions are highly complex and multifactorial. In general, there is much evidence on different factors associated with complications in microvascular flap surgery. Some studies, however, have quite small sample sizes, making them less reliable for recommendations, and most are retrospective in nature, making them more prone to selection and misclassification bias. The evidence on some factors, such as the assessment of nutritional status, correction of preoperative anemia [28], postoperative analgesia [102,103], and the use of PNB [61,65,66], is clear and generally accepted. In these areas, the main concepts have been agreed upon, yet more studies on their application and specific guidelines are necessary. Preoperatively, albumin supplementation [41], chronic inflammation [47,48], and hypercoagulability assessment [10,45,47] currently lack sufficient data and are highly promising avenues for further research. For intraoperative care, further studies are crucially needed to refine optimal body temperature management [71–73], as well as fluid [69,81] and vasopressor [84–86] strategies. With regard to postoperative care, continuous instrumental monitoring for free flap transfer is preferable, although the major issue of optimal postoperative antithrombotic regimen [110–114] is yet to be elucidated.

5. Conclusions

The main focus of perioperative physicians in microvascular flap surgery is to optimize the patient's preoperative risks and provide optimal intraoperative and postoperative care. Optimal perioperative care in microvascular flap surgery is increasingly being accepted as a multidisciplinary team-based process. Despite the scientific progress achieved in the field, further exploration is required. This narrative review outlines possible avenues for future research to refine perioperative care and improve outcomes in microvascular flap surgery.

Author Contributions: A.M.O., R.P.R., B.M., S.D. and I.V. conceived and planned the study. A.M.O., R.P.R., I.M. and E.B. participated in the selection of included studies. A.M.O., R.P.R., J.Z., E.B., B.M., S.D., I.M. and I.V. interpreted the results, formulated the discussion, and prepared the draft. All authors have read and agreed to the published version of the manuscript.

Funding: The authors declare that Riga Stradiņš University kindly covered the publication fee for this article (6-DN-20/3/2024). J.Z. would like to acknowledge financial support from the European Union's Horizon 2020 research and innovation program under agreement No. 857287. None of the funders were involved in the study design, collection, analysis, interpretation of the data, or writing of this article.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data sets used during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Min, K.; Hong, J.P.; Suh, H.P. Risk Factors for Partial Flap Loss in a Free Flap: A 12-Year Retrospective Study of Anterolateral Thigh Free Flaps in 303 Lower Extremity Cases. *Plast. Reconstr. Surg.* **2022**, *150*, 1071e–1081e. [CrossRef] [PubMed]
2. Wei, F.C.; Lin Tay, S.K.; Al Deek, N.F. Principles and techniques of microvascular surgery. *Plast. Surg.* **2024**, *25*, 414–415.
3. Lese, I.; Biedermann, R.; Constantinescu, M.; Grobbelaar, A.O.; Olariu, R. Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *J. Plast. Reconstr. Aesthet. Surg.* **2021**, *4*, 512–522. [CrossRef] [PubMed]
4. Rocans, R.P.; Zarins, J.; Bine, E.; Deksnis, R.; Citovica, M.; Donina, S.; Mamaja, B. The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery. *J. Clin. Med.* **2023**, *12*, 4794. [CrossRef] [PubMed]
5. Yu, J.; Hong, J.P.; Suh, H.P.; Park, J.Y.; Kim, D.H.; Ha, S.; Lee, J.; Hwang, J.H.; Kim, Y.K. Prognostic Nutritional Index is a Predictor of Free Flap Failure in Extremity Reconstruction. *Nutrients* **2020**, *12*, 562. [CrossRef]

6. Ishimaru, M.; Ono, S.; Suzuki, S.; Matsui, H.; Fushimi, K.; Yasunaga, H. Risk Factors for Free Flap Failure in 2,846 Patients with Head and Neck Cancer: A National Database Study in Japan. *J. Oral. Maxillofac. Surg.* **2016**, *74*, 1265–1270. [CrossRef]
7. Bishop, J.L.; Vasudev, M.; Garcia, N.; Heslop, G.; Pham, T.T.; Hicks, M.D.; Chowdhury, F.; Grayson, J.W.; Goddard, J.A.; Tjoa, T.; et al. Effect of Perioperative Antithrombotics on Head and Neck Microvascular Free Flap Survival After Anastomotic Revision. *Otolaryngol. Head Neck Surg.* **2023**, *168*, 1353–1361. [CrossRef]
8. Stevens, M.N.; Freeman, M.H.; Shinn, J.R.; Kloosterman, N.; Carr, S.; Mannion, K.; Rohde, S.L. Preoperative Predictors of Free Flap Failure. *Otolaryngol. Head Neck Surg.* **2022**, *168*, 180–187. [CrossRef]
9. Tsai, M.H.; Chuang, H.C.; Lin, Y.T.; Lu, H.; Chen, W.C.; Fang, F.M.; Chien, C.Y. Clinical impact of albumin in advanced head and neck cancer patients with free flap reconstruction-a retrospective study. *PeerJ* **2018**, *6*, e4490. [CrossRef]
10. Drizlionoka, K.; Zarinš, J.; Ozolina, A.; Nikitina-Zake, L.; Mamaja, B. Polymorphism rs2066865 in the Fibrinogen Gamma Chain (FGG) Gene Increases Plasma Fibrinogen Concentration and Is Associated with an Increased Microvascular Thrombosis Rate. *Med.* **2019**, *55*, 563. [CrossRef]
11. Motakef, S.; Mountziaris, P.M.; Ismail, I.K.; Agag, R.L.; Patel, A. Emerging paradigms in perioperative management for microsurgical free tissue transfer: Review of the literature and evidence-based guidelines. *Plast. Reconstr. Surg.* **2015**, *135*, 290–299. [CrossRef] [PubMed]
12. Dort, J.C.; Farwell, D.G.; Findlay, M.; Huber, G.F.; Kerr, P.; Shea-Budgell, M.A.; Simon, C.; Uppington, J.; Zygun, D.; Ljungqvist, O.; et al. 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 Otolaryngol. Head Neck Surg.* **2017**, *143*, 292–303. [CrossRef] [PubMed]
13. Liu, Y.J.; Hirsch, B.P.; Shah, A.A.; Reid, M.A.; Thomson, J.G. Mild intraoperative hypothermia reduces free tissue transfer thrombosis. *J. Reconstr. Microsurg.* **2011**, *27*, 121–126. [CrossRef] [PubMed]
14. Lou, F.; Sun, Z.; Huang, N.; Hu, Z.; Cao, A.; Shen, Z.; Shao, Z.; Yu, P.; Miao, C.; Wu, J. Epidural Combined with General Anesthesia versus General Anesthesia Alone in Patients Undergoing Free Flap Breast Reconstruction. *Plast. Reconstr. Surg.* **2016**, *137*, 502e–509e. [CrossRef]
15. Sayal, N.R.; Militsakh, O.; Aurit, S.; Hufnagle, J.; Hubble, L.; Lydiatt, W.; Lydiatt, D.; Lindau, R.; Coughlin, A.; Osmolak, A.; et al. Association of multimodal analgesia with perioperative safety and opioid use following head and neck microvascular reconstruction. *Head Neck* **2020**, *42*, 2887–2895. [CrossRef]
16. Dawoud, B.E.S.; Kent, S.; Tabbenor, O.; Markose, G.; Java, K.; Kyzas, P. Does anticoagulation improve outcomes of microvascular free flap reconstruction following head and neck surgery: A systematic review and meta-analysis. *Br. J. Oral. Maxillofac. Surg.* **2022**, *60*, 1292–1302. [CrossRef]
17. Chorath, K.; Go, B.; Shinn, J.R.; Mady, L.J.; Poonia, S.; Newman, J.; Cannady, S.; Revenaugh, P.C.; Moreira, A.; Rajasekaran, K. Enhanced recovery after surgery for head and neck free flap reconstruction: A systematic review and meta-analysis. *Oral. Oncol.* **2021**, *113*, 105117. [CrossRef]
18. Sanati-Mehrizy, P.; Massenburg, B.B.; Rozehnal, J.M.; Ingargiola, M.J.; Hernandez Rosa, J.; Taub, P.J. Risk Factors Leading to Free Flap Failure: Analysis from the National Surgical Quality Improvement Program Database. *J. Craniofac. Surg.* **2016**, *20*, 1956–1964. [CrossRef]
19. Bui, D.T.; Cordeiro, P.G.; Hu, Q.Y.; Disa, J.J.; Pusic, A.; Mehrara, B.J. Free flap reexploration: Indications, treatment, and outcomes in 1193 free flaps. *Plast. Reconstr. Surg.* **2007**, *119*, 2092–2100. [CrossRef]
20. Ooms, M.; Puladi, B.; Houschyar, K.S.; Heitzer, M.; Rashad, A.; Bickenbach, J.; Hölzle, F.; Modabber, A. Smoking and microvascular free flap perfusion in head and neck reconstruction: Radial free forearm flaps and anterolateral thigh flaps. *Sci. Rep.* **2022**, *12*, 13902. [CrossRef]
21. Wong, J.; An, D.; Urman, R.D.; Warner, D.O.; Tønnesen, H.; Raveendran, R.; Abdullah, H.R.; Pfeifer, K.; Maa, J.; Finegan, B.; et al. Society for Perioperative Assessment and Quality Improvement (SPAQI) Consensus Statement on Perioperative Smoking Cessation. *Anesth. Analg.* **2019**, *131*, 955–968. [CrossRef] [PubMed]
22. Weimann, A.; Braga, M.; Carli, F.; Higashiguchi, T.; Hübner, M.; Klek, S.; Laviano, A.; Ljungqvist, O.; Lobo, D.N.; Martindale, R.; et al. ESPEN guideline: Clinical nutrition in surgery. *Clin. Nutr.* **2017**, *36*, 623–650. [CrossRef] [PubMed]
23. Stechmiller, J.K. Understanding the role of nutrition and wound healing. *Nutr. Clin. Pract.* **2010**, *25*, 61–68. [CrossRef] [PubMed]
24. Hwang, T.Z.; Wang, Y.M.; Jeng, S.F.; Lee, Y.C.; Chen, T.S.; Su, S.Y.; Huang, C.C.; Lam, C.F. Intraoperative Enteral Nutrition Feeding in Free-Flap Healing after Reconstruction Surgery for Head and Neck Cancers. *Otolaryngol. Head Neck Surg.* **2023**, *169*, 843–851. [CrossRef] [PubMed]
25. Martínez-Ortega, A.J.; Piñar-Gutiérrez, A.; Serrano-Aguayo, P.; González-Navarro, I.; Remón-Ruíz, P.J.; Pereira-Cunill, J.L.; García-Luna, P.P. Perioperative Nutritional Support: A Review of Current Literature. *Nutrients* **2022**, *14*, 1601. [CrossRef]
26. Jie, B.; Jiang, Z.M.; Nolan, M.T.; Zhu, S.N.; Yu, K.; Kondrup, J. Impact of preoperative nutritional support on clinical outcome in abdominal surgical patients at nutritional risk. *Nutrition* **2012**, *28*, 1022–1027. [CrossRef]
27. Sigurdsson, G.H. Perioperative fluid management in microvascular surgery. *J. Reconstr. Microsurg.* **1995**, *11*, 57–65. [CrossRef]
28. Hill, J.B.; Patel, A.; Del Corral, G.A.; Sexton, K.W.; Ehrenfeld, J.M.; Guillamondegui, O.D.; Shack, R.B. Preoperative anemia predicts thrombosis and free flap failure in microvascular reconstruction. *Ann. Plast. Surg.* **2012**, *69*, 364–367. [CrossRef]
29. Sigaux, N.; Philouze, P.; Boucher, F.; Jacquemart, M.; Frobert, P.; Breton, P. Efficacy of the postoperative management after microsurgical free tissue transfer. *J. Stomatol. Oral. Maxillofac. Surg.* **2017**, *118*, 173–177. [CrossRef]

30. Danan, D.; Smolkin, M.E.; Varhegyi, N.E.; Bakos, S.R.; Jameson, M.J.; Shonka, D.C., Jr. Impact of blood transfusions on patients with head and neck cancer undergoing free tissue transfer. *Laryngoscope* **2015**, *125*, 86–91. [CrossRef]
31. Munting, K.E.; Klein, A.A. Optimisation of pre-operative anaemia in patients before elective major surgery—Why, who, when and how? *Anaesthesia* **2019**, *74*, 49–57. [CrossRef] [PubMed]
32. Ferrer-Barceló, L.; Artero, L.S.; García-Argüelles, J.S.; Gamir, P.C.; Gisbert, J.P.; Ferrer-Arranz, L.M.; Gallego, A.M.; Campos, L.P.; Malavés, J.M.H.; Sanchis, M.L.; et al. Randomised clinical trial: Intravenous vs. oral iron for the treatment of anaemia after acute gastrointestinal bleeding. *Aliment. Pharmacol. Ther.* **2019**, *50*, 258–268. [CrossRef] [PubMed]
33. Soeters, P.B.; Wolfe, R.R.; Shenkin, A. Hypoalbuminemia: Pathogenesis and Clinical Significance. *J. Parenter. Enteral Nutr.* **2019**, *43*, 181–193. [CrossRef] [PubMed]
34. Eckart, A.; Struja, T.; Kutz, A.; Baumgartner, A.; Baumgartner, T.; Zurfluh, S.; Neeser, O.; Huber, A.; Stanga, Z.; Mueller, B.; et al. Relationship of Nutritional Status, Inflammation, and Serum Albumin Levels during Acute Illness: A Prospective Study. *Am. J. Med.* **2020**, *133*, 713–722. [CrossRef]
35. da Silva, A.C.O.; Silva, G.B.; Cho, A.B.; Wei, T.H.; Mattar, R.; Iamaguchi, R.B. Hypoalbuminemia in microsurgical flaps of the musculoskeletal apparatus. *Acta Ortop. Bras.* **2020**, *28*, 168–171. [CrossRef]
36. Shum, J.; Markiewicz, M.R.; Park, E.; Bui, T.; Lubek, J.; Bell, R.B.; Dierks, E.J. Low prealbumin level is a risk factor for microvascular free flap failure. *J. Oral. Maxillofac. Surg.* **2014**, *72*, 169–177. [CrossRef]
37. Xu, H.; Han, Z.; Ma, W.; Zhu, X.; Shi, J.; Lin, D. Perioperative Albumin Supplementation is Associated with Decreased Risk of Complications Following Microvascular Head and Neck Reconstruction. *J. Oral. Maxillofac. Surg.* **2021**, *79*, 2155–2161. [CrossRef]
38. Juang, L.J.; Hur, W.S.; Silva, L.M.; Strilchuk, A.W.; Francisco, B.; Leung, J.; Robertson, M.K.; Groeneveld, D.J.; La Prairie, B.; Chun, E.M.; et al. Suppression of fibrin(ogen)-driven pathologies in disease models through controlled knockdown by lipid nanoparticle delivery of siRNA. *Blood* **2022**, *139*, 1302–1311. [CrossRef]
39. Handschel, J.; Burghardt, S.; Naujoks, C.; Kübler, N.R.; Giers, G. Parameters predicting complications in flap surgery. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol.* **2013**, *115*, 589–594. [CrossRef]
40. Kaplangoray, M.; Toprak, K.; Cicek, O.F.; Deveci, E. Relationship between the Fibrinogen/Albumin Ratio and Microvascular Perfusion in Patients Undergoing Primary Percutaneous Coronary Intervention for ST-Elevated Myocardial Infarction: A Prospective Study. *Arq. Bras. Cardiol.* **2023**, *120*, e20230002. [CrossRef]
41. Tomita, K.; Ochiai, S.; Gunji, T.; Hikita, K.; Kobayashi, T.; Sano, T.; Chiba, N.; Kawachi, S. Prognostic Significance of Plasma Fibrinogen/Serum Albumin Ratio in the Postoperative Outcome of Pancreatic Ductal Adenocarcinoma. *Anticancer. Res.* **2020**, *40*, 7017–7023. [CrossRef] [PubMed]
42. Xu, J.; Li, S.; Feng, Y.; Zhang, J.; Peng, Y.; Wang, X.; Wang, H. The Fibrinogen/Albumin Ratio Index as an Independent Prognostic Biomarker for Patients with Combined Hepatocellular Cholangiocarcinoma After Surgery. *Cancer Manag. Res.* **2022**, *14*, 1795–1806. [CrossRef] [PubMed]
43. Yang, S.; Pi, J.; Ma, W.; Gu, W.; Zhang, H.; Xu, A.; Liu, Y.; Shi, T.; Yang, F.; Chen, L. Prognostic value of the fibrinogen-to-albumin ratio (FAR) in patients with chronic heart failure across the different ejection fraction spectrum. *Libyan J. Med.* **2024**, *19*, 2309757. [CrossRef] [PubMed]
44. Zhang, D.P.; Mao, X.F.; Wu, T.T.; Chen, Y.; Hou, X.-G.; Yang, Y.; Ma, X.; Zhang, J.-Y.; Ma, Y.-T.; Xie, X.; et al. The Fibrinogen-to-Albumin Ratio Is Associated with Outcomes in Patients With Coronary Artery Disease Who Underwent Percutaneous Coronary Intervention. *Clin. Appl. Thromb. Hemost.* **2020**, *26*, 1076029620933008. [CrossRef] [PubMed]
45. Vanags, I.; Stepanovs, J.; Ozolina, A.; Mukans, M.; Bjertnaes, L.J.; Mamaja, B. Thromboelastometry for Assessing Risks of Free Flap Thrombosis in Patients Undergoing Microvascular Surgery. *Front Med.* **2020**, *7*, 289. [CrossRef]
46. Leandro-Merhi, V.A.; Costa, C.L.; Saragiotto, L.; Aquino, J.L.B. Nutritional indicators of malnutrition in hospitalized patients. *Arq. Gastroenterol.* **2019**, *56*, 447–450. [CrossRef]
47. Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Deksnis, R.; Citovica, M.; Donina, S.; Vanags, I.; Gravelsina, S.; Vilmane, A.; et al. Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery. *J. Clin. Med.* **2024**, *13*, 5411. [CrossRef]
48. Chargi, N.; Breik, O.; Forouzanfar, T.; Martin, T.; Praveen, P.; Idle, M.; Parmar, S.; de Bree, R. Association of low skeletal muscle mass and systemic inflammation with surgical complications and survival after microvascular flap reconstruction in patients with head and neck cancer. *Head Neck* **2022**, *44*, 2077–2094. [CrossRef]
49. Pereira, C.M.; Figueiredo, M.E.; Carvalho, R.; Catre, D.; Assunção, J.P. Anesthesia and surgical microvascular flaps. *Rev. Bras. Anestesiol.* **2012**, *62*, 563–579. [CrossRef]
50. Jayaram, K.; Rao, P.; Gurajala, I.; Ramachandran, G. Evaluation of the Effect of Regional Anaesthesia on Microvascular Free Flaps. *Turk. J. Anaesthesiol. Reanim.* **2018**, *46*, 441–446. [CrossRef]
51. Balakrishnan, M.; Kuriakose, R.; Koshy, R.C. Radiation induced changes in the airway—Anaesthetic implications. *South. Afr. J. Anaesth. Analg.* **2004**, *10*, 19–21. [CrossRef]
52. Goswami, U.; Jain, A. Anaesthetic implications of free-flap microvascular surgery for head and neck malignancies—A relook. *J. Anaesthesiol. Clin. Pharmacol.* **2021**, *37*, 499–504. [CrossRef] [PubMed]
53. Chappell, D.; Heindl, B.; Jacob, M.; Annecke, T.; Chen, C.; Rehm, M.; Conzen, P.; Becker, B.F. Sevoflurane reduces leukocyte and platelet adhesion after ischemia-reperfusion by protecting the endothelial glycocalyx. *Anesthesiology* **2011**, *115*, 483–491. [CrossRef] [PubMed]

54. Eryilmaz, T.; Sencan, A.; Camgoz, N.; Ak, B.; Yavuzer, R. A challenging problem that concerns the aesthetic surgeon: Postoperative nausea and vomiting. *Ann. Plast. Surg.* **2008**, *61*, 489–491. [CrossRef] [PubMed]
55. Weibel, S.; Rücker, G.; Eberhart, L.H.; Pace, N.L.; Hartl, H.M.; Jordan, O.L.; Mayer, D.; Riemer, M.; Schaefer, M.S.; Raj, D.; et al. Drugs for preventing postoperative nausea and vomiting in adults after general anaesthesia: A network meta-analysis. *Cochrane Database Syst. Rev.* **2020**, *10*, CD012859.
56. Kaushal, J.; Gupta, M.C.; Kaushal, V.; Bhutani, G.; Dhankar, R.; Atri, R.; Verma, S. Clinical evaluation of two antiemetic combinations palonosetron dexamethasone versus ondansetron dexamethasone in chemotherapy of head and neck cancer. *Singap. Med. J.* **2010**, *51*, 871–875.
57. Ciudad, P.; Escandón, J.M.; Manrique, O.J.; Escobar, H.; Pejerrey, M.B.; Arredondo, M.A. Efficacy of Combined Spinal-Epidural Anesthesia for Lower Extremity Microvascular Reconstruction. *J. Surg. Res.* **2023**, *291*, 700–710. [CrossRef]
58. Owen, A.R.; Amundson, A.W.; Larson, D.R.; Duncan, C.M.; Smith, H.M.; Johnson, R.L.; Taunton, M.J.; Pagnano, M.W.; Berry, D.J.; Abdel, M.P. Spinal Versus General Anesthesia in Contemporary Revision Total Knee Arthroplasties. *J. Arthroplast.* **2023**, *38*, S271–S274. [CrossRef]
59. Galitzine, S.; Wilson, K.; Edington, M.; Burumdayal, A.; McNally, M. Patients' reported experiences and outcomes following surgical excision of lower limb osteomyelitis and microvascular free tissue reconstruction under 'awake' epidural anaesthesia and sedation. *Surgeon* **2021**, *19*, 193–199. [CrossRef]
60. Erni, D.; Banic, A.; Signer, C.; Sigurdsson, G.H. Effects of epidural anaesthesia on microcirculatory blood flow in free flaps in patients under general anaesthesia. *Eur. J. Anaesthesiol.* **1999**, *16*, 692–698. [CrossRef]
61. Koster, I.T.; Os, M.M.D.; Rutten, M.V.; Dungen, T.R.v.D.; de Jong, T.; Winters, H.A.; Driessen, C. The Effect of Regional Anaesthesia on Free Flap Survival in Lower Extremity Reconstructions. *Strateg. Trauma. Limb Reconstr.* **2024**, *19*, 15–20. [CrossRef] [PubMed]
62. Kim, N.Y.; Lee, K.Y.; Bai, S.J.; Hong, J.H.; Lee, J.; Park, J.M.; Kim, S.H. Comparison of the effects of remifentanil-based general anesthesia and popliteal nerve block on postoperative pain and hemodynamic stability in diabetic patients undergoing distal foot amputation: A retrospective observational study. *Medicine* **2016**, *95*, e4302. [CrossRef] [PubMed]
63. Quinlan, J. Anaesthesia for reconstructive surgery. *Anaesth. Intensive Care Med.* **2006**, *7*, 31–35. [CrossRef]
64. Wang, J.C.; Piple, A.S.; Mayfield, C.K.; Chung, B.C.; Oakes, D.A.; Gucev, G.; Lieberman, J.R.; Christ, A.B.; Heckmann, N.D. Peripheral Nerve Block Utilization is Associated with Decreased Postoperative Opioid Consumption and Shorter Length of Stay Following Total Knee Arthroplasty. *Arthroplast. Today* **2023**, *20*, 101101. [CrossRef] [PubMed]
65. Salibian, A.A.; Frey, J.D.; Karp, N.S.; Choi, M. Abstract: Transversus Abdominis Plane Blocks in Microsurgical Breast Reconstruction: An Analysis of Pain, Narcotic Consumption, Length of Stay and Cost Implications. *Plast. Reconstr. Surg. Glob. Open* **2018**, *6*, 174–175. [CrossRef]
66. Egan, R.J.; Hopkins, J.C.; Beamish, A.J.; Shah, R.; Edwards, A.G.; Morgan, J.D. Randomized clinical trial of intraoperative superficial cervical plexus block versus incisional local anaesthesia in thyroid and parathyroid surgery. *Br. J. Surg.* **2013**, *100*, 1732–1738. [CrossRef]
67. Mak, Q.H.Y.; Chan, H.T.; Irwin, M.G. Anaesthesia for plastic and reconstructive surgery. *Anaesth. Intensive Care Med.* **2021**, *24*, 800–805. [CrossRef]
68. Karamanos, E.; Walker, R.; Wang, H.T.; Shah, A.R. Perioperative Fluid Resuscitation in Free Flap Breast Reconstruction: When Is Enough Enough? *Plast. Reconstr. Surg. Glob. Open.* **2020**, *8*, e2662. [CrossRef]
69. Tapia, B.; Garrido, E.; Cebrian, J.L.; Del Castillo, J.L.; Gonzalez, J.; Losantos, I.; Gilsanz, F. Impact of Goal Directed Therapy in Head and Neck Oncological Surgery with Microsurgical Reconstruction: Free Flap Viability and Complications. *Cancers* **2021**, *13*, 545. [CrossRef]
70. Kouz, K.; Thiele, R.; Michard, F.; Saugel, B. Haemodynamic monitoring during noncardiac surgery: Past, present, and future. *J. Clin. Monit. Comput.* **2024**, *38*, 565–580. [CrossRef]
71. Moellhoff, N.; Broer, P.N.; Heidekrueger, P.I.; Ninkovic, M.; Ehrl, D. Impact of Intraoperative Hypothermia on Microsurgical Free Flap Reconstructions. *J. Reconstr. Microsurg.* **2021**, *37*, 174–180. [CrossRef] [PubMed]
72. Young, V.L.; Watson, M.E. Prevention of perioperative hypothermia in plastic surgery. *Aesthet. Surg. J.* **2006**, *26*, 551–571. [CrossRef] [PubMed]
73. Laitman, B.M.; Ma, Y.; Hill, B.; Teng, M.; Genden, E.; DeMaria, S.; Miles, B.A. Mild hypothermia is associated with improved outcomes in patients undergoing microvascular head and neck reconstruction. *Am. J. Otolaryngol.* **2019**, *40*, 418–422. [CrossRef] [PubMed]
74. Schraven, S.P.; Kossack, B.; Strüder, D.; Jung, M.; Skopnik, L.; Gross, J.; Hilsmann, A.; Eisert, P.; Mlynski, R.; Wisotzky, E.L. Continuous intraoperative perfusion monitoring of free microvascular anastomosed fasciocutaneous flaps using remote photoplethysmography. *Sci. Rep.* **2023**, *13*, 1532. [CrossRef]
75. Bombardelli, J.; Farhat, S.; De La Fuente Hagopian, A.; Hua, J.; Schusterman, M.A.; Echo, A. Evaluation of Intraoperative Anastomotic Patency with Angiography in Microsurgical Breast Reconstruction. *Plast. Reconstr. Surg. Glob. Open.* **2023**, *11*, e5230. [CrossRef]
76. Chen, K.-C.; Lin, C.-H.; Ma, H.; Wang, T.-H.; Shih, Y.-C.; Chen, M.-C.; Chiu, Y.-J.; Chen, C.-E. Outcome analysis of free flap reconstruction for head and neck cancer with intraoperative indocyanine green angiography. *J. Plast. Reconstr. Aesthet. Surg.* **2023**, *85*, 387–392. [CrossRef]

77. Abdou, S.A.; Sharif-Askary, B.; Zolper, E.G.; Evans, K.K. Intraoperative Utility of the Implantable Doppler in Lower Extremity Reconstruction: A Matched Case-control Study. *Plast. Reconstr. Surg. Glob. Open* **2020**, *8*, e3229. [CrossRef]
78. Huang, T.C.T.; Ciudad, P.; Manrique, O.J.; Agko, M.; Chen, S.H.; Tang, P.Y.; Sabbagh, M.D.; Chen, H.C. Staged inset of free flaps for complex microsurgical head and neck reconstruction to ensure total flap survival. *Microsurgery* **2018**, *38*, 844–851. [CrossRef]
79. Chang, E.I. My first 100 consecutive microvascular free flaps: Pearls and lessons learned in first year of practice. *Plast. Reconstr. Surg. Glob. Open* **2013**, *1*, e27. [CrossRef]
80. Elia, J.; Diwan, M.; Deshpande, R.; Brainard, J.C.; Karamchandani, K. Perioperative Fluid Management and Volume Assessment. *Anesthesiol. Clin.* **2023**, *41*, 191–209. [CrossRef]
81. Haughey, B.H.; Wilson, E.; Kluwe, L.; Piccirillo, J.; Fredrickson, J.; Sessions, D.; Spector, G. Free flap reconstruction of the head and neck: Analysis of 241 cases. *Otolaryngol. Head Neck Surg.* **2001**, *125*, 10–17. [CrossRef] [PubMed]
82. Dooley, B.J.; Karassawa Zanoni, D.; Mcgill, M.R.; Awad, M.I.; Shah, J.P.; Wong, R.J.; Broad, C.; Mehrara, B.J.; Ganly, I.; Patel, S.G. Intraoperative and postanesthesia care unit fluid administration as risk factors for postoperative complications in patients with head and neck cancer undergoing free tissue transfer. *Head Neck* **2020**, *42*, 14–24. [CrossRef] [PubMed]
83. Clark, J.R.; McCluskey, S.A.; Hall, F.; Lipa, J.; Neligan, P.; Brown, D.; Irish, J.; Gullane, P.; Gilbert, R. Predictors of morbidity following free flap reconstruction for cancer of the head and neck. *Head Neck* **2007**, *29*, 1090–1101. [CrossRef] [PubMed]
84. Chang, C.S.; Chu, M.W.; Nelson, J.A.; Basta, M.; Gerety, P.; Kanchwala, S.K.; Wu, L.C. Complications and Cost Analysis of Intraoperative Arterial Complications in Head and Neck Free Flap Reconstruction. *J. Reconstr. Microsurg.* **2017**, *33*, 318–327.
85. Chen, C.; Nguyen, M.D.; Bar-Meir, E.; Hess, P.A.; Lin, S.; Tobias, A.M.; Upton, J.I.; Lee, B.T. Effects of vasopressor administration on the outcomes of microsurgical breast reconstruction. *Ann. Plast. Surg.* **2010**, *65*, 28–31. [CrossRef]
86. Ibrahim, A.M.; Kim, P.S.; Rabie, A.N.; Lee, B.T.; Lin, S.J. Vasopressors and reconstructive flap perfusion: A review of the literature comparing the effects of various pharmacologic agents. *Ann. Plast. Surg.* **2014**, *73*, 245–248. [CrossRef]
87. Cordeiro, P.G.; Santamaria, E.; Hu, Q.Y.; Heerdt, P. Effects of vasoactive medications on the blood flow of island musculocutaneous flaps in swine. *Ann. Plast. Surg.* **1997**, *39*, 524–531. [CrossRef]
88. Kelly, D.A.; Reynolds, M.; Crantford, C.; Pestana, I.A. Impact of intraoperative vasopressor use in free tissue transfer for head, neck, and extremity reconstruction. *Ann. Plast. Surg.* **2014**, *72*, S135–S138. [CrossRef]
89. Suominen, S.; Svartling, N.; Silvasti, M.; Niemi, T.; Kuokkanen, H.; Asko-Seljavaara, S. The effect of intravenous dopamine and dobutamine on blood circulation during a microvascular TRAM flap operation. *Ann. Plast. Surg.* **2004**, *53*, 425–431. [CrossRef]
90. Eley, K.A.; Young, J.D.; Watt-Smith, S.R. Power spectral analysis of the effects of epinephrine, norepinephrine, dobutamine and dopexamine on microcirculation following free tissue transfer. *Microsurgery* **2013**, *33*, 275–281. [CrossRef]
91. Eley, K.A.; Young, J.D.; Watt-Smith, S.R. Epinephrine, norepinephrine, dobutamine, and dopexamine effects on free flap skin blood flow. *Plast. Reconstr. Surg.* **2012**, *130*, 564–570. [CrossRef] [PubMed]
92. Sanchez-Porro Gil, L.; Leon Vintro, X.; Lopez Fernandez, S.; Vega Garcia, C.; Pons Playa, G.; Fernandez Garrido, M.; Masia Ayala, J. The Effect of Perioperative Blood Transfusions on Microvascular Anastomoses. *J. Clin. Med.* **2021**, *10*, 1333. [CrossRef] [PubMed]
93. Karamanos, E.; Shah, A.R.; Kim, J.N.; Wang, H.T. Impact of Blood Transfusion in Free Flap Breast Reconstruction Using Propensity Score Matching. *J. Reconstr. Microsurg.* **2021**, *37*, 315–321. [CrossRef] [PubMed]
94. Remy, K.E.; Hall, M.W.; Cholette, J.; Juffermans, N.P.; Nicol, K.; Doctor, A.; Blumberg, N.; Spinella, P.C.; Norris, P.J.; Dahmer, M.K.; et al. Mechanisms of red blood cell transfusion-related immunomodulation. *Transfusion* **2018**, *58*, 804–815. [CrossRef]
95. Mantoani, C.C.; Margatho, A.S.; Dantas, R.A.S.; Galvão, C.M.; de Campos Pereira Silveira, R.C. Perioperative Blood Transfusion and Occurrence of Surgical Site Infection: An Integrative Review. *AORN J.* **2019**, *110*, 626–634. [CrossRef]
96. Higgins, R.M.; Helm, M.C.; Kindel, T.L.; Gould, J.C. Perioperative blood transfusion increases risk of surgical site infection after bariatric surgery. *Surg. Obes. Relat. Dis.* **2019**, *15*, 582–587. [CrossRef]
97. Kim, M.J.; Woo, K.J.; Park, B.Y.; Kang, S.R. Effects of Transfusion on Free Flap Survival: Searching for an Optimal Hemoglobin Threshold for Transfusion. *J. Reconstr. Microsurg.* **2018**, *34*, 610–615. [CrossRef]
98. Mashrah, M.A.; Aldhohrah, T.; Abdelrehem, A.; Sabri, B.; Ahmed, H.; Al-Rawi, N.H.; Yu, T.; Zhao, S.; Wang, L.; Ge, L. Postoperative care in ICU versus non-ICU after head and neck free-flap surgery: A systematic review and meta-analysis. *BMJ Open* **2022**, *12*, e053667. [CrossRef]
99. Yalamanchi, P.; Thomas, W.W.; Workman, A.D.; Rajasekaran, K.; Chalian, A.A.; Shanti, R.M.; Newman, J.G.; Cannady, S.B. Value of Intensive Care Unit-Based Postoperative Management for Microvascular Free Flap Reconstruction in Head and Neck Surgery. *Facial Plast. Surg. Aesthet. Med.* **2021**, *23*, 49–53. [CrossRef]
100. Stevens, M.N.; Prasad, K.; Sharma, R.K.; Gallant, J.N.; Habib, D.R.S.; Langerman, A.; Mannion, K.; Rosenthal, E.; Topf, M.C.; Rohde, S.L. Comparative Outcomes for Microvascular Free Flap Monitoring Outside the Intensive Care Unit. *Otolaryngol. Head Neck Surg.* **2024**, *171*, 381–386. [CrossRef]
101. Puppo Moreno, A.M.; Abella Alvarez, A.; Morales Conde, S.; Pérez Flecha, M.; García Ureña, M.Á. The intensive care unit in the postoperative period of major abdominal surgery. *Med. Intensiv.* **2019**, *43*, 569–577. [CrossRef] [PubMed]
102. Lee, T.S.; Wang, L.L.; Yi, D.I.; Prasanna, P.D.; Kandl, C. Opioid sparing multimodal analgesia treats pain after head and neck microvascular reconstruction. *Laryngoscope* **2020**, *130*, 1686–1691. [CrossRef] [PubMed]
103. Go, B.C.; Go, C.C.; Chorath, K.; Moreira, A.; Rajasekaran, K. Multimodal Analgesia in Head and Neck Free Flap Reconstruction: A Systematic Review. *Otolaryngol. Head Neck Surg.* **2022**, *166*, 820–831. [CrossRef] [PubMed]

104. Mao, Y.; Wu, L.; Ding, W. The efficacy of preoperative administration of gabapentin/pregabalin in improving pain after total hip arthroplasty: A meta-analysis. *BMC Musculoskelet. Disord.* **2016**, *7*, 373. [CrossRef] [PubMed]
105. Biermann, N.; Chak, J.C.; Wiesmeier, A.; Klein, S.M.; Ruewe, M.; Spoerl, S.; Kruppa, P.; Prantl, L.; Anker, A.M. Evidence-Based Approaches to Anticoagulation in Reconstructive Microsurgery-A Systematic Literature Review. *Life* **2024**, *14*, 82. [CrossRef]
106. Schug, S.A. Do NSAIDs Really Interfere with Healing after Surgery? *J. Clin. Med.* **2021**, *10*, 2359. [CrossRef]
107. Ridha, H.; Jallali, N.; Butler, P.E. The use of dextran post free tissue transfer. *J. Plast. Reconstr. Aesthet. Surg.* **2006**, *59*, 951–954. [CrossRef]
108. Kearns, M.C.; Baker, J.; Myers, S.; Ghanem, A. Towards standardization of training and practice of reconstructive microsurgery: An evidence-based recommendation for anastomosis thrombosis prophylaxis. *Eur. J. Plast. Surg.* **2018**, *41*, 379–386. [CrossRef]
109. Disa, J.J.; Polvora, V.P.; Pusic, A.L.; Singh, B.; Cordeiro, P.G. Dextran-related complications in head and neck microsurgery: Do the benefits outweigh the risks? A prospective randomized analysis. *Plast. Reconstr. Surg.* **2003**, *112*, 1534–1539. [CrossRef]
110. Bashir, M.M.; Yousaf, N.; Khan, F.A. The outcome of microvascular free flap surgery with or without the use of postoperative heparin. *J. Coll. Physicians Surg. Pak.* **2014**, *24*, 412–415.
111. Lee, K.T.; Mun, G.H. The efficacy of postoperative antithrombotics in free flap surgery: A systematic review and meta-analysis. *Plast Reconstr Surg.* **2015**, *135*, 1124–1139. [CrossRef] [PubMed]
112. Lighthall, J.G.; Cain, R.; Ghanem, T.A.; Wax, M.K. Effect of postoperative aspirin on outcomes in microvascular free tissue transfer surgery. *Otolaryngol. Head Neck Surg.* **2013**, *148*, 40–466. [CrossRef] [PubMed]
113. Rothweiler, R.; Gerlach, V.; Voss, P.; Poxleitner, P.; Ermer, M.; Gross, C.; Schwer, C.; Vach, K.; Kalbhenn, J.; Metzger, M. Aspirin, heparin and ischemia time in microvascular free flap surgery—Their influence and an optimal anticoagulation protocol. *J. Stomatol. Oral. Maxillofac. Surg.* **2022**, *123*, e556–e562. [CrossRef] [PubMed]
114. Karimi, E.; Ardestani, S.H.; Jafari, M.; Hagh, A.B. Testing a New Anticoagulation Method for Free Flap Reconstruction of Head and Neck Cancers. *Clin. Exp. Otorhinolaryngol.* **2016**, *9*, 370–373. [CrossRef]

Second Publication

Journal of *Clinical Medicine*

MDPI

Article

The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery

Rihards P. Rocans [1,2,*], Janis Zarins [3,4], Evita Bine [1], Renars Deksnis [5], Margarita Citovica [6], Simona Donina [7] and Biruta Mamaja [2]

[1] Intensive Care Clinic, Riga East Clinical University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; evitabine@gmail.com
[2] Department of Anaesthesia and Intensive Care, Riga Stradins University, Dzirciema Street 16, LV-1007 Riga, Latvia; biruta.mamaja@aslimnica.lv
[3] Department of Hand and Plastic Surgery, Microsurgery Centre of Latvia, Brivibas Street 410, LV-1024 Riga, Latvia; janis.zarins@mcl.lv
[4] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Pulka Street 3, LV-1007 Riga, Latvia
[5] Surgical Oncology Clinic, Riga East Clinical University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia; renars.deksnis@gmail.com
[6] Laboratory Department, Riga East Clinical University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; margarita.citovica@aslimnica.lv
[7] Institute of Microbiology and Virology, Riga Stradins University, Ratsupites Street 5, LV-1067 Riga, Latvia; donsimon@inbox.lv
* Correspondence: rihards.rocans@gmail.com

Abstract: Microvascular flap surgery is a widely acknowledged procedure for significant defect reconstruction. Multiple flap complication risk factors have been identified, yet there are limited data on laboratory biomarkers for the prediction of flap loss. The controlling nutritional status (CONUT) score has demonstrated good postoperative outcome assessment ability in diverse surgical populations. We aim to assess the predictive value of the CONUT score for complications in microvascular flap surgery. This prospective cohort study includes 72 adult patients undergoing elective microvascular flap surgery. Preoperative blood draws for analysis of full blood count, total plasma cholesterol, and albumin concentrations were collected on the day of surgery before crystalloid infusion. Postoperative data on flap complications and duration of hospitalization were obtained. The overall complication rate was 15.2%. True flap loss with vascular compromise occurred in 5.6%. No differences in flap complications were found between different areas of reconstruction, anatomical flap types, or indications for surgery. Obesity was more common in patients with flap complications ($p = 0.01$). The CONUT score had an AUC of 0.813 (0.659–0.967, $p = 0.012$) for predicting complications other than true flap loss due to vascular compromise. A CONUT score > 2 was indicated as optimal during cut-off analysis ($p = 0.022$). Patients with flap complications had a longer duration of hospitalization (13.55, 10.99–16.11 vs. 25.38, 14.82–35.93; $p = 0.004$). Our findings indicate that the CONUT score has considerable predictive value in microvascular flap surgery.

Keywords: controlling nutritional status; microvascular flap complications; reconstructive surgery

Citation: Rocans, R.P.; Zarins, J.; Bine, E.; Deksnis, R.; Citovica, M.; Donina, S.; Mamaja, B. The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery. *J. Clin. Med.* **2023**, *12*, 4794. https://doi.org/10.3390/jcm12144794

Academic Editor: Alexandre Bozec

Received: 6 June 2023
Revised: 14 July 2023
Accepted: 17 July 2023
Published: 20 July 2023

1. Introduction

Microvascular flap surgery has become a generally acknowledged procedure for significant defect reconstruction. Complex microvascular techniques and in-depth knowledge of blood rheology and microanastomosis function are required for this kind of surgery. Although substantial progress has been achieved in preventing complications, the rate of flap loss is still significant (1–7.1%) and can have significant adverse effects on the patient [1,2].

Flap thrombosis, flap hematoma, and flap loss are the most frequent and severe major surgical complications [3]. Mechanical problems comprise the most frequent causes of late flap failure (>48 h), and impaired arterial and venous blood supply is the most widespread cause of early flap failure (<48 h) [4]. Problematic and delayed healing, wound dehiscence, infection, fistula, and donor site problems are considered minor surgical complications. Even though microvascular flap transplantation relies on greatly specific surgical concepts, the issue of systemic reaction to surgical trauma and tissue healing is just as relevant here as in other types of surgery [5].

The most common indications for microvascular flap surgery are primary oncology or trauma, as well as defects related to previous surgery or infection [1]. Malnutrition may be common in patients requiring microvascular flap surgery [6], as many indications for microvascular flap surgery are also risk factors for poor nutritional status [7]. Previous studies show that the presence of malnutrition is a considerable risk factor for surgical complications in different patient populations [6–10]. Malnourished patients are at a higher risk of surgical complications such as wound dehiscence, infection, and fistula formation [9,11]. Most of these complications require reoperation, which can further increase patient morbidity and hospital costs [12]. Screening, assessing, and managing these patients is important because malnutrition is a modifiable pre-operative risk factor that, if addressed early, can reduce the risk of post-operative complications [13]. Given the complexity of microvascular flap transplantation and the availability of nutritional treatment strategies, a systematic approach to addressing nutrition risk significantly improves surgical outcomes in microvascular flap surgery [6,7].

The objective measurement of nutritional status can be performed with a wide range of tools, although there is no "gold standard" approach for measuring malnutrition [14]. The use of laboratory biomarkers for screening and assessing nutrition risk may be convenient, since laboratory evaluation is already routinely performed for preoperative assessment. Multiple studies have elucidated the link between laboratory biomarkers of poor nutritional status and surgical complications [6–8]. Studies have shown that lymphocyte count, albumin, prealbumin, and total plasma cholesterol are markers for poor nutritional status and can be quantified using nutritional assessment tools [15,16]. The controlling nutritional status (CONUT) score is an evolving tool that has demonstrated good postoperative outcome assessment ability in diverse surgical populations [9,17]. It is intended for inpatient assessment and is relatively simple to use, as it is calculated using only three values: serum albumin level, total cholesterol level, and total lymphocyte count [16]. A CONUT score of 0–1 is defined as no nutrition risk, and higher scores are defined as higher degrees of nutrition risk [16]. CONUT could be applied for assessment of nutrition risk in microvascular flap surgery due to its broad applicability and previous evidence for predicting complications in various surgical populations. The purpose of this study is to assess the predictive value of the CONUT score for predicting complications in elective microvascular flap surgery.

2. Materials and Methods

The study protocol and the informed consent form were approved by the Ethics Committee of Riga Stradins University (Approval Number 22-2/399/2021), and by the Science Department of Riga East University hospital (Approval Number Nr.AP/08-08/22/135).

2.1. Patient Selection

This prospective cohort study included 72 patients undergoing elective microvascular flap transplantation surgery at Riga East University Hospital from the 1 October 2021 to the 31 January 2023. Given the observational nature of our study, all surgical, anesthesia, and clinical management decisions were made by the attending physicians. The inclusion criterion was adult patients undergoing elective microvascular flap transplantation. The exclusion criteria were patients with sepsis or severe systemic bacterial infection; patients with autoimmune disorders; patients with blood-borne viral infections (Hepatitis B; Hep-

atitis C and HIV); pregnant patients and patients during lactation period; and patients with congenital hypercoagulability or any clotting disorder.

2.2. Anaesthesia and Surgical Protocol

All patients received general anesthesia (GA). Starting at the induction of anesthesia electrocardiography, pulse oximetry, noninvasive blood pressure, and end-tidal carbon dioxide concentration were monitored in all patients. Induction was performed using fentanyl (Fentanyl-Kalceks® 0.05 mg/mL, A/S Kalceks, Riga, Latvia) 1.5–2 μg/kg, and propofol (Propofol® 10 mg/mL, Fresenius Kabi AG, Bad Homburg, Germany) 1–2 mg/kg intravenously (iv). GA was maintained using sevoflurane (Sevorane®, AbbVie S.r.l., Campoverde, Italy) 0.8–1.2 MAC, and continuous analgesia was provided with fentanyl 1–1.5 μg/kg/h. Cisatracurium (Nimbex 2 mg/mL, Aspen Pharma Ltd., Dublin, Ireland) 0.15 mg/kg iv was used for tracheal intubation, followed by a continuous infusion of 1–2 μg/kg/min for muscle relaxation. Crystalloid infusion (RiLac, B. Braun Melsungen AG, Melsungen, Germany) was administered at a rate of 3.5 to 6.0 mL/kg iv per hour during surgery and the early postoperative period, with a target urine output of 1–2 mL/kg/h. Colloid fluid (Gelofusine, B. Braun Melsungen AG, Melsungen, Germany) was administered when an estimated blood loss of >500 mL occurred during surgery. Patients received both peripheral and central temperature monitoring during surgery to avoid hypothermia. Patients were administered vasopressors, such as ephedrine (Ephedrine Sintetica, Sintetica GmbH, Münster, Germany) or norepinephrine (Norepinephrine Sopharma, Sopharma AD, Sofia, Bulgaria), when their mean arterial blood pressure was below 65 mmHg for more than 5 min. Peripheral nerve blocks with ultrasound and neurostimulation guidance were performed when indicated. Patients received close postoperative monitoring of vital signs, fluid balance, and postoperative pain management in the post-anesthesia care unit. Postoperative thromboprophylaxis was provided with enoxaparin (Clexane®, Sanofi-Aventis S.A., Barcelona, Spain) 40 mg once daily from the first postoperative day for all patients. During and after surgery, patients with clinical symptoms of excessive blood loss or those with hemoglobin < 7 g/dL received blood product transfusions. All operations were performed by a team of highly experienced surgeons. The selection of flap type was based on the tissue type necessary for defect site reconstruction, the size of defect, the length of the pedicle, and the patient's positioning during surgery. The flaps used in the study were the anterolateral thigh flap, deep inferior epigastric artery perforator flap, fibular flap; radial free forearm flap, gracilis muscle flap, temporal artery flap, serratus anterior flap, latissimus dorsi flap, and medial condyle flap. The team of surgeons closely monitored the microvascular flap for the first five postoperative days. Flap patency was assessed using clinical assessment of flap color, temperature, tissue turgor, and capillary refill.

2.3. Data Collection

Blood draws were obtained on the day of surgery immediately upon the first arrival in the operating room before initiation of the first crystalloid infusion. Full blood count analysis was performed using the XN-1000 system (Sysmex Europe SE, Norderstedt, Germany). Concentrations of albumin were analyzed using the colorimetric method (Cobas C, Roche, Manheim, Germany). Concentrations of total plasma cholesterol were analyzed using the Enzymatic colorimetric method (Cobas C, Roche, Manheim, Germany). The serum albumin concentration, total peripheral lymphocyte count, and serum total cholesterol concentration were used to assign the CONUT score. As seen in Table 1, the CONUT score was determined by assigning laboratory values according to the tool first used by Ignacio de Ulíbarri and coauthors [16].

Table 1. The evaluation of the controlling nutritional status (CONUT) score; the controlling nutritional status (CONUT) score tool as first described by Ignacio de Ullibarri and coauthors [16].

Variable	Undernutrition Degree			
	Normal	Mild	Moderate	Severe
Serum albumin (g/dL)	≥3.50	3.00–3.49	2.50–2.99	<2.50
Score	0	2	4	6
Total lymphocyte count (/mm^3)	≥1600	1200–1599	800–1199	<800
Score	0	1	2	3
Total cholesterol (mg/dL)	≥180	140–179	100–139	<100
Score	0	1	2	3

Demographic data, comorbidities, data on perioperative course, anesthesia care, surgical outcome, length of stay in the intensive care unit (ICU), and total duration of hospitalization were obtained from written and electronic health records according to a previously defined protocol. Patients received postoperative daily follow up until discharge from the hospital.

2.4. Definitions

True flap loss was defined as flap blood supply deficiency due to arterial or venous anastomosis dysfunction or thrombosis that leads to complete loss of the transplanted flap. Other flap complications were defined as any of the following: hematoma (without interfering with flap blood supply), flap wound infection, secondary or incomplete flap wound healing, and partial flap loss. Partial flap loss was defined as the presence of distal marginal flap necrosis with no anastomosis dysfunction. Any flap complication was defined as the presence of either true flap loss or any other flap complication. ICU length of stay was the timing between admission to the ICU and discharge from the ICU to the ward. Hospital length of stay was the timing between admission to the hospital and discharge from the hospital.

2.5. Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 26.0. (IBM Corp. Armonk, NY, USA). The Kolmogorov–Smirnov test was used to evaluate whether the datasets conformed to a normal distribution. Continuous variables conforming to normal distribution were presented as mean and CI95, while categorical variables were presented as median ± interquartile range (IQR). Differences in data distribution between the groups were evaluated using the Mann–Whitney U test for non-parametric datasets and the two-sample t-test or ANOVA for datasets conforming with normal distribution. A Chi-square test was applied for nominal variable sets. Binary logistic regression models were used to obtain odds ratios for specific variables. The receiver operator curve (ROC) and area under curve (AUC) were used for evaluating the diagnostic ability of a binary classifier system. Youden's Index (YI) and the Concordance Probability Method (CZ) was used for defining optimal cut-off values [18]. Statistical significance was assumed if two-tailed $p < 0.05$.

3. Results

In total, 72 patients—40 (55.6%) men and 32 (44.4%) women—were included. The mean age was 55.3 years (95% CI95 51.5–59.1). The overall complication rate was 15.2% ($n = 11$). True flap loss with vascular compromise occurred in 5.6% ($n = 4$), with two of these cases being late flap loss (>72 h). Both cases of early true flap loss underwent urgent anastomosis revision. Both cases of late flap loss underwent repeated elective microvascular flap transplantation. Other flap complications occurred in seven cases, with difficult flap healing or partial flap loss occurring in 5.6% ($n = 4$), flap infection occurring in one, and hematoma occurring in two cases. The median number of revisions in patients with true

flap loss was 1.5 (IQR 1). The median number of revisions in patients with other flap complications was 1 (IQR 0.75, $p = 0.223$).

As seen in Table 2, there were no significant differences in age or gender distribution in patients with any flap complications or flap loss, and in patients without complications. No significant differences in true flap failure or other flap complications were found between different areas of reconstruction and different anatomical flap types. No significant differences in true flap failure or other flap complications were found between different indications for reconstruction. Of the included comorbidities, obesity was found to be more common in patients with any flap complications ($p = 0.01$). Only two patients had a BMI < 20 kg/m^2, and there was no statistically significant link between decreased BMI and any flap complications. No statistically significant link was found between BMI and CONUT score. No significant differences in the rates of true flap failure or other flap complications were found in patients with other comorbidities.

Table 2. Demographic characteristics, surgical considerations, and comorbidities; data are presented as mean (CI95) or count (percentage). Abbreviations—BMI (body mass index); ENT (ear, nose, and throat surgery); DIEP (deep inferior epigastric artery perforator flap); ALT (anterolateral thigh flap).

Patient Group	Overall $n = 72$	No Complications $n = 61$	True Flap Loss $n = 4$	Any Flap Complications $n = 11$	p-Value
Demographical data					
Mean age, years	55.3 (51.5–59.1)	56.9 (61.0–65.4)	65.0 (63.5–66.5)	49.6 (37.7–56.1)	0.057
Sex (female), *n* (%)	32 (44.4%)	25 (40.1%)	2 (50.0%)	5 (45.5%)	0.418
Area of reconstruction					
Extremity, *n* (%)	15 (20.8%)	12 (19.6%)	-	3 (27.3%)	0.289
ENT, *n* (%)	26 (36.1%)	22 (36.1%)	2 (50.0%)	4 (36.4%)	0.496
Head and neck, *n* (%)	16 (22.2%)	14 (30.0%)	1 (25.0%)	2 (18.2%)	0.322
Breast, *n* (%)	15 (20.8%)	13 (21.3%)	1 (25.0%)	2 (18.2%)	0.457
Microvascular flap type					
ALT, (%)	32 (44.4%)	27 (44.3%)	2 (50.0%)	5 (45.5%)	0.828
Fibular flap, (%)	9 (12.5%)	8 (13.1%)	1 (25.0%)	1 (9.1%)	0.478
DIEP, *n* (%)	9 (12.5%)	7 (11.5%)	-	2 (18.2%)	0.528
Radial artery flap, *n* (%)	6 (8.3%)	6 (9.8%)	-	-	-
Other, *n* (%)	16 (22.2%)	13 (21.3%)	1 (25.0%)	3 (27.3%)	0.413
Indication for surgery					
Trauma, *n* (%)	8 (11.1%)	6 (10.1%)	-	1 (9.1%)	0.918
Oncology, *n* (%)	40 (55.6%)	32 (58.2%)	3 (75.0%)	6 (54.5%)	0.469
Defect, *n* (%)	19 (26.4%)	11 (20.0%)	1 (25.0%)	4 (36.4%)	0.511
Infection, *n* (%)	5 (6.9%)	5 (8.2%)	-	-	-
Comorbidities					
Coronary artery disease, *n* (%)	4 (5.6%)	3 (4.9%)	1 (25.0%)	1 (9.1%)	0.059
Diabetes mellitus, *n* (%)	5 (6.9%)	4 (6.6%)	-	1 (9.1%)	0.691
Hypertension, *n* (%)	28 (38.8%)	19 (31.1%)	3 (75.0%)	6 (54.5%)	0.133
Dyslipidemia, *n* (%)	16 (22.2%)	13 (21.3%)	1 (25.0%)	3 (27.3%)	0.624
Smoking history, *n* (%)	13 (18.1%)	11 (18.0%)	1 (25.0%)	2 (18.2%)	0.249
Obesity (BMI > 30 kg/m^2), *n* (%)	12 (16.6%)	8 (13.1%)	2 (50.0%)	5 (45.5%)	0.010 **
Cerebrovascular accident, *n* (%)	4 (5.6%)	4 (6.6%)	-	-	0.620

The ** symbol is used to indicate statistical significance when comparing the group without complications to both the true flap loss group and the any flap complications group.

As seen in Table 3, no significant links were found between the duration of surgery and anesthesia factors and any flap complications. A higher intraoperative hematocrit was associated with flap complications, with the highest intraoperative hematocrit found in cases with subsequent true flap loss ($p = 0.009$). Only one patient received intraoperative

hemotransfusion, and five patients received hemotransfusion in the early postoperative period. There was no significant link between the presence of hemotransfusion and any flap complications.

Table 3. Intraoperative and anesthesia considerations; data are presented as mean (CI95) or count (percentage).

Patient Group	Overall $n = 72$	No Complications $n = 61$	True Flap Loss $n = 4$	Any Flap Complications $n = 11$	p-Value
Duration of surgery, hours	6.39 (5.75–7.02)	6.33 (5.59–7.07)	7.63 (5.86–9.39)	6.66 (5.29–8.04)	0.235
Volume of intraoperative crystalloid, mL	2345.83 (2141.39–2550.28)	2352.50 (2133.31–2571.69)	2875.00 (1681.58–4068.42)	2312.50 (1608.14–3016.86)	0.145
Volume of intraoperative colloid, mL	506.25 (401.74–610.76)	482.50 (367.10–597.90)	500.00 (-)	625.00 (329.42–920.58)	0.471
Intraoperative colloid to crystalloid ratio	0.22 (0.17–0.27)	0.20 (0.15–0.25)	0.18 (0.10–0.27)	0.33 (0.09–0.56)	0.306
Intraoperative hematocrit, %	30.60 (29.20–32.00)	29.58 (27.70–31.45)	31.50 (25.15–37.85)	34.40 (30.32–38.48)	0.009 *
Use of vasopressors/ sympathomimetics, n (%)	41 (56.90%)	36 (59.00%)	2 (50.00%)	6 (54.50%)	0.549

The * symbol is used to indicate statistical significance when comparing the group without complications to the any flap complications group.

As seen in Table 4, patients with any flap complications had a significantly lower plasma lymphocyte count ($p = 0.001$). Multivariate regression analysis revealed that an increase in lymphocyte count decreases the incidence of all complications (OR 0.998 CI95 0.996–0.999). Patients with any flap complications had a significantly lower plasma monocyte count ($p = 0.021$). No differences in plasma lymphocyte/monocyte ratio, plasma albumin, and total plasma cholesterol were found in patients with any flap complications.

Table 4. Biomarkers and nutritional systems for predicting any flap complications; data are presented as mean (CI95), median (IQR), or count (percentage).

Patient Group	Overall $n = 72$	No Complications $n = 61$	Any Flap Complications $n = 11$	p-Value
Biomarkers				
Lymphocyte count 10^9/L	1.59 (1.39–1.79)	1.71 (1.49–1.92)	0.97 (0.67–1.26)	0.001 *
Monocyte count 10^9/L	0.55 (0.48–0.62)	0.58 (0.51–0.66)	0.37 (0.22–0.51)	0.021 *
Lymphocyte/monocyte ratio	3.46 (2.91–4.02)	3.55 (2.90–4.20)	2.97 (2.28–3.65)	0.830
Mean plasma albumin, g/dL	3.94 (3.81–4.06)	3.96 (3.84–4.09)	3.79 (3.28–4.30)	0.631
Mean total plasma cholesterol, mg/dL	196.58 (185.21–207.95)	198.44 (186.43–210.45)	186.73 (147.93–225.53)	0.310
Nutritional assessment systems				
CONUT score	2(2)	2 (3)	3 (6)	0.013 *
CONUT ≤ 2	50 (69.4%)	46 (75.4%)	4 (36.4%)	0.009 *

The * symbol is used to indicate statistical significance when comparing the group without complications to the any flap complications group.

As seen in Figure 1, analysis on the predictive accuracy of CONUT score of other surgical complications found that CONUT score had an AUC of 0.813 (0.659–0.967, $p = 0.012$). A CONUT score of >2 was found to be optimal during cut-off analysis (Sensitivity 21.1%, Specificity 95.6%, PPV 66.7%, NPV 74.1%, $p = 0.022$). CONUT score of >2 increases the odds of other flap complications (OR 5.4, CI95 1.38–20.90, $p = 0.015$). Univariate regression revealed that any increase in CONUT score increased the odds of other flap complications (OR 1.43 1.09–1.85). Patients with any flap complications had a longer duration of hospitalization (13.55, 10.99–16.11 vs. 25.38, 14.82–35.93; $p = 0.004$). There was no difference in duration of ICU stay between patients with flap complications and patients with no flap complications (1.13, 0.03–2.26 vs. 1.50 1.00–2.00, $p = 0.471$).

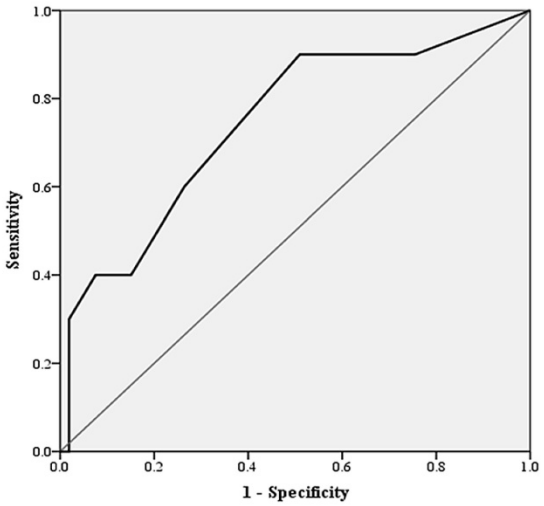

Figure 1. ROC curve characteristics of CONUT score for predicting complications in microvascular flap surgery; receiver operator curve characteristics and area under curve of CONUT score for predicting the presence of flap complications other than true flap loss. CONUT scores had an AUC of 0.813 (CI95 0.659–0.967, $p = 0.012$).

4. Discussion

The main findings of the present study were that an increase in the preoperative CONUT index is a reliable predictor for flap complications, with a CONUT score of >2 being the optimal cut-off for predicting complication risk. Flap complications were found to be linked to lymphocytopenia, monocytopenia, hematocrit, and obesity. The incidence of true flap loss was 6.2%, and the incidence of other less severe complications was 9.2%. The duration of hospitalization was significantly longer in patients who had flap complications.

Microvascular flap transplantation requires complex microvascular techniques, and flap success relies on the function of microanastomosis and adequate flap perfusion [4]. While these are greatly specific concepts, the issue of systemic reaction to surgical trauma and tissue healing and nutrition is just as relevant here as in other types of surgery [5]. Malnourished patients are more likely to experience complications during and after surgery, longer hospital stays, and a slower recovery time both in the general surgical population [8–10] and in microvascular flap surgery [6,7]. Given the complexity of the procedure and severity of the complications, clinical prediction tools regarding nutrition risk may be used during preoperative assessments to identify patients who may require more extensive evaluation or preparation before surgery [6,7]. A study by Yu and co-authors suggests that the prognostic nutritional index (PNI), a score including some of the same parameters as CONUT, can be simply and effectively used to predict free flap failure in extremity reconstruction [6]. Our results indicate that an increased CONUT score significantly increases the odds of postoperative complications. To the best of our knowledge, no previous studies have elucidated the predictive value of CONUT in microvascular flap surgery. However, our findings coincide with data from different surgical populations wherein CONUT has been shown to reliably predict complications and mortality [8,19]. Additionally, our results suggest that patients with flap complications had longer hospital stays. This coincides with previous studies that report longer hospital stays and increased hospital costs in patients who experience free flap failure in breast and head and neck reconstruction [20,21]. Considering that any increase in the CONUT score increases the risk of flap complications it can also consequently lead to longer hospital stays and increased costs.

In our study, we found CONUT > 2 to be the most optimal cut-off value, which also coincides with some data from previous studies in other surgical populations [9,17,22]. It must be noted that we found a CONUT > 2 cut-off value to have a relatively low sensitivity (21.1%) and a high specificity (95.6%). These results imply that a cut-off value of CONUT > 2 is best utilized for excluding patients who are at a low nutrition risk and low risk of subsequent flap complications.

Interestingly, while our data showed CONUT to be a reliable predictor for flap complications, it was not a reliable predictor specifically for true flap loss. This indicates that the pathophysiology of true flap loss due to anastomosis compromise [23] may be separate from the pathophysiology of other surgical complications in microvascular flap surgery. Most minor complications in microvascular flap surgery, such as wound dehiscence, infection, and fistula formation, occur due to inadequate tissue healing and regeneration [24]. These complications may be linked to undernutrition [7] instead of being a direct result of early anastomosis compromise. Notably, even minor complications place the patient at an increased risk of re-exploration or repeated microvascular flap transplantation [25]. Furthermore, patients receiving microvascular flap transplantation are predisposed to difficult wound healing, both at the site of reconstruction and at the donor site [25].

Plasma lymphocyte count is a component of CONUT that may have a substantial role in the pathophysiology of microvascular flap complications. Studies in various surgical populations show that patients with preoperative lymphocytopenia had a significantly higher incidence of complications compared to those with a normal lymphocyte level at admission [5,26,27]. Lymphocyte recovery in the first postoperative days could play an important role in the mechanisms of tissue repair, and a primary role in wound healing [28]. Monocytes are the most responsive leukocytes in response to trauma [29] and multiple monocyte immunophenotypic alterations are observed upon surgery [30,31]. In contrast to our findings, Kosec and co-authors did not find a link between preoperative monocyte count and postoperative complications in microvascular flap surgery [5].

Multiple patient-related risk factors, including coronary artery disease, diabetes, smoking, peripheral arterial vascular disease, arterial hypertension, and higher ASA score, are related to flap failure [1]. Obesity has been deemed to be a risk factor for poor surgical outcomes in medical care, but the majority of published studies in various surgical populations have been uncertain [32–34]. Some previous studies found obesity to be associated with increased perioperative risk in free abdominally based autologous breast reconstruction, which coincides with our findings [35,36]. Conversely, multiple studies have also evidenced that obesity does not increase the risk of postoperative complications in microvascular flap surgery [37–39]. However, it must be noted that the presence of obesity does not exclude the presence of double-burden malnutrition, which can also have detrimental effects on overall health [40,41]. Furthermore, the study by Ignacio de Ulíbarri and coauthors found no relationship between BMI and undernutrition in their study population, as BMI is not a reliable indicator for acute malnutrition [16]. Our data indicate that both obesity and nutrition risk increase the rate of flap complications, which indicates that both conditions should be assessed and treated to improve outcomes in microvascular flap surgery.

This study had several limitations. Firstly, given the observational nature of our study, individual surgical, anesthesia, and nutritional management decision-making was performed by the clinicians, and may have varied between cases. Secondly, ours was a single-center study, which affects the possible generalizability of the findings. Notably, a considerable part of our study population has oncology as a primary diagnosis, which likely introduces additional confounding risk factors for surgical complications. Conversely, it must be noted that patients with oncology as a primary diagnosis are very likely to benefit from an assessment of nutrition risk [8,9,15,17]. It should be noted that the presence of radiotherapy, which can present confounding factors, was not considered in this study. Finally, it is important to note that serum albumin, which is an important item in both the CONUT and PNI scores, is not a part of current definitions of malnutrition [42]. Therefore, CONUT score results are considered to be indicators of nutrition risk rather than an

assessment of nutritional status. Further studies are needed to clarify the use of nutrition risk assessment tools to predict complications in different patient populations, and to specify the use of specific nutritional interventions to improve outcomes in microvascular flap surgery.

5. Conclusions

Assessment of nutritional risk to estimate the risk of microvascular flap complications using the CONUT score has considerable predictive value. Patients undergoing this type of surgery can be evaluated in terms of predicting nutritional risk to optimize decision-making in perioperative care.

Author Contributions: R.P.R., B.M., S.D. and M.C. conceived and planned the study. J.Z., R.P.R., R.D. and M.C. participated in data collection. R.P.R. and E.B. performed data curation and statistical analysis. R.P.R., J.Z., B.M., S.D. and E.B. interpreted the results and prepared the draft. All authors have read and agreed to the published version of the manuscript.

Funding: The authors declare that Riga Stradiņš University kindly covered the publication fee for this article (Grant Reference Number 6-DN-20/2/2023). The funder was not involved in the study design, collection, analysis, interpretation of data or writing of this article.

Institutional Review Board Statement: The study protocol and the informed consent form were approved by the Ethics Committee of Riga Stradins University (Approval Number 22-2/399/2021; Approval date 8 July 2021), and by the Science Department of Riga East University Hospital (Approval Number AP/08-08/22/135; Approval date 8 November 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. The corresponding author will ensure individual privacy is not compromised during the transfer of datasets.

Acknowledgments: We would like to acknowledge the help of Vita Kalnberzina, Department of English studies, University of Latvia, for reviewing and revising this manuscript's English grammar and syntax.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lese, I.; Biedermann, R.; Constantinescu, M.; Grobbelaar, A.O.; Olariu, R. Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *J. Plast. Reconstr. Aesthet. Surg.* **2021**, *74*, 512–522. [CrossRef] [PubMed]
2. Hanasono, M.M.; Butler, C.E. Prevention and treatment of thrombosis in microvascular surgery. *J. Reconstr. Microsurg.* **2008**, *24*, 305–314. [CrossRef] [PubMed]
3. Lo, S.L.; Yen, Y.H.; Lee, P.J.; Liu, C.C.; Pu, C.M. Factors Influencing Postoperative Complications in Reconstructive Microsurgery for Head and Neck Cancer. *J. Oral Maxillofac. Surg.* **2017**, *75*, 867–873. [CrossRef]
4. Novakovic, D.; Patel, R.S.; Goldstein, D.P.; Gullane, P.J. Salvage of failed free flaps used in head and neck reconstruction. *Head Neck Oncol.* **2009**, *1*, 33. [CrossRef] [PubMed]
5. Košec, A.; Solter, D.; Ribić, A.; Knežević, M.; Vagić, D.; Pegan, A. Systemic Inflammatory Markers as Predictors of Postoperative Complications and Survival in Patients with Advanced Head and Neck Squamous Cell Carcinoma Undergoing Free-Flap Reconstruction. *J. Oral Maxillofac. Surg.* **2022**, *80*, 744–755. [CrossRef] [PubMed]
6. Yu, J.; Hong, J.P.; Suh, H.P.; Park, J.Y.; Kim, D.H.; Ha, S.; Lee, J.; Hwang, J.H.; Kim, Y.K. Prognostic Nutritional Index is a Predictor of Free Flap Failure in Extremity Reconstruction. *Nutrients* **2020**, *12*, 562. [CrossRef]
7. Shum, J.; Markiewicz, M.R.; Park, E.; Bui, T.; Lubek, J.; Bell, R.B.; Dierks, E.J. Low prealbumin level is a risk factor for microvascular free flap failure. *J. Oral Maxillofac. Surg.* **2014**, *72*, 169–177. [CrossRef]
8. Takagi, K.; Domagala, P.; Polak, W.G.; Buettner, S.; Wijnhoven, B.P.L.; Ijzermans, J.N.M. Prognostic significance of the controlling nutritional status (CONUT) score in patients undergoing gastrectomy for gastric cancer: A systematic review and meta-analysis. *BMC Surg.* **2019**, *19*, 129. [CrossRef]
9. Qian, Y.; Liu, H.; Pan, J.; Yu, W.; Lv, J.; Yan, J.; Gao, J.; Wang, X.; Ge, X.; Zhou, W. Preoperative Controlling Nutritional Status (CONUT) score predicts short-term outcomes of patients with gastric cancer after laparoscopy-assisted radical gastrectomy. *World J. Surg. Oncol.* **2021**, *19*, 25. [CrossRef]

10. Venianaki, M.; Andreou, A.; Nikolouzakis, T.K.; Chrysos, E.; Chalkiadakis, G.; Lasithiotakis, K. Factors Associated with Malnutrition and Its Impact on Postoperative Outcomes in Older Patients. *J. Clin. Med.* **2021**, *10*, 2550. [CrossRef]
11. Abela, G. The potential benefits and harms of early feeding post-surgery: A literature review. *Int. Wound J.* **2017**, *14*, 870–873. [CrossRef]
12. Jones, N.F.; Jarrahy, R.; Song, J.I.; Kaufman, M.R.; Markowitz, B. Postoperative medical complications—Not microsurgical complications—Negatively influence the morbidity, mortality, and true costs after microsurgical reconstruction for head and neck cancer. *Plast. Reconstr. Surg.* **2007**, *119*, 2053–2060. [CrossRef]
13. Jie, B.; Jiang, Z.M.; Nolan, M.T.; Zhu, S.N.; Yu, K.; Kondrup, J. Impact of preoperative nutritional support on clinical outcome in abdominal surgical patients at nutritional risk. *Nutrition* **2012**, *28*, 1022–1027. [CrossRef]
14. Skipper, A.; Coltman, A.; Tomesko, J.; Charney, P.; Porcari, J.; Piemonte, T.A.; Cheng, F.W. Adult Malnutrition (Undernutrition) Screening: An Evidence Analysis Center Systematic Review. *J. Acad. Nutr. Diet.* **2020**, *120*, 669–708. [CrossRef]
15. Smale, B.F.; Mullen, J.L.; Buzby, G.P.; Rosato, E.F. The efficacy of nutritional assessment and support in cancer surgery. *Cancer* **1981**, *47*, 2375–2381. [CrossRef]
16. de Ulíbarri, J.I.; González-Madroño, A.; de Villar, N.G.; González, P.; González, B.; Mancha, A.; Rodríguez, F.; Fernández, G. CONUT: A tool for controlling nutritional status. First validation in a hospital population. *Nutr. Hosp.* **2005**, *20*, 38–45.
17. Toyokawa, T.; Kubo, N.; Tamura, T.; Sakurai, K.; Amano, R.; Tanaka, H.; Muguruma, K.; Yashiro, M.; Hirakawa, K.; Ohira, M. The pretreatment Controlling Nutritional Status (CONUT) score is an independent prognostic factor in patients with resectable thoracic esophageal squamous cell carcinoma: Results from a retrospective study. *BMC Cancer* **2016**, *16*, 722. [CrossRef]
18. Unal, I. Defining an Optimal Cut-Point Value in ROC Analysis: An Alternative Approach. *Comput. Math. Methods Med.* **2017**, *2017*, 3762651. [CrossRef]
19. Lee, S.C.; Lee, J.G.; Lee, S.H.; Kim, E.Y.; Chang, J.; Kim, D.J.; Paik, H.C.; Chung, K.Y.; Jung, J.Y. Prediction of postoperative pulmonary complications using preoperative controlling nutritional status (CONUT) score in patients with resectable non-small cell lung cancer. *Sci. Rep.* **2020**, *10*, 12385. [CrossRef]
20. Lindeborg, M.M.; Sethi, R.K.V.; Puram, S.V.; Parikh, A.; Yarlagadda, B.; Varvares, M.; Emerick, K.; Lin, D.; Durand, M.L.; Deschler, D.G. Predicting length of stay in head and neck patients who undergo free flap reconstruction. *Laryngoscope Investig. Otolaryngol.* **2020**, *5*, 461–467. [CrossRef]
21. O'Neill, A.C.; Mughal, M.; Saggaf, M.M.; Wisniewski, A.; Zhong, T.; Hofer, S.O.P. A structured pathway for accelerated postoperative recovery reduces hospital stay and cost of care following microvascular breast reconstruction without increased complications. *J. Plast. Reconstr. Aesthet. Surg.* **2020**, *73*, 19–26. [CrossRef] [PubMed]
22. Iseki, Y.; Shibutani, M.; Maeda, K.; Nagahara, H.; Ohtani, H.; Sugano, K.; Ikeya, T.; Muguruma, K.; Tanaka, H.; Toyokawa, T.; et al. Impact of the Preoperative Controlling Nutritional Status (CONUT) Score on the Survival after Curative Surgery for Colorectal Cancer. *PLoS ONE* **2015**, *10*, e0132488. [CrossRef] [PubMed]
23. Adams, J.; Charlton, P. Anaesthesia for microvascular free tissue transfer. *BJA CEPD Rev.* **2003**, *3*, 33–37. [CrossRef]
24. Felekis, D.; Eleftheriadou, A.; Papadakos, G.; Bosinakou, I.; Ferekidou, E.; Kandiloros, D.; Katsaragakis, S.; Charalabopoulos, K.; Manolopoulos, L. Effect of perioperative immuno-enhanced enteral nutrition on inflammatory response, nutritional status, and outcomes in head and neck cancer patients undergoing major surgery. *Nutr. Cancer* **2010**, *62*, 1105–1112. [CrossRef] [PubMed]
25. Pohlenz, P.; Klatt, J.; Schön, G.; Blessmann, M.; Li, L.; Schmelzle, R. Microvascular free flaps in head and neck surgery: Complications and outcome of 1000 flaps. *Int. J. Oral Maxillofac. Surg.* **2012**, *41*, 739–743. [CrossRef]
26. Chiarelli, M.; Achilli, P.; Tagliabue, F.; Brivio, A.; Airoldi, A.; Guttadauro, A.; Porro, F.; Fumagalli, L. Perioperative lymphocytopenia predicts mortality and severe complications after intestinal surgery. *Ann. Transl. Med.* **2019**, *7*, 311. [CrossRef]
27. Lee, Y.Y.; Choi, C.H.; Sung, C.O.; Do, I.G.; Hub, S.J.; Kim, H.J.; Kim, T.J.; Lee, J.W.; Bae, D.S.; Kim, B.G. Clinical significance of changes in peripheral lymphocyte count after surgery in early cervical cancer. *Gynecol. Oncol.* **2012**, *127*, 107–113. [CrossRef]
28. Vulliamy, P.E.; Perkins, Z.B.; Brohi, K.; Manson, J. Persistent lymphopenia is an independent predictor of mortality in critically ill emergency general surgical patients. *Eur. J. Trauma Emerg. Surg.* **2016**, *42*, 755–760. [CrossRef]
29. Chen, T.; Delano, M.J.; Chen, K.; Sperry, J.L.; Namas, R.A.; Lamparello, A.J.; Deng, M.; Conroy, J.; Moldawer, L.L.; Efron, P.A.; et al. A road map from single-cell transcriptome to patient classification for the immune response to trauma. *JCI Insight* **2021**, *6*, e145108. [CrossRef]
30. Edomskis, P.P.; Dik, W.A.; Sparreboom, C.L.; Nagtzaam, N.M.A.; van Oudenaren, A.; Lambrichts, D.P.V.; Bayon, Y.; van Dongen, N.N.N.; Menon, A.G.; de Graaf, E.J.R.; et al. Monocyte response after colorectal surgery: A prospective cohort study. *Front. Immunol.* **2022**, *13*, 1031216. [CrossRef]
31. Klava, A.; Windsor, A.; Boylston, A.W.; Reynolds, J.V.; Ramsden, C.W.; Guillou, P.J. Monocyte activation after open and laparoscopic surgery. *Br. J. Surg.* **1997**, *84*, 1152–1156.
32. Malietzis, G.; Johns, N.; Al-Hassi, H.O.; Knight, S.C.; Kennedy, R.H.; Fearon, K.C.; Aziz, O.; Jenkins, J.T. Low Muscularity and Myosteatosis Is Related to the Host Systemic Inflammatory Response in Patients Undergoing Surgery for Colorectal Cancer. *Ann. Surg.* **2016**, *263*, 320–325. [CrossRef]
33. Pedrazzani, C.; Conti, C.; Zamboni, G.A.; Chincarini, M.; Turri, G.; Valdegamberi, A.; Guglielmi, A. Impact of visceral obesity and sarcobesity on surgical outcomes and recovery after laparoscopic resection for colorectal cancer. *Clin. Nutr.* **2020**, *39*, 3763–3770. [CrossRef]
34. Ri, M.; Aikou, S.; Seto, Y. Obesity as a surgical risk factor. *Ann. Gastroenterol. Surg.* **2017**, *2*, 13–21. [CrossRef]

35. Fischer, J.P.; Nelson, J.A.; Sieber, B.; Cleveland, E.; Kovach, S.J.; Wu, L.C.; Serletti, J.M.; Kanchwala, S. Free tissue transfer in the obese patient: An outcome and cost analysis in 1258 consecutive abdominally based reconstructions. *Plast. Reconstr. Surg.* **2013**, *131*, 681e–692e. [CrossRef]
36. Sinha, S.; Ruskin, O.; D'Angelo, A.; McCombe, D.; Morrison, W.A.; Webb, A. Are overweight and obese patients who receive autologous free-flap breast reconstruction satisfied with their postoperative outcome? A single-centre study. *J. Plast. Reconstr. Aesthet. Surg.* **2016**, *69*, 30–36. [CrossRef]
37. Chang, E.I.; Liu, J. Prospective Evaluation of Obese Patients Undergoing Autologous Abdominal Free Flap Breast Reconstruction. *Plast. Reconstr. Surg.* **2018**, *142*, 120e–125e. [CrossRef]
38. de la Garza, G.; Militsakh, O.; Panwar, A.; Galloway, T.L.; Jorgensen, J.B.; Ledgerwood, L.G.; Kaiser, K.; Kitzerow, C.; Shnayder, Y.; Neumann, C.A.; et al. Obesity and perioperative complications in head and neck free tissue reconstruction. *Head Neck* **2016**, *38* (Suppl. S1), E1188–E1191. [CrossRef]
39. Crippen, M.M.; Brady, J.S.; Mozeika, A.M.; Eloy, J.A.; Baredes, S.; Park, R.C.W. Impact of Body Mass Index on Operative Outcomes in Head and Neck Free Flap Surgery. *Otolaryngol. Head Neck Surg.* **2018**, *159*, 817–823. [CrossRef]
40. Popkin, B.M.; Corvalan, C.; Grummer-Strawn, L.M. Dynamics of the double burden of malnutrition and the changing nutrition reality. *Lancet* **2020**, *395*, 65–74. [CrossRef]
41. Wells, J.C.; Sawaya, A.L.; Wibaek, R.; Mwangome, M.; Poullas, M.S.; Yajnik, C.S.; Demaio, A. The double burden of malnutrition: Aetiological pathways and consequences for health. *Lancet* **2020**, *395*, 75–88. [CrossRef] [PubMed]
42. Evans, D.C.; Corkins, M.R.; Malone, A.; Miller, S.; Mogensen, K.M.; Guenter, P. The Use of Visceral Proteins as Nutrition Markers: An ASPEN Position Paper. *Nutr. Clin. Pract.* **2021**, *36*, 22–28. [CrossRef] [PubMed]

Third Publication

JPRAS Open 44 (2025) 414–423

Contents lists available at ScienceDirect

JPRAS Open

journal homepage: www.elsevier.com/locate/jpra

Original Article

Fibrinogen-to-albumin ratio (FAR) for predicting microvascular flap complications in reconstructive surgery

Rihards Peteris Rocans[a,b,*], Janis Zarins[c,d], Evita Bine[a], Insana Mahauri[b], Renars Deksnis[e], Margarita Citovica[f], Simona Donina[g,h], Indulis Vanags[b], Biruta Mamaja[b]

[a] Intensive Care Clinic, Riga East Clinical University Hospital, Riga, Latvia
[b] Department of Anaesthesia and Intensive Care, Riga Stradiņš University, Riga, Latvia
[c] Department of Hand and Plastic Surgery, Microsurgery Centre of Latvia, Riga, Latvia
[d] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia
[e] Surgical Oncology Clinic, Riga East Clinical University Hospital, Riga, Latvia
[f] Laboratory Department, Riga East Clinical University Hospital, Riga, Latvia
[g] Institute of Microbiology and Virology, Riga Stradiņš University, Riga, Latvia
[h] Outpatient Department, Riga East Clinical University Hospital, Riga, Latvia

ARTICLE INFO

Article history:
Received 21 January 2025
Accepted 30 March 2025
Available online 2 April 2025

Keywords:
fibrinogen-to-albumin ratio
FAR
microvascular flap surgery
microvascular flap complications
reconstructive surgery

ABSTRACT

Background: Microvascular flap surgery is a widely used procedure for the reconstruction of various defects. Data on laboratory biomarkers for the prediction of flap complications are currently limited. We aimed to investigate the link between preoperative fibrinogen-to-albumin ratio (FAR) and various flap complications.
Methods: This prospective cohort study included 130 adult patients who underwent elective microvascular flap surgery. Preoperative blood samples for the analysis of plasma fibrinogen (g/L) and albumin (g/L) were collected on the day of surgery before initiating crystalloid infusion. Post-operative data on various flap complications were obtained.
Results: The overall complication rate was 17.7% with true flap loss occurring in 5.4%. Binary logistic regression revealed that the patients with FAR<0.08 and FAR<0.06 had increased odds of flap hematoma or flap loss (OR 3.68 [1.04-13.03], p=0.044 and 6.01

* Corresponding author.
E-mail address: rihardspeteris.rocans@rsu.lv (R.P. Rocans).

https://doi.org/10.1016/j.jpra.2025.03.022

[1.71-21.08], p=0.005). Patients with FAR>0.10 had increased odds of minor flap complications (OR 5.47 [1.33-22.50], p=0.019). Patients with FAR<0.06 had increased odds of any flap complications (OR 4.71 [1.27-18.03], p=0.021). Patients with FAR>0.10 also had increased odds of any flap complications (OR 3.09 [1.08-8.81], p=0.035), implying a U-shaped link.

Conclusions: Assessment of FAR to estimate the risk of complications has considerable predictive value in microvascular flap surgery. Patients undergoing this surgery can be evaluated to predict their nutrition and coagulation risks and optimize decision-making in their perioperative care.

Introduction

Microvascular flap surgery is a complex procedure commonly used in reconstructive surgery to repair or replace damaged tissues, often following trauma, cancer resection, or preceding defects. Although significant progress has been made toward improving outcomes in microvascular flap surgery, flap loss continues to occur.[1] Flap thrombosis, flap hematoma, and subsequent flap loss are the most severe major surgical complications.[2] Minor complications, such as problematic and delayed healing, wound dehiscence, infection, fistula, and donor site problems can also have a negative impact on patient recovery.[3] Even though microvascular flap transplantation relies greatly on specific surgical concepts, the issue of systemic inflammation, hemostasis, and the rheological properties of blood are highly relevant.[4] These complex interactions between the flap and patient's various systemic characteristics may open new avenues for preoperative risk assessment.[3] Multiple studies have proposed the in-depth analysis of preoperative biomarkers to predict complications in microvascular flap surgery.[5,6] Using laboratory biomarkers for screening and assessing flap complication risk is convenient and reasonable as laboratory evaluation is routinely performed for preoperative assessment.[7] Furthermore, reduced blood albumin levels are associated with higher levels of proinflammatory mediators and poor tissue regeneration.[8] High fibrinogen is also linked to tissue inflammation and repair[9] and is frequently used as a predictor of complications for numerous clinical manifestations.[9,10] Although individual laboratory measures of various biomarkers can predict surgical complications and patient outcomes,[8-10] recent studies demonstrated the improved predictive capacity of the combined measures.[11,12] The fibrinogen-to-albumin ratio (FAR) is a novel combined measurement that has displayed satisfactory post-operative outcome assessment ability in different surgical populations.[11-14] The FAR may be considered as an indicator of the balance between procoagulant and anticoagulant factors, as well as the inflammatory state in the body.[15] Using FAR has been suggested in other surgical populations[16,17]; however, the specific use of FAR in the context of microvascular flap surgery is yet to be elucidated. The purpose of this study was to assess the predictive value of FAR in predicting complications in elective microvascular flap surgery.

Materials and Methods

Patient selection

This prospective cohort study included 130 patients undergoing elective microvascular flap transplantation surgery at the Riga East University Hospital from the October 1, 2021 to January 31, 2024. The study was observational in nature, all surgical, anesthesia, and perioperative care decisions were made by a multidisciplinary team of attending physicians. The inclusion criteria were adult patients

undergoing elective microvascular flap transplantation. The exclusion criteria were liver failure, kidney failure, disseminated tumor metastasis; preoperative neoadjuvant treatment (radiotherapy and chemotherapy); multiple concomitant malignancies; incomplete data; sepsis or severe systemic bacterial infection; autoimmune disorders; blood-borne viral infections (Hepatitis B, Hepatitis C, and HIV); pregnant patients and lactating women; or patients with congenital coagulation disorder.

Surgical protocol

The selection of flap type was based on the defect characteristics, pedicle length, patient positioning during surgery, patient body mass index (BMI), and body fat distribution. All operations were performed by a team of experienced surgeons. The following flap types were used in the study: anterolateral thigh flap; fibular flap; deep inferior epigastric artery perforator flap; radial free forearm flap, gracilis muscle flap, temporal artery flap, serratus anterior flap, and latissimus dorsi flap. Attending surgeons closely monitored the microvascular flap for the first 3-5 post-operative days. Clinical assessment of the flap color, temperature, tissue turgor, and capillary refill was used to monitor flap patency. In all cases of early flap loss, urgent surgical re-exploration was performed.

Anesthesia protocol

All patients received general anesthesia with monitoring of electrocardiography, pulse oximetry, blood pressure, end-tidal carbon dioxide concentration, body temperature, and diuresis. Fentanyl (Fentanyl-Kalceks® 0.05 mg/ml, A/S Kalceks, Riga, Latvia) 1.5–2 μg/kg and propofol (Propofol® 10 mg/ml, Fresenius Kabi AG, Bad Homburg, Germany) 1–2 mg/kg were used intravenously for induction. General anesthesia was maintained using sevoflurane (Sevorane®, AbbVie S.r.l., Campoverde, Italy), and intraoperative analgesia was provided with fentanyl 1–1.5 μg/kg/h. Cisatracurium (Nimbex 2 mg/ml, Aspen Pharma Ltd, Dublin, Ireland) 0.15 mg/kg iv was used for intubation, followed by a continuous infusion of 1–2 μg/kg/min. Crystalloid (RiLac, B. Braun Melsungen AG, Melsungen, Germany) was administered at a rate of 3.0 to 6.0 ml/kg iv per hour during surgery and was adjusted according to the urine output by the anesthesiologist. Vasopressors were administered when the mean arterial blood pressure was <65 mmHg for more than 5 min. Colloid fluid (Gelofusine, B. Braun Melsungen AG, Melsungen, Germany) was administered if indicated by the anesthesiologist. Peripheral nerve blocks were performed when applicable. Patients with excessive blood loss or those with hemoglobin <7 g/dl received blood product transfusions. Post-operatively, patients were monitored for vital signs, supportive treatment, and pain management in the post-anesthesia care unit. Post-operative thromboprophylaxis was provided with 40 mg of enoxaparin (Clexane®, Sanofi-Aventis S.A., Barcelona, Spain) once daily starting on the first post-operative day.

Data collection

Blood samples were obtained on the day of surgery immediately upon the first arrival in the operating room before initiation of the first crystalloid infusion. Full blood count analysis was performed using the XN-1500 system (Sysmex Europe SE, Norderstedt, Germany). Concentrations of albumin were analyzed using the colorimetric method (Cobas C, Roche/Hitachi, Manheim, Germany). Concentrations of fibrinogen were analyzed using the CS 5100 system (Sysmex Corporation, Kobe, Japan). FAR was defined as the proportion of the plasma fibrinogen (g/l) to the plasma albumin level (g/l). The written and electronic health records were used to obtain information on the patient's demographics, flap type, location and duration of surgery, comorbidities, anesthesia and post-operative care, flap and donor site outcomes, duration of intensive care unit stay, and duration of hospitalization. All data collection was performed according to a previously defined protocol.

Definitions

True flap loss was defined as the impairment of flap blood supply due to any arterial or venous anastomosis dysfunction. Flap hematoma or loss was defined as the presence of either hematoma or

true flap loss. Minor flap complications were defined as the presence of flap wound infection, slow or incomplete flap wound healing, and partial flap loss. Partial flap loss was defined as distal necrosis of flap margins with no anastomosis dysfunction. Any flap complication was defined as the presence of either true flap loss, flap hematoma, or minor flap complications. Gracilis muscle flap, temporal artery flap, serratus anterior flap, or latissimus dorsi flap were categorized as other flap types. Duration of hospitalization was defined as the time between admission to the hospital and discharge. Duration of intensive care unit (ICU) stay was defined as the time between admission to the ICU and discharge to the ward. Complication predictability index (low, moderate, and high risk) was calculated based on the defect etiology and presence of coronary heart disease, diabetes, smoking, peripheral arterial vascular disease, and arterial hypertension.[18]

Ethics

The study protocol and the informed consent form were approved by the Science Department of the Riga East University Hospital (Approval Number Nr.AP/08-08/22/135) and by the Ethics Committee of Riga Stradiņš University (Approval Number 22-2/399/2021).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 26.0. (IBM Corp. Armonk, NY, USA). The Kolmogorov–Smirnov test was used to evaluate whether the datasets conformed to a normal distribution. Continuous variables conforming to normal distribution were presented as mean and CI95. Differences in data distribution between the groups were evaluated using the Mann–Whitney U test for non-parametric datasets and two-sample t-test or one-way analysis of variance for datasets conforming with normal distribution. Correlation between parameters was investigated using the Spearman rank correlation test for non-parametric datasets. The Chi-squared and Fisher's exact tests were applied for nominal variable sets. Binary logistic regression models were used to obtain odds ratios for specific variables. Statistical significance was assumed if two-tailed $p<0.05$.

Results

In total, 130 patients—69 (53.1%) men and 61 (46.9%) women—were included. The mean age was 56.5 years (95% CI95 53.9-59.1). The overall complication rate was 17.7% (N=23). True flap loss with vascular compromise occurred in 5.4% (N=7) with 3 of these cases being late flap loss (>72 h). All 4 cases of early true flap loss underwent urgent anastomosis revision. Two cases of late flap loss underwent repeated microvascular flap transplantation, and 1 case underwent necrectomy and reconstruction with a regional flap. Other flap complications occurred in 16 cases (12.3%), with flap hematoma occurring in 5 cases.

No significant differences in age or gender distribution were noted in patients with any flap complications and in patients without complications (Table 1). No significant differences in the rates of true flap failure or any flap complications were found between different areas of reconstruction. Multiple logistic regression analysis revealed that using other flap types increased the odds of any flap complications (OR 5.23 [1.86-14.70], p=0.002). No significant differences in true flap failure or any flap complications were found between different indications for reconstruction. Among the included comorbidities, obesity was found to be common in patients with any flap complications (p=0.028). Patients with high complication probability index had increased rates of true flap loss ($p<0.001$) and any flap complications (p=0.011).

Higher intraoperative hematocrit was associated with flap complications, with the highest intraoperative hematocrit found in cases with subsequent true flap loss (p=0.032; Table 1). No significant links were found between other perioperative factors and the rate of flap complications. Patients with any flap complications had a longer duration of hospitalization (13.72 [11.79-16.15] vs. 23.83 [16.92-30.75], p=0.001). There was no difference in the duration of ICU stay between patients with any flap complications and patients with no flap complications (1.17 [0.23-2.10] vs. 1.46 [0.96-1.96], p=0.537). FAR was positively correlated with the duration of hospitalization ($r=0.422$, $p<0.001$).

Table 1
Demographic Characteristics, Perioperative Considerations, and Comorbidities

Patient group	Overall N = 130	No complications N = 107	True flap loss N = 7	Any flap complications N = 23	p - value
Demographical data					
Mean age, years	56.5(53.9-59.1)	57.1 (54.4-59.8)	50.1 (45.9-55.5)	50.6 (47.7-53.1)	0.123
Women, n (%)	61 (46.9%)	54 (50.5%)	2 (28.6%)	8 (34.7%)	0.109
Area of reconstruction					
Extremity, n (%)	28 (21.5%)	20 (18.7%)	2 (28.6%)	8 (34.8%)	0.217
ENT, n (%)	59 (45.4%)	50 (46.7%)	3 (42.9%)	9 (39.1%)	0.795
Head and Neck, n (%)	24 (18.5%)	21 (19.6%)	1 (14.3%)	3 (13.0%)	0.731
Breast, n (%)	19 (14.6%)	16 (15.0%)	1 (14.3%)	3 (13.0%)	0.972
Microvascular flap type					
ALT, (%)	61 (46.9%)	55 (51.4%)	2 (28.6%)	6 (26.1%)	0.055
Fibular flap, (%)	12 (9.2%)	8 (7.5%)	2 (28.6%)	4 (17.4%)	0.093
DIEP, n (%)	15 (11.5%)	13 (12.2%)	-	2 (8.7%)	0.639
Radial artery flap, n (%)	9 (6.9%)	9 (8.4%)	-	-	-
Other, n (%)	33 (25.4%)	22 (20.6%)	3 (42.9%)	11 (47.8%)	0.015
Indication for surgery					
Trauma, n (%)	10 (7.7%)	8 (7.5%)	-	2 (8.7%)	0.843
Oncology, n (%)	84 (64.6%)	72 (67.3%)	4 (57.1%)	12 (52.2%)	0.360
Defect, n (%)	23 (17.7%)	18 (16.8%)	3 (42.9%)	5 (21.7%)	0.220
Infection, n (%)	13 (10.0%)	9 (8.4%)	-	4 (17.4%)	0.195
Comorbidities					
Coronary artery disease, n (%)	9 (6.9%)	7 (6.5%)	1 (14.3%)	2 (8.7%)	0.872
Diabetes mellitus, n (%)	8 (6.2%)	6 (5.6%)	-	2 (8.7%)	0.578
Hypertension, n (%)	35 (26.9%)	27 (25.2%)	3 (42.8%)	8 (34.7%)	0.427
Dyslipidemia, n (%)	20 (15.4%)	14 (13.1%)	1 (14.3%)	6 (26.1%)	0.624
Smoking history, n (%)	18 (13.8.1%)	14 (13.1%)	1 (14.3%)	4 (17.4%)	0.863
Obesity (BMI>30 kg/m^2), n (%)	15 (11.5%)	9 (8.4%)	2 (28.6%)	6 (26.1%)	0.028
Cerebrovascular accident, n (%)	6 (4.6%)	6 (5.6%)	-	-	-
Complication predictability index					
Low risk ()	74 (56.9%)	62 (57.9%)	2 (28.5%)	10 (43.5%)	0.172
Moderate risk ()	56 (43.1%)	43 (40.2%)	3 (43.0%)	10 (43.5%)	0.952
High risk ()	7 (5.4%)	2 (1.9%)	2 (28.5%)	3 (13.0%)	0.001
Anesthesia and Surgical factors					
Duration of surgery, h	6.25 (5.74-6.76)	6.35 (5.76-6.94)	7.25 (6.11-8.39)	5.79 (4.67-6.91)	0.324
Volume of intraoperative crystalloid, ml	2346.97 (2190.41-2450.00)	2340.74 (2167.75-2513.74)	2875.00 (1681.58-4068.42)	2375.00 (1943.96-2086.04)	0.845
Volume of intraoperative colloid, ml	507.58 (426.18-588.77)	481.48 (390.71-572.25)	500.00 (-)	625.00 (427.53-822.46)	0.564
Intraoperative colloid to crystalloid ratio	0.22 (0.18-0.26)	0.20 (0.16-0.24)	0.22 (0.12-0.32)	0.30 (0.15-0.44)	0.364
Intraoperative hematocrit, %	30.94 (29.69-32.20)	31.02 (29.66-32.38)	33.00 (29.33-36.67)	30.55 (26.58-34.538)	0.032
Use of vasopressors/sympathomimetics, n (%)	67 (51.54%)	51 (47.66%)	5 (71.43%)	16 (69.56%)	0.095

Data are presented as mean (CI95) or count (percentage); Abbreviations - BMI (body mass index); ENT (ear, nose, and throat surgery); DIEP (deep inferior epigastric artery perforator flap); ALT (anterolateral thigh flap).

Patients with any flap complications had a significantly lower plasma lymphocyte count (p=0.001) and significantly lower mean plasma albumin (p=0.045; Table 2). Multiple logistic regression analysis revealed that increased lymphocyte count decreases the odds of any flap complications (OR 0.39 [0.17-0.92], p=0.031). Upon further analysis of the specific complication groups, patients with flap hematoma or flap loss had a lower mean fibrinogen than patients with no complications (2.75 [1.84-3.67] vs. 3.44 [3.25-3.64], p=0.014). Patients with minor flap complications had a significantly

Table 2
Biomarkers for predicting flap complications

Patient group	Overall N = 130	No complications N = 107	True flap loss N = 7	Any flap complications N = 23	p-value
Biomarkers					
Leukocyte count 10^9/l	6.31 (5.89-6.73)	6.42 (5.94-6.91)	6.16 (2.64-9.70)	5.77 (4.99-6.55)	0.379
Lymphocyte count 10^9/l	1.65 (1.52-1.77)	1.71 (1.58-1.84)	1.56 (0.43-2.71)	1.36 (1.08-1.63)	0.018
Red blood cell count 10^9/l	4.08 (3.97-4.19)	4.08 (3.96-4.19)	4.24 (3.35-5.12)	4.10 (3.77-4.43)	0.717
Platelet count 10^9/l	244.97 (230.81-259.14)	244.00 (228.21-259.79)	202.00 (148.94-255.05)	249.52 (214.99-284.04)	0.770
Hemoglobin g/dl	12.31 (11.99-12.64)	12.31 (11.97-12.66)	13.06 (10.44-15.68)	12.30 (11.34-13.27)	0.632
Mean plasma albumin, g/l	38.90 (38.12-39.69)	39.34 (38.54-40.14)	41.16 (34.67-47.65)	36.91 (34.50-39.32)	0.045
Mean plasma fibrinogen, g/l	3.47 (3.27-3.66)	3.44 (3.25-3.64)	2.66 (1.65-3.68)	3.58 (2.98-4.18)	0.892
Fibrinogen-to-albumin ratio	0.09 (0.08-0.10)	0.09 (0.08-0.10)	0.09 (0.05-0.13)	0.11 (0.09-0.14)	0.016

Data are presented as mean (CI95).

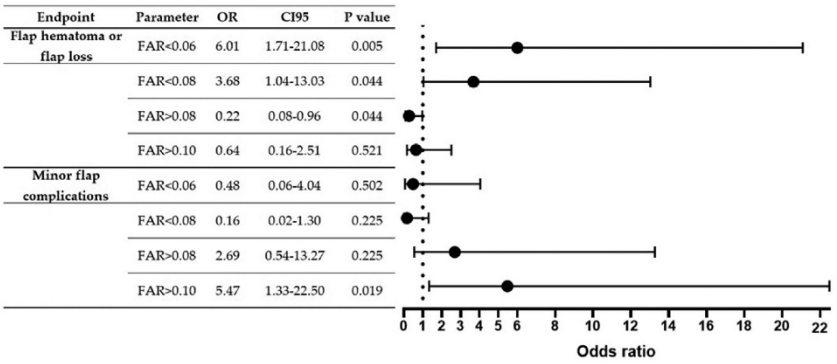

Figure 1. Association between the fibrinogen-to-albumin ratio (FAR) and flap complication groups at various cutoff levels. Logistic regression analysis of the association between FAR at different cutoff levels and flap hematoma, flap loss, or minor flap complications. Patients with FAR<0.08 and FAR<0.06 had increased odds of flap hematoma or flap loss (OR 3.68 [1.04-13.03], p=0.044 and 6.01 [1.71-21.08], p=0.005). Patients with FAR>0.10 had increased odds of minor flap complications (OR 5.47 [1.33-22.50], p=0.019).

higher plasma fibrinogen than patients with no flap complications (4.19 [3.41-4.98] vs. 3.44 [3.25-3.64], p=0.043). Patients with minor flap complications had lower mean albumin levels than patients with no complications (33.30 [30.18-36.42] vs. 39.34 [38.12-39.69], p<0.001). No difference in plasma albumin level was found between patients with flap hematoma or flap loss and patients with no complications (p=0.394). Increased FAR was specifically linked to minor flap complications when compared to patients with no flap complications (0.12 [0.09-0.15] vs. 0.09 [0.08-0.10], p=0.002). Decreased FAR was in turn specifically linked to flap loss or flap hematoma when compared to patients with no flap complications (0.07 [0.04-0.09] vs. 0.09 [0.08-0.10], p=0.046).

Logistic regression analysis revealed the association between FAR and various flap complications at various cutoff levels (Figure 1). Patients with FAR<0.08 and FAR<0.06 had increased odds of flap hematoma or flap loss (OR 3.68 [1.04-13.03], p=0.044 and 6.01 [1.71-21.08], p=0.005). Patients with FAR>0.08 had decreased odds of flap hematoma and flap loss (OR 0.22 [0.08-0.96], p=0.044). Patients with FAR>0.10 had increased odds of minor flap complications (OR 5.47 [1.33-22.50], p=0.019).

After adjustment for lymphocyte count and the presence of other flap types, multiple logistic regression analysis revealed that patients with FAR<0.06 had increased odds of any flap complications

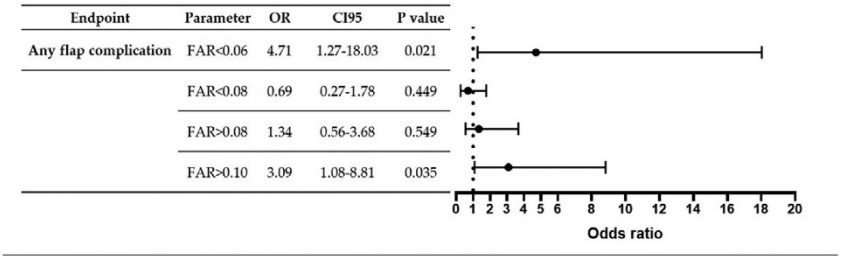

Figure 2. U-shaped association between fibrinogen-to-albumin ratio (FAR) and any flap complications.
Multiple logistic regression analysis of the association between FAR and any flap complications at different cut-off levels. Multiple logistic regression analysis adjusted for lymphocyte count and the presence of other flap types. Patients with FAR<0.06 had increased odds of any flap complications (OR 4.71 [1.27-18.03], p=0.021). Patients with FAR>0.10 also had increased odds of any flap complications (OR 3.09 [1.08-8.81], p=0.035).

(OR 4.71 [1.27-18.03], p=0.021; Figure 2). Patients with FAR>0.10 also had increased odds of any flap complications (OR 3.09 [1.08-8.81], p=0.035).

Discussion

The main findings of the present study were that increased and decreased preoperative FAR is a predictor for flap complications, implying a U-shaped association. Decreased FAR is a predictor for flap hematoma or flap loss and an increased FAR is a predictor for minor flap complications. FAR was positively linked to the duration of hospitalization. Flap complications were also found to be linked to low lymphocyte count, high intraoperative hematocrit, obesity, high complication probability index, and the use of other flap types. The overall complication rate was 17.7%. True flap loss occurred in 5.4%. Duration of hospitalization was significantly longer in patients with flap complications.

The literature currently available on microvascular flap surgery highlights the importance of a thorough risk assessment that goes beyond established measurements.[5,11,16] The link between increased plasma fibrinogen and decreased plasma albumin in patients with flap complications is consistent with the findings from studies that highlight the role of inflammatory and coagulation markers in predicting the adverse outcomes in various surgical settings.[4,5,14]

Plasma albumin is frequently evaluated and corrected prior to surgical procedures to promote tissue healing and improve results.[14] Our results revealed a link between low plasma albumin levels and increased risk of minor surgical complications. According to several previous studies, lower preoperative albumin levels are related to a higher incidence of surgical complications.[5,6] Reduced albumin levels may limit the efficiency of the inflammatory response and hinder the healing process, resulting in delayed or incomplete wound closure.[19] Previous studies have linked low plasma albumin as an indicator of malnutrition risk, which increases the rate of complications in microvascular flap surgery.[5,6] Albumin is also crucial for maintaining oncotic pressure and fluid balance inside the tissues[20] and its deficiency might lead to insufficient perfusion and oxygenation of the flaps, resulting in flap complications.[21] Chronic inflammation and hypoalbuminemia are present in double burden malnutrition[22] which is also associated with flap complications.[6]

Fibrinogen, a key component of the coagulation cascade, plays a varied role in the complex processes of wound healing and tissue regeneration.[9] In our study, we found that patients with minor flap complications had significantly higher plasma fibrinogen levels, whereas patients with flap hematoma or flap loss had lower plasma fibrinogen levels. Studies have shown that variations in fibrinogen levels have a significant impact on the success of flap surgery, with hypofibrinogenemia[23] and hyperfibrinogenemia[24] being linked to flap complications. Low fibrinogen levels can impair the formation of a strong hemostatic clot, predisposing patients to increased bleeding and a higher risk of flap failure.[10] Conversely, increased fibrinogen might be associated with increased microvascular flap thrombosis incidence rate[24] and is linked to acute[9] and chronic[25] inflammation which has been proven to be detrimental to flap success.[26]

Intriguingly, we found FAR to have a U-shaped association with flap complications. Patients with FAR>0.13 had increased odds of complications, and detailed analysis revealed that increased FAR was specifically linked to minor flap complications. Numerous previous studies have indicated a link between elevated FAR and the presence of complications in various surgical populations.[14,16] The pattern of increased fibrinogen and decreased albumin coincides with chronic inflammation[27,28] and decreased albumin is an indicator of malnutrition risk.[29] Malnutrition[5,6] and inflammation[30] have been shown to be detrimental for outcomes in free flap surgery. Moreover, the presence of malnutrition risk is specifically predictive of minor surgical complications,[6] which coincides with our results.

All reviewed articles on FAR in other surgical populations point to poor outcomes in high FAR patients.[11-14,17] Surprisingly, our study found that patients with FAR<0.05 also had a higher risk of flap complications, more specifically, flap hematoma or true flap loss. This indicates that the pathophysiology of true flap loss or hematoma is separate from the pathophysiology of minor surgical complications in microvascular flap surgery[31] and therefore may have separate interaction with FAR. Among all reviewed articles, only one study revealed similar patterns of pathophysiology, showing that hypoalbuminemia increases the risk of minor surgical complications, but has no impact on flap thrombosis or bleeding.[31] Low FAR states may be indicative of coagulopathy as low fibrinogen has been shown to increase the rates of bleeding in microvascular flap surgery.[23] Furthermore, increased albumin has been shown to have anticoagulant effects in experimental settings.[32] Moreover, albumin-induced coagulopathy has been shown to be effectively reversed by the addition of fibrinogen concentrate.[33] These aforementioned studies, while not evaluating FAR directly, may explain the pathophysiological link between low FAR and increased rates of bleeding.

The cutoff values of FAR in microvascular surgery requires further elucidation. Studies in other surgical populations show that FAR is highly dependent on the underlying diseases and indications of surgery.[14,16] Nevertheless, our study showed the potential application of FAR in preoperative risk assessment for microvascular flap surgery and outlined the different patterns of flap complication pathophysiology.

This study had some limitations. First, the single-center design prevents generalizability across populations and may have institutional bias. Second, although our sample size of 130 individuals was adequate for the current analysis, it may not have adequately captured the diversity of clinical circumstances or potential effects of subgroups within the study population. Several of our patients had malignancy as their primary indication for surgery and FAR is linked to overall poor prognosis in patients with cancer,[12] which could affect our results. Furthermore, our study focused on the predictive advantage of FAR rather than directly comparing it to other acknowledged biomarkers. Future research should strive to build on these findings by including multicenter designs, investigating subgroup effects, and using experimental approaches to improve our understanding of the relevance of FAR in different clinical settings.

Conclusions

Preoperative FAR has a U-shaped relationship with complications in microvascular flap surgery. Low FAR has considerable predictive value in case of minor flap complications whereas high FAR is predictive of flap hematoma and true flap loss. These findings enhance our understanding of the pathophysiological patterns associated with microvascular flap complications and may improve decision-making in perioperative care.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

R.P.R., B.M., S.D., I.V., and M.C. conceived and planned the study. J.Z., R.P.R., R.D., I.M., and M.C., participated in data collection. R.P.R. and E.B. performed data curation and statistical analysis. R.P.R.,

J.Z., B.M., S.D., I.M., I.V., R.D., M.C. and E.B. interpreted the results and prepared the draft. All authors have read and agreed to the published version of the manuscript.

Funding

The authors declare that Riga Stradiņš University kindly covered the publication fee for this article (Grant number Nr. 6-DN-20/1/2024). The funder was not involved in the study design, collection, analysis, interpretation of data or writing of this article.

Acknowledgments

We would like to acknowledge the help of Vita Kalnberzina, Dr. phil., Department of English studies, University of Latvia in reviewing and revising the manuscript for English grammar and syntax. We would like to thank Irina Olehnovica (Riga East University Hospital, Anesthesiology Clinic) and Anastasija Maksaja (Riga East University Hospital, Anesthesiology Clinic) for their abundant support throughout the creation of this study.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Science Department of Riga East University hospital (Approval Number Nr.AP/08-08/22/135; 08/11/2022) and by the Ethics Committee of Riga Stradins University (Ap-proval Number 22-2/399/2021; 08/07/2021).

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. The corresponding author will ensure individual privacy is not compromised during the transfer of datasets.

References

1. Zhang Y, Gazyakan E, Bigdeli AK, Will-Marks P, Kneser U, Hirche C. Soft tissue free flap for reconstruction of upper extremities: A meta-analysis on outcome and safety. *Microsurgery*. 2019;39:463–475.
2. Lo SL, Yen YH, Lee PJ, Liu CC. Pu CM. Factors influencing postoperative complications in reconstructive microsurgery for head and neck cancer. *J Oral Maxillofac Surg*. 2017;75:867–873.
3. Vincent A, Sawhney R, Ducic Y. Perioperative care of free flap patients. *Semin Plast Surg*. 2019;33:5–12.
4. Du W, Wu PF, Qing LM, Wang CY, Liang JY, Yu F, et al. Systemic and flap inflammatory response associates with thrombosis in flap venous crisis. *Inflammation*. 2015;38:298–304.
5. Yu J, Hong JP, Suh HP, Park JY, Kim DH, Ha S, et al. Prognostic nutritional index is a predictor of free flap failure in extremity reconstruction. *Nutrients*. 2020;12:562.
6. Rocans RP, Zarins J, Bine E, Deksnis R, Citovica M, Donina S, et al. The controlling nutritional status (CONUT) score for prediction of microvascular flap complications in reconstructive surgery. *J Clin Med*. 2023;12:4794.
7. Ekin Y, Günüşen İ, Özdemir ÖY, Tiftikçioğlu YÖ. Effect of coagulation status and co-morbidity on flap success and complications in patients with reconstructed free flap. *Turk J Anaesthesiol Reanim*. 2019;47:98–106.
8. Eckart A, Struja T, Kutz A, Baumgartner A, Baumgartner T, Zurfluh S, et al. Relationship of nutritional status, inflammation, and serum albumin levels during acute illness: A prospective study. *Am J Med*. 2020;133:713–722.
9. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood*. 2019;133:511–520.
10. Vanags I, Stepanovs J, Ozolina A, Mukans M, Bjertnaes LJ, Mamaja B. Thromboelastometry for assessing risks of free flap thrombosis in patients undergoing microvascular surgery. *Front Med (Lausanne)*. 2020;7:289.
11. You X, Zhou Q, Song J, Gan L, Chen J, Shen H. Preoperative albumin-to-fibrinogen ratio predicts severe postoperative complications in elderly gastric cancer subjects after radical laparoscopic gastrectomy. *BMC Cancer*. 2019;19:931.
12. Tomita K, Ochiai S, Gunji T, Hikita K, Kobayashi T, Sano T, et al. Prognostic significance of plasma fibrinogen/serum albumin ratio in the postoperative outcome of pancreatic ductal adenocarcinoma. *Anticancer Res*. 2020;40:7017–7023.
13. Li F, Ren Y, Fan J, Zhou J. The predictive value of the preoperative albumin-to-fibrinogen ratio for postoperative hospital length of stay in liver cancer patients. *Cancer Med*. 2023;12:20321–20331.
14. La Vaccara V, Cammarata R, Coppola A, Farolfi T, Cascone C, Angeletti S, et al. Data of postoperative complications related to fibrinogen-to-albumin ratio in pancreatic resections. *Data Brief*. 2022;42:108064.
15. Zhang DP, Mao XF, Wu TT, Chen Y, Hou XG, Yang Y, et al. The fibrinogen-to-albumin ratio is associated with outcomes in patients with coronary artery disease who underwent percutaneous coronary intervention. *Clin Appl Thromb Hemost*. 2020;26:1076029620933008.

16. Xu J, Li S, Feng Y, Zhang J, Peng Y, Wang X, et al. The fibrinogen/albumin ratio index as an independent prognostic biomarker for patients with combined hepatocellular cholangiocarcinoma after surgery. *Cancer Manag Res*. 2022;14:1795–1806.
17. Liu H, Qiu G, Hu F, Wu H. Fibrinogen/albumin ratio index is an independent predictor of recurrence-free survival in patients with intrahepatic cholangiocarcinoma following surgical resection. *World J Surg Oncol*. 2021;19:218.
18. Lese I, Biedermann R, Constantinescu M, Grobbelaar AO, Olariu R. Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *J Plast Reconstr Aesthet Surg*. 2021 Mar;74(3):512–522.
19. Utariani A, Rahardjo E, Perdanakusuma DS. Effects of albumin infusion on serum levels of albumin, proinflammatory cytokines (TNF-α, IL-1, and IL-6), CRP, and MMP-8; tissue expression of EGRF, ERK1, ERK2, TGF-β, collagen, and MMP-8; and wound healing in sprague dawley rats. *Int J Inflam*. 2020;2020:3254017.
20. Wiedermann CJ. Phases of fluid management and the roles of human albumin solution in perioperative and critically ill patients. *Curr Med Res Opin*. 2020;36:1961–1973.
21. Xu H, Han Z, Ma W, Zhu X, Shi J, Lin D. Perioperative albumin supplementation is associated with decreased risk of complications following microvascular head and neck reconstruction. *J Oral Maxillofac Surg*. 2021;79:2155–2161.
22. Merrill RD, Burke RM, Northrop-Clewes CA, Rayco-Solon P, Flores-Ayala R, Namaste SM, et al. Factors associated with inflammation in preschool children and women of reproductive age: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *Am J Clin Nutr*. 2017;106:348S–358S.
23. Kolbenschlag J, Diehm Y, Daigeler A, Kampa D, Fischer S, Kapalschinski N, et al. Insufficient fibrinogen response following free flap surgery is associated with bleeding complications. *GMS Interdiscip Plast Reconstr Surg DGPW*. 2016;5:Doc22.
24. Drizlionoka K, Zariņš J, Ozoliņa A, Ņikitina-Zaķe L, Mamaja B. Polymorphism rs2066865 in the fibrinogen gamma chain (FGG) gene increases plasma fibrinogen concentration and is associated with an increased microvascular thrombosis rate. *Medicina (Kaunas)*.. 2019;55:563.
25. Fibrinogen Studies Collaboration, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis [published correction appears in JAMA. 2005; 294;848. *JAMA*. 2005;294:1799–1809.
26. Košec A, Solter D, Ribić A, Knežević M, Vagić D, Pegan A. Systemic inflammatory markers as predictors of postoperative complications and survival in patients with advanced head and neck squamous cell carcinoma undergoing free-flap reconstruction. *J Oral Maxillofac Surg*. 2022;80:744–755.
27. Bender M, Haferkorn K, Tajmiri-Gondai S, Uhl E, Stein M. Fibrinogen to albumin ratio as early serum biomarker for prediction of intra-hospital mortality in neurosurgical intensive care unit patients with spontaneous intracerebral hemorrhage. *J Clin Med*. 2022;11:4214.
28. Chen X, Zhao J, You Y, Li Z, Chen S. The ratio of fibrinogen to albumin is related to the occurrence of retinopathy in type 2 diabetic patients. *Diabetes Metab Syndr Obes*. 2023;16:1859–1867.
29. Ignacio de Ulíbarri J, González-Madroño A, de Villar NG, González P, González B, Mancha A, et al. CONUT: A tool for controlling nutritional status. First validation in a hospital population. *Nutr Hosp*. 2005;20:38–45.
30. Tarle M, Čvrljević I, Raguž M, Lukšić I. Hemoglobin-Albumin-Lymphocyte-Platelet (HALP) score as a predictive model for the success of reconstruction of head and neck defects with free microvascular flaps. *J Clin Med*. 2023;12:5314.
31. Shum J, Markiewicz MR, Park E, Bui T, Lubek J, Bell RB, et al. Low prealbumin level is a risk factor for microvascular free flap failure. *J Oral Maxillofac Surg*. 2014;72:169–177.
32. Paar M, Rossmann C, Nusshold C, Wagner T, Schlagenhauf A, Leschnik B, Oettl K, et al. Anticoagulant action of low, physiologic, and high albumin levels in whole blood. *PLoS One*. 2017;12:e0182997.
33. Winstedt D, Hanna J, Schött U. Albumin-induced coagulopathy is less severe and more effectively reversed with fibrinogen concentrate than is synthetic colloid-induced coagulopathy. *Scand J Clin Lab Invest*. 2013;73:161–169.

Fourth Publication

Journal of *Clinical Medicine*

MDPI

Article

Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery

Rihards Peteris Rocans [1,2,*], Janis Zarins [3,4], Evita Bine [1], Insana Mahauri [2], Renars Deksnis [5], Margarita Citovica [6], Simona Donina [7,8], Indulis Vanags [2], Sabine Gravelsina [7], Anda Vilmane [7], Santa Rasa-Dzelzkaleja [7] and Biruta Mamaja [2]

1 Intensive Care Clinic, Riga East University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; evitabine@gmail.com
2 Department of Anaesthesia and Intensive Care, Riga Stradiņš University, Dzirciema Street 16, LV-1007 Riga, Latvia; insana.mahauri@rsu.lv (I.M.); indulis.vanags@rsu.lv (I.V.); biruta.mamaja@aslimnica.lv (B.M.)
3 Department of Hand and Plastic Surgery, Microsurgery Centre of Latvia, Brivibas Street 410, LV-1024 Riga, Latvia; janis.zarins@mcl.lv
4 Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Pulka Street 3, LV-1007 Riga, Latvia
5 Surgical Oncology Clinic, Riga East University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia; renars.deksnis@gmail.com
6 Laboratory Department, Riga East University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; margarita.citovica@aslimnica.lv
7 Institute of Microbiology and Virology, Riga Stradins University, Ratsupites Street 5, LV-1067 Riga, Latvia; donsimon@inbox.lv (S.D.); sabine.gravelsina@rsu.lv (S.G.); anda.vilmane@rsu.lv (A.V.); santa.rasa-dzelzkaleja@rsu.lv (S.R.-D.)
8 Outpatient Department, Riga East University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia
* Correspondence: rihardspeteris.rocans@rsu.lv

check for updates

Citation: Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Deksnis, R.; Citovica, M.; Donina, S.; Vanags, I.; Gravelsina, S.; Vilmane, A.; et al. Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery. *J. Clin. Med.* **2024**, *13*, 5411. https://doi.org/10.3390/jcm13185411

Academic Editors: Pier Camillo Parodi, Michele Riccio, Luca Vaienti, Francesco De Francesco and Nicola Zingaretti

Received: 16 July 2024
Revised: 4 September 2024
Accepted: 10 September 2024
Published: 12 September 2024

Abstract: Background: Microvascular flap surgery has become a routine option for defect correction. The role of von Willebrand factor antigen (VWF:Ag) in the pathophysiology of flap complications is not fully understood. We aim to investigate the predictive value of VWF:Ag for microvascular flap complications and explore the relationship between chronic inflammation and VWF:Ag. **Methods**: This prospective cohort study included 88 adult patients undergoing elective microvascular flap surgery. Preoperative blood draws were collected on the day of surgery before initiation of crystalloids. The plasma concentration of VWF:Ag as well as albumin, neutrophil-to-lymphocyte ratio (NLR), interleukin-6, and fibrinogen were determined. **Results**: The overall complication rate was 27.3%, and true flap loss occurred in 11.4%. VWF:Ag levels were higher in true flap loss when compared to patients without complications (217.94 IU/dL [137.27–298.45] vs. 114.14 [95.67–132.71], $p = 0.001$). Regression analysis revealed the association between VWF:Ag and true flap loss at the cutoff of 163.73 IU/dL (OR 70.22 [10.74–485.28], $p = 0.043$). Increased VWF:Ag concentrations were linked to increases in plasma fibrinogen ($p < 0.001$), C-reactive protein ($p < 0.001$), interleukin-6 ($p = 0.032$), and NLR ($p = 0.019$). **Conclusions**: Preoperative plasma VWF:Ag concentration is linked to biomarkers of inflammation and may be valuable in predicting complications in microvascular flap surgery.

Keywords: von Willebrand factor antigen; neutrophil-to-lymphocyte ratio; flap loss; microvascular flap complications; microvascular flap thrombosis; reconstructive surgery

1. Introduction

Microvascular flap surgery has become a routine option for the correction of various defects during the past decades. Despite improvements in surgical reconstructive techniques, flap loss still occurs, and the rate of flap loss varies from 6 to 10% [1–3]. The success of microvascular flaps depends on a multitude of variables, including technical

factors, blood rheology, and coagulogy, as well as patient comorbidities [4]. Many recent studies have outlined the use of preoperative biomarkers to predict complications in microvascular flap surgery [5,6]. There is potential to improve perioperative care, assess the risk of flap loss, and understand the pathophysiology of microvascular flap problems through the use of laboratory biomarkers [5]. In recent studies, different patterns of pathophysiology for distinct complications have been outlined [7]. For example, secondary flap complications have been associated with the risk of malnutrition [8,9] as well as chronic inflammation [8–10]. The main biomarkers of chronic inflammation that have been associated with flap complications are low albumin [9,11], increased C-reactive protein (CRP) [12], and increased neutrophil-to-lymphocyte ratio (NLR) [13]. Given the pathophysiological distinctions between secondary flap complications and flap thrombosis, the exact role of chronic inflammation in the pathophysiology of flap thrombosis is not fully understood. True flap loss has been associated with increased fibrinogen [14,15], von Willebrand factor (VWF) function [15], and VWF antigen [15–17]. Elevated levels of VWF antigen have been linked to a variety of thrombotic conditions [15–19], suggesting that it could be used as a thrombosis marker in reconstructive surgery [15–17]. Previous studies indicate a complex interaction between VWF and inflammatory biomarkers in the context of thrombotic events [18–20]. Elevated VWF antigen levels can be caused by endothelial damage or inflammation [18,19], both of which often occur during surgery [16]. VWF, once released, can bind to platelets and collagen, causing platelet adhesion and aggregation at the injury site [20]. Specifically, for microvascular flap surgery, the study by Handschel et al. [15] and the case report by Rothweiler et al. [16] indicate a significant impact of VWF antigen concentration on true flap loss. Moreover, the relationship between elevated VWF antigen and other risk factors associated with the underlying indications for reconstruction, as well as chronic inflammation, is not fully understood [21]. It is also uncertain how VWF antigen concentrations, minor flap complication, and true flap loss are related to one another [15]. CRP [22] and interleukin-6 (IL-6) [23] levels are also considerably elevated during surgery, indicating an inflammatory response that may influence thrombus formation. Specifically for microvascular flap surgery, Du et al. found elevated VWF antigen and CRP levels postoperatively after flap venous crisis in animal models [17]. The aim of this study is to determine the predictive value of VWF antigen for microvascular flap complications and to investigate the relationship between chronic inflammation and increased VWF antigen in various complication types.

2. Materials and Methods

2.1. Patient Selection, Perioperative Considerations and Outcome Definitions

This prospective observational cohort study included 88 patients undergoing elective microvascular flap transplantation surgery at Riga East University Hospital from 1 October 2021 to 31 March 2024. The study protocol and the informed consent form were approved by the Science Department of Riga East University hospital (Approval Number Nr.AP/08-08/22/135) and by the Ethics Committee of Riga Stradins University (Approval Number 22-2/399/2021). The study included adult patients undergoing elective microvascular flap transplantation. The study excluded patients with severe chronic liver or kidney diseases, with cardiovascular and autoimmune diseases, patients with pre-existing coagulopathies or any clotting and bleeding disorders, patients with inherited or acquired von Willebrand disease, patients receiving hormonal contraception or estrogen therapy, patients after recent thrombotic or thromboembolic events, patients currently taking anticoagulants or antiplatelet agents, patients with active systemic infections or inflammatory conditions, pregnant patients and patients during the lactation period, and children under the age of 18. Patients with medication-related osteonecrosis of the jaw, osteoradionecrosis of the jaw, and recent radiotherapy were excluded from the study. Patients with missing or incomplete data were also excluded. The type of flap was chosen based on the type of defect, the length of the pedicle, the positioning during surgery, the body mass index (BMI), and the composition of the patient. Preoperative evaluation, general anesthesia, and postoperative care were

provided by a team of experienced attending anesthesiologists. The following flap types were used in the study: anterolateral thigh flap, fibular flap, deep inferior epigastric artery perforator flap, radial free forearm flap, gracilis muscle flap, temporal artery flap, serratus anterior flap, and latissimus dorsi flap. The surgical team closely monitored the microvascular flap for the first 5–7 postoperative days. To monitor flap patency, a clinical assessment of flap color, temperature, tissue turgor, and capillary refill were used. Trauma patients who were operated upon within 30 days of injury were denoted early surgery, and patients who were operated upon later than 30 days from injury were denoted late surgery [21]. True flap loss was defined as the impairment of flap blood supply due to anastomosis dysfunction or thrombosis that leads to a complete loss of the transposed flap. Flap hematoma was defined as the presence of a hematoma adjacent to the flap recipient site without interfering with the flap blood supply. Minor flap complications were defined as the presence of flap wound infection, slow or difficult flap wound healing, marginal flap necrosis, or difficult healing at the donor site. All flap complications were defined as the presence of either true flap loss, flap hematoma or minor flap complications. In all cases of true flap loss, urgent surgical re-exploration was performed.

2.2. General Patient Data, Sample Collection, and Laboratory Analysis

General patient data collection was performed according to a previously defined protocol. Written and electronic health records were used to obtain information on the demographic characteristics of the patient, the flap type, the indication for surgery, the recipient location, the duration of surgery, the blood type, perioperative care, and surgical outcomes. Blood draws were obtained on the day of surgery immediately upon the first arrival in the operating room prior to the initiation of the first crystalloid infusion. Full blood count analysis was performed using the XN-1500 system (Sysmex Europe SE, Norderstedt, Germany). Albumin concentrations were analyzed using the colorimetric method (Cobas C, Roche/Hitachi, Mannheim, Germany). Bilirubin concentrations were analyzed using the colorimetric method (Cobas C, Roche/Hitachi, Mannheim, Germany). Interleukin-6 concentrations were analyzed by electrochemiluminescence immunoassay (ECLIA) (Cobas e, Roche/Hitachi, Mannheim, Germany). CRP concentrations were analyzed using the method of immunoturbidimetry (Cobas C, Roche/Hitachi, Mannheim, Germany). The fibrinogen concentrations were analyzed using the CS 5100 system (Sysmex Corporation, Kobe, Japan). The albumin–bilirubin score was calculated using the following formula: albumin–bilirubin score = (log10 bilirubin [μmol/L] × 0.66) + (albumin [g/L] × −0.0852) [24]. The NLR was defined as the proportion of neutrophil count (count/mm^3) and lymphocyte count (count/mm^3). All full blood count and clinical chemistry analyses were performed in a clinical laboratory within 8 h after blood draw. All blood samples for VWF antigen analysis were stored within 6 h from blood draw. Prior to storage, blood samples for VWF antigen analysis (collected in citrate tubes) were spun at 3500×/g for 10 min. Plasma samples were then stored at −80 °C until analysis. VWF antigen analysis was performed after a single thaw using the human von Willebrand factor ELISA kit according to the manufacturer's protocol from Abcam (Cambridge, United Kingdom). All reagents, working standards, and samples were prepared as directed in the product protocol datasheet. The assay was performed at room temperature (20–25 °C) according to the manufacturer's protocol. The absorbance was read on a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 450 nm immediately after the stop solution was added. The obtained readings were grouped for further statistical analysis.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 26.0. (IBM Corp. Armonk, NY, USA) and data visualization. GraphPad Prism version 5.03 (GraphPad Software Inc., Palo Alto, CA, USA) was used to present the data graphically. The normality of all variables was tested with visual inspection of the Q-Q plot. The Kolmogorov–Smirnov test was also used to assess whether the datasets conformed to a

normal distribution. The Chi-square test and Fisher's exact test were applied to nominal variable sets. Differences in data distribution between the two groups were evaluated using the Mann–Whitney U test for non-parametric datasets and the two-sample *t*-test for datasets conforming to the normal distribution. Spearman's rho was used to evaluate correlations between two non-parametric variables. Data sets for Il-6, CRP, fibrinogen, and NLRwere divided into four quartiles. The data distribution differences in VWF antigen were further compared between quartiles using the Kruskal–Wallis H test. Youden's index (YI) and the concordance probability method (CZ) were used to define optimal cut-off values [25]. Binary logistic regression models were used to obtain odds ratios for specific variables. Continuous variables that conform to normal distribution were presented as a mean and CI95. Statistical significance was assumed if two-tailed $p < 0.05$.

3. Results

In total, 88 patients were included, 43 (48.9%) men and 45 (51.1%) women. The mean age was 57.9 years (95% CI95 54.8–61.0). The overall rate of complication was 27.3% ($n = 24$). True flap loss with vascular compromise occurred in 11.4% ($n = 10$), with 4 of these cases being late flap loss (>72 h). Minor flap complications occurred in 10 cases (11.4%), and flap hematoma occurred in 4 (4.5%) cases. All the cases of early true flap loss underwent urgent anastomosis revision. Three cases of late flap loss underwent repeated microvascular flap surgery, and one case underwent necrectomy and reconstruction with a rotated local flap. One patient received intraoperative hemotransfusion, while three patients received hemotransfusion during the early postoperative period. There was no significant relationship between the presence of hemotransfusion and VWF antigen levels.

As depicted in Table 1, there were no significant distinctions in age or gender distribution between the patients with flap complications and the patients without complications. No significant differences in the rates of any flap complications were found between different areas of reconstruction, indications for surgery, or the flap type used.

Table 1. Demographic characteristics, perioperative considerations, comorbidities and laboratory results; data are presented as mean (95% CI) or count (percentage); abbreviations—ENT (ear, nose, and throat surgery); ALT (anterolateral thigh flap); DIEP (deep inferior epigastric artery perforator flap); NLR (neutrophil-to-lymphocyte ratio); CRP (C-reactive protein); VWF (von Willebrand factor).

Patient Group	Overall $n = 88$	No Complications $n = 64$	Any Flap Complications $n = 24$	*p*-Value
Demographic data				
Mean age, years	57.9 (54.8–61.0)	57.1 (54.4–59.8)	50.6 (47.7–53.1)	0.204
Women, *n* (%)	45 (51.1%)	31 (48.4%)	10 (41.67%)	0.271
Location				
Extremity, *n* (%)	18 (20.5%)	12 (18.8%)	6 (25.0%)	0.603
ENT, *n* (%)	40 (45.5%)	30 (46.9%)	10 (41.7%)	0.787
Head and neck, *n* (%)	17 (19.3%)	12 (18.8%)	5 (20.8%)	0.857
Breast, *n* (%)	13 (14.8%)	10 (15.6%)	3 (12.5%)	0.750
Flap type				
ALT, (%)	40 (45.5%)	33 (51.6%)	7 (29.2%)	0.232
Fibular flap, (%)	9 (10.2%)	5 (7.8%)	4 (16.7%)	0.279
DIEP, *n* (%)	10 (11.4%)	8 (12.5%)	2 (8.3%)	0.622
Radial artery flap, *n* (%)	7 (8.0%)	5 (7.8%)	2 (8.3%)	0.941
Other, *n* (%)	22 (25.0%)	13 (20.3%)	9 (37.5%)	0.212

Table 1. *Cont.*

Patient Group	Overall $n = 88$	No Complications $n = 64$	Any Flap Complications $n = 24$	p-Value
Indication				
Trauma, *n* (%)	9 (10.2%)	7 (10.9%)	2 (8.3%)	0.106
Oncology, *n* (%)	55 (62.5%)	42 (47.7%)	13 (54.2%)	0.629
Defect, *n* (%)	16 (18.2%)	10 (11.4%)	6 (25.0%)	0.406
Blood type				
Blood type, O, *n* (%)	35 (39.8%)	26 (40.6%)	9 (37.5%)	0.539
Laboratory values				
Leukocyte count 10^9/L	6.24 (5.76–6.73)	6.16 (5.56–6.75)	6.50 (5.62–7.38)	0.309
Lymphocyte count 10^9/L	1.74 (1.53–1.94)	1.83 (1.58–2.09)	1.41 (1.14–1.68)	0.020
Neutrophil count 10^9/L	3.66 (3.23–4.09)	3.54 (3.02–4.07)	4.06 (3.28–4.84)	0.109
NLR	2.57 (2.11–3.02)	2.32 (1.85–2.80)	3.4 (2.16–4.64)	0.006
Monocyte count 10^9/L	0.56 (0.51–0.61)	0.56 (0.50–0.61)	0.56 (0.46–0.66)	0.839
Red blood cell count 10^9/L	4.08 (3.97–4.19)	4.18 (4.03–4.32)	4.27 (4.06–4.49)	0.625
Platelet count 10^9/L	246.75 (229.25–264.24)	232.98 (216.00–249.96)	288.37 (238.35–338.40)	0.021
Hemoglobin g/dL	12.58 (12.22–12.94)	12.45 (12.03–12.87)	12.86 (12.13–13.61)	0.208
Mean total plasma protein g/L	64.72 (63.40–66.05)	64.84 (63.27–66.40)	64.08 (61.32–66.83)	0.543
Mean plasma albumin, g/L	38.90 (38.12–39.69)	39.21 (38.25–40.16)	36.58 (34.33–38.84)	0.049
Mean plasma bilirubin, mg/dl	0.47 (0.42–0.53)	0.48 (0.41–0.55)	0.43 (0.31–0.55)	0.447
Mean albumin–bilirubin score	−2.78 (−2.87–−2.69)	−2.80 (−3.02–−2.56)	−2.77 (−2.87–−2.67)	0.551
CRP, mg/L	7.10 (4.67–9.54)	6.76 (3.67–9.86)	7.93 (4.32–11.54)	0.058
Mean plasma fibrinogen, g/L	3.59 (3.35–3.84)	3.52 (3.24–3.81)	3.82 (3.30–4.35)	0.505
Interleukin-6, pg/mL	13.9 (10.09–16.29)	13.03 (9.33–16.7)	13.73 (7.26–20.20)	0.893
VWF antigen, IU/dL	129.61 (111.93–147.27)	120.44 (99.43–141.36)	157.59 (123.62–191.61)	0.014

Trauma was the indication for surgery in nine patients, and the mean time from trauma to surgery was 35.8 days. There was no statistically significant link between time from trauma and VWF antigen concentration (r = −0.67, $p = 0.865$). Regarding time from trauma, no significant differences in the rates of any flap complications were found between early surgery and late surgery groups ($p = 0.571$).

The mean duration of surgery was 6.41 [5.77–7.04] hours. There were no significant differences in the duration of surgery between patients with flap complications and patients without complications (6.68 [5.31–8.06] vs. 6.35 [5.61–7.09], $p = 0.135$). Patients with any flap complications had a significantly lower plasma lymphocyte count ($p = 0.020$); a lower mean plasma albumin ($p = 0.049$); a higher platelet count ($p = 0.021$); and a higher VWF antigen ($p = 0.014$). There was no link between albumin–bilirubin score and VWF antigen concentration (r = 0.89, $p = 0.430$). There were no statistically significant differences in mean bilirubin concentration and albumin–bilirubin score between patients with flap complications and patients without complications (−2.77 [−2.87–−2.67] vs. −2.80 [−3.02–−2.56], $p = 0.551$).

When comparing different surgical indications, trauma had the highest preoperative VWF antigen, followed by oncology, and patients with defects had the lowest VWF antigen (138.62 [107.48–150.62] vs. 129.03 [107.52–150.63] vs. 89.92 [68.09–118.14], $p = 0.029$).

As seen in Figure 1, VWF antigen concentrations were positively linked to preoperative plasma fibrinogen ($p < 0.001$), plasma CRP ($p < 0.001$), plasma IL-6 ($p = 0.032$), and NLR ($p = 0.019$).

VWF antigen levels were higher in true flap loss when compared to patients without complications (217.94 [137.27–298.45] vs. 114.14 [95.67–132.71], $p = 0.001$). Preoperative NLR was the highest in the patients with subsequent secondary flap complications when compared to patients without flap complications (4.36 [2.60–6.13] vs. 2.32 [2.03–2.32], $p = 0.024$). Fibrinogen levels were higher in the patients with subsequent true flap loss compared to patients without complications (5.00 [4.29–5.70] vs. 3.46 [3.18–3.73], $p < 0.001$) (Figure 2).

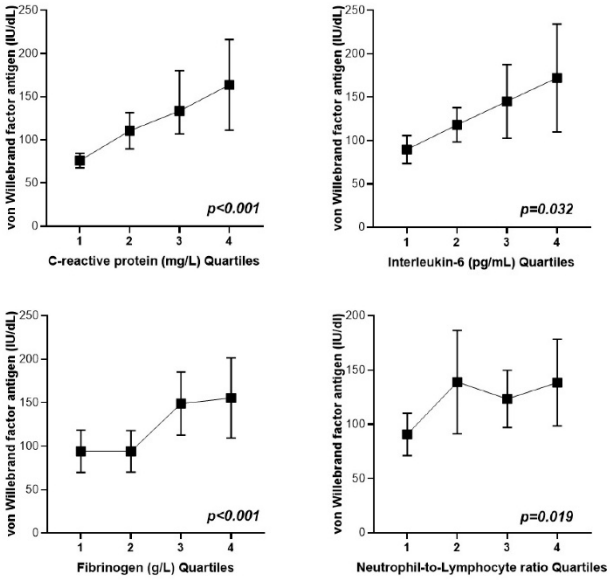

Figure 1. Association between preoperative von Willebrand factor antigen and different preoperative markers of inflammation in microvascular flap surgery patients. Quartiles of C-reactive protein (mg/L): quartile 1 < 0.83; quartile 2 0.84–2.37; quartile 3 2.38–7.81; quartile 4 > 7.82. Quartiles of interleukin-6 (pg/mL): quartile 1 < 5.11; quartile 2 5.12–8.08; quartile 3 8.09–14.80; quartile 4 > 14.81. Quartiles of fibrinogen (g/L): quartile 1 < 2.81; quartile 2 2.82–3.46; quartile 3 3.47–4.27; quartile 4 > 4.28. Quartiles of neutrophil-to-lymphocyte ratio: quartile 1 < 1.47; quartile 2 1.48–1.93; quartile 3 1.94–2.77; quartile 4 > 2.78. Data are presented as mean (CI95).

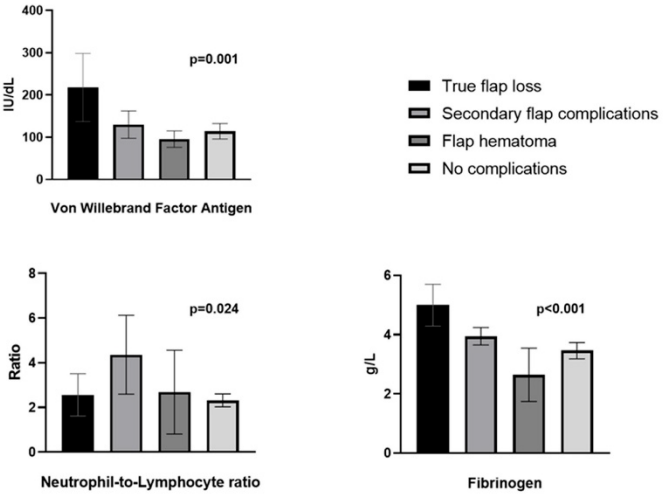

Figure 2. Association between preoperative von Willebrand factor antigen, neutrophil-to-lymphocyte ratio, fibrinogen, and different flap complication types and Kruskal–Wallis H test distribution comparisons of preoperative von Willebrand factor antigen, neutrophil-to lymphocyte ratio and fibrinogen levels in different surgical outcome groups.

After adjustment for fibrinogen and the presence of other types of flaps, logistic regression analysis revealed the association between VWF antigen and true flap loss at the selected cutoff level of 163.73 IU/dL (OR 70.22 [10.74–485.28], $p = 0.043$).

4. Discussion

The main findings of the present study were that biomarkers of inflammation and VWF antigen levels are linked to complications in microvascular flap surgery. The central finding is that elevated preoperative VWF antigen concentrations increase the odds of true flap loss. Flap complications were also found to be related to decreased lymphocyte count, decreased plasma albumin, increased platelet count, increased plasma fibrinogen, and increased NLR. Increases in CRP, fibrinogen, IL-6, and NLR were also directly linked to increases in VWF antigen concentration.

Recent studies have demonstrated that certain aspects of overall health of patients, such as malnutrition [7] and inflammation [10,26], are associated with flap complications. Inflammatory reactions of various kinds, such as infection, malignancy, and autoimmune diseases, also generally show increased fibrinogen activity and increased VWF antigen concentrations [27–31]. VWF is a large glycoprotein produced by endotheliocytes and megakaryocytes [32], and it plays a crucial role in normal hemostasis by enabling platelet plug formation at the sites of vascular injury [33]. VWF mediates platelet adhesion by binding to the collagen and platelet receptors [34]. VWF is highly shear-sensitive and is therefore known to promote platelet aggregation at post-stenotic sites that have a negative shear rate gradient [35]. Studies on atherosclerotic plaques reveal that interactions between the plaque geometries, local endothelial VWF release, and plasma levels of VWF antigen can promote thrombosis [36]. The concept of VWF elongation and subsequent contribution to platelet aggregation at post-stenotic sites may be applicable to the anastomosis site in microvascular flap surgery [15–17].

Although specific mechanisms govern VWF-mediated platelet aggregation and subsequent thrombosis, the overall concentration of VWF antigen in plasma has been shown to predict thrombotic events. Specifically for microvascular flap surgery, Handschel et al. found higher levels of plasma VWF antigen concentration in microvascular flap surgery patients with thrombosis of the anastomosed vein [15], which coincides with our findings.

Among the multitude of factors influencing VWF antigen concentrations, chronic inflammation seems to be very prevalent in the microvascular flap surgery patient population [10,13]. Hypercoagulable states may arise due to the primary indication for reconstructive surgery [21], and therefore, all indications for surgery may not imply the same risk of flap loss. The two main indications for reconstruction that can increase the risk of thrombosis are oncology [37,38] and trauma [39,40]. Both aforementioned indications have been associated with increased plasma VWF antigen concentrations [18,27,41]. This coincides with our findings, as our data revealed that trauma has the highest VWF antigen, followed by oncology. Our data revealed that patients with uncomplicated defects as an indication for reconstruction had the lowest VWF antigen concentrations, although all three indication groups had mean values comparable to the previously described normal ranges [42].

Increases in plasma VWF antigen concentrations have previously been associated with indicators of inflammatory states such as increased Il-6 [42], decreased albumin [43], increased CRP [42], increased platelet count [44] and chronic liver disease [45]. Our data revealed that VWF antigen concentration is not linked to albumin–bilirubin score, although no patients in our cohort had chronic liver disease, and the mean albumin–bilirubin score of our patient cohort was expectedly correlated with low risk of hepatic decompensation [46]. We found that microvascular flap complications are related to all included biomarkers of inflammation, except for CRP and IL-6. Furthermore, we found that increased NLR and decreased albumin are linked to secondary flap complications, and increased fibrinogen is linked to true flap loss. These findings further support the notion that true flap loss and secondary flap complications have different patterns of pathophysiology [7]. Chronic

inflammation may cause secondary flap complications through impaired healing, much like in any other surgical population [47,48]. However, our data revealed that the presence of chronic inflammation is also associated with an increase in fibrinogen and VWF antigen concentrations. Both fibrinogen and VWF antigen have been shown to increase the risk of true flap loss [15], which coincides with our findings.

This study has several limitations. First, the single-center design prevents generalizability across populations and may have institutional bias. Second, while our sample size of 88 patients is sufficient to provide statistically significant results, due to the limited sample size, multiple risk factors described in the previous literature could not be accounted for in the regression models. Our analysis of the link between true flap loss and VWF antigen concentration did not include the distinction between arterial and venous thrombosis of the anastomosis, which has been reviewed in previous studies [15,16]. Furthermore, collecting blood samples before surgery does not account for changes in biomarker levels before and after surgery. Continuous monitoring of VWF antigen and inflammatory markers during the perioperative phase would provide a more complete understanding of flap complication pathophysiology. The exclusion criteria, which excluded patients with various comorbidities and conditions, may have created selection bias. This could have had an impact on the external validity of the study because excluded populations may have different levels of VWF antigen and inflammatory biomarkers. Specifically, patients with osteoradionecrosis of the jaw were excluded to avoid confounding factors, although inclusion of these cases would have provided valuable insight into the pathophysiology of flap failure after radiotherapy [49]. Our study design and cohort size did not allow for evaluating the predictive power and cost efficiency of VWF antigen when compared with other commonly proposed biomarkers. Larger, multi-center investigations are required to validate the applicability of preoperative VWF antigen analysis to a wider population and to propose specific recommendations for treatment strategies.

5. Conclusions

The preoperative level of VWF antigen is associated with true flap loss, while markers of chronic inflammation are linked to both secondary flap complications and increased plasma VWF antigen. Assessment of the preoperative plasma VWF antigen concentration may be valuable for predicting complications in reconstructive surgery. Understanding the pathophysiological link between chronic inflammation, preoperative plasma VWF antigen, and true flap loss may improve decision making in the perioperative care of microvascular flap surgery patients.

Author Contributions: R.P.R., B.M., S.D., I.V. and M.C. conceived and planned the study. J.Z., R.P.R., R.D., I.M. and M.C., participated in data collection. R.P.R., M.C., S.G., A.V. and S.R.-D. participated in laboratory analysis. R.P.R., S.G. and E.B. performed data curation and statistical analysis. R.P.R., J.Z., R.D., M.C., B.M., S.D., I.M., I.V., S.G., A.V., S.R.-D. and E.B. interpreted the results and prepared the draft. All authors have read and agreed to the published version of the manuscript.

Funding: The authors declare that Riga Stradiņš University kindly covered the publication fee for this article. J.Z. would like to acknowledge financial support from the European Union's Horizon 2020 research and innovation program under agreement No. 857287. None of the funders were involved in the study design, collection, analysis, interpretation of the data, or writing of this article.

Institutional Review Board Statement: The study protocol and the informed consent form were approved by the Science Department of Riga East University Hospital (Approval Number Nr.AP/08-08/22/135; 8 November 2022) and by the Ethics Committee of Riga Stradins University (Approval Number 22-2/399/2021; 8 July 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. The corresponding author will ensure that individual privacy is not compromised during the transfer of datasets.

Acknowledgments: We would like to acknowledge the help of Vita Kalnberzina from the Department of English studies at the University of Latvia for reviewing and revising the manuscript for English grammar and syntax.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lese, I.; Biedermann, R.; Constantinescu, M.; Grobbelaar, A.O.; Olariu, R. Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *J. Plast. Reconstr. Aesthet. Surg.* **2021**, *74*, 512–522. [CrossRef]
2. Kohlert, S.; Quimby, A.E.; Saman, M.; Ducic, Y. Postoperative Free-Flap Monitoring Techniques. *Semin Plast Surg.* **2019**, *33*, 13–16. [CrossRef] [PubMed]
3. Koster, I.T.S.; Borgdorff, M.P.; Jamaludin, F.S.; de Jong, T.; Botman, M.; Driessen, C. Strategies Following Free Flap Failure in Lower Extremity Trauma: A Systematic Review. *JPRAS Open* **2023**, *29*, 94–104. [CrossRef] [PubMed]
4. Ekin, Y.; Günüşen, İ.; Özdemir, Ö.Y.; Tiftikçioğlu, Y.Ö. Effect of Coagulation Status and Co-Morbidity on Flap Success and Complications in Patients with Reconstructed Free Flap. *Turk. J. Anaesthesiol. Reanim.* **2019**, *47*, 98–106. [CrossRef] [PubMed]
5. Gunusen, I.; Ozdemir, O.Y.; Gur, E.; Tiftikcioglu, Y.O.; Ekin, Y.C.; Ozturk, K. Blood Count Parameters can be Useful in Predicting Vascular Events in Microvascular Surgery: Results of a Preliminary Study. *Niger. J. Clin. Pract.* **2021**, *24*, 1343–1349. [CrossRef] [PubMed]
6. Tarle, M.; Čvrljević, I.; Raguž, M.; Lukšić, I. Hemoglobin–Albumin–Lymphocyte–Platelet (HALP) Score as a Predictive Model for the Success of Reconstruction of Head and Neck Defects with Free Microvascular Flaps. *J. Clin. Med.* **2023**, *12*, 5314. [CrossRef]
7. Rocans, R.P.; Zarins, J.; Bine, E.; Deksnis, R.; Citovica, M.; Donina, S.; Mamaja, B. The Controlling Nutritional Status (CONUT) Score for Prediction of Microvascular Flap Complications in Reconstructive Surgery. *J. Clin. Med.* **2023**, *12*, 4794. [CrossRef]
8. Huang, C.Y.; Lee, S.L.; Chiu, W.K.; Chen, C.; Chen, J.H.; Wang, H.J. Determinants of the success in flap reconstruction-Outcome analysis of 120 flaps in 484 procedures for pressure injury. *Int. Wound J.* **2023**, *20*, 3105–3115. [CrossRef]
9. da Silva, A.C.O.; Silva, G.B.; Cho, A.B.; Wei, T.H.; Mattar, R.; Iamaguchi, R.B. Hypoalbuminemia in microsurgical flaps of the musculoskeletal apparatus. *Acta Ortop. Bras.* **2020**, *28*, 168–171. [CrossRef]
10. Košec, A.; Solter, D.; Ribić, A.; Knežević, M.; Vagić, D.; Pegan, A. Systemic Inflammatory Markers as Predictors of Postoperative Complications and Survival in Patients With Advanced Head and Neck Squamous Cell Carcinoma Undergoing Free-Flap Reconstruction. *J. Oral Maxillofac. Surg.* **2022**, *80*, 744–755. [CrossRef]
11. Yu, J.; Hong, J.P.; Suh, H.P.; Park, J.Y.; Kim, D.H.; Ha, S.; Lee, J.; Hwang, J.H.; Kim, Y.K. Prognostic Nutritional Index is a Predictor of Free Flap Failure in Extremity Reconstruction. *Nutrients* **2020**, *12*, 562. [CrossRef]
12. Schmidt, F.; Ward, M.; Repanos, C. Postoperative serum C-reactive protein dynamics after pharyngolaryngectomy with jejunal free-flap reconstruction. *Ann. R. Coll. Surg. Engl.* **2023**, *105*, 263–268. [CrossRef] [PubMed]
13. Chargi, N.; Breik, O.; Forouzanfar, T.; Martin, T.; Praveen, P.; Idle, M.; Parmar, S.; de Bree, R. Association of low skeletal muscle mass and systemic inflammation with surgical complications and survival after microvascular flap reconstruction in patients with head and neck cancer. *Head Neck* **2022**, *44*, 2077–2094. [CrossRef] [PubMed]
14. Drizlionoka, K.; Zarins, J.; Ozolina, A.; Nikitina-Zake, L.; Mamaja, B. Polymorphism rs2066865 in the Fibrinogen Gamma Chain (FGG) Gene Increases Plasma Fibrinogen Concentration and Is Associated with an Increased Microvascular Thrombosis Rate. *Medicina* **2019**, *55*, 563. [CrossRef] [PubMed]
15. Handschel, J.; Burghardt, S.; Naujoks, C.; Kübler, N.R.; Giers, G. Parameters predicting complications in flap surgery. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2013**, *115*, 589–594. [CrossRef]
16. Rothweiler, R.M.; Metzger, M.C.; Zieger, B.; Huber-Schumacher, S.; Schmelzeisen, R.; Kalbhenn, J. The Role of von Willebrand Factor in Microvascular Surgery in Severely Injured Patients. *Plast. Reconstr. Surg. Glob. Open* **2021**, *9*, e3836. [CrossRef]
17. Du, W.; Wu, P.F.; Qing, L.M.; Wang, C.Y.; Liang, J.Y.; Yu, F.; Tang, J.Y. Systemic and flap inflammatory response associates with thrombosis in flap venous crisis. *Inflammation* **2015**, *38*, 298–304. [CrossRef]
18. Comerford, C.; Glavey, S.; Quinn, J.; O'Sullivan, J.M. The role of VWF/FVIII in thrombosis and cancer progression in multiple myeloma and other hematological malignancies. *J. Thromb. Haemost.* **2022**, *20*, 1766–1777. [CrossRef]
19. Edvardsen, M.S.; Hindberg, K.; Hansen, E.S.; Morelli, V.M.; Ueland, T.; Aukrust, P.; Brækkan, S.K.; Evensen, L.H.; Hansen, J.B. Plasma levels of von Willebrand factor and future risk of incident venous thromboembolism. *Blood Adv.* **2021**, *5*, 224–232. [CrossRef]
20. Neubauer, K.; Zieger, B. Endothelial cells and coagulation. *Cell Tissue. Res.* **2022**, *387*, 391–398. [CrossRef]
21. Vanags, I.; Stepanovs, J.; Ozolina, A.; Mukans, M.; Bjertnaes, L.J.; Mamaja, B. Thromboelastometry for Assessing Risks of Free Flap Thrombosis in Patients Undergoing Microvascular Surgery. *Front. Med.* **2020**, *7*, 289. [CrossRef] [PubMed]
22. De Cicco Nardone, C.; De Luca, C.; Plotti, F.; Fabris, S.; Rossini, G.; Montera, R.; Luvero, D.; Angioli, R.; Terranova, C. C-reactive protein "at first sight": Standard postoperative trend in gynecological surgery and a comparative analysis with white blood cell levels. *Int. J. Gynaecol. Obstet.* **2023**, *160*, 237–243. [CrossRef] [PubMed]
23. Finke, J.C.; Yang, J.; Bredell, M.; Fritschen, U.; Glocker, M.O. Plasma cytokine and growth factor profiling during free flap transplantation. In *Issues in Flap Surgery*; IntechOpen: London, UK, 2017; pp. 3–24.
24. Deng, M.; Ng, S.W.Y.; Cheung, S.T.; Chong, C.C.N. Clinical application of Albumin-Bilirubin (ALBI) score: The current status. *Surgeon* **2020**, *18*, 178–186. [CrossRef] [PubMed]

25. Unal, I. Defining an Optimal Cut-Point Value in ROC Analysis: An Alternative Approach. *Comput. Math. Methods Med.* **2017**, *2017*, 3762651. [CrossRef]
26. Mangialardi, M.L.; Barone Adesi, L.; Salgarello, M.; Baldelli, I.; Raposio, E. Warning on coagulopathy COVID-19 related in microsurgical procedures. *Microsurgery* **2021**, *41*, 503–504. [CrossRef]
27. Colonne, C.K.; Favaloro, E.J.; Pasalic, L. The Intriguing Connections between von Willebrand Factor, ADAMTS13 and Cancer. *Healthcare* **2022**, *10*, 557. [CrossRef]
28. Favaloro, E.J.; Pasalic, L.; Lippi, G. Autoimmune Diseases Affecting Hemostasis: A Narrative Review. *Int. J. Mol. Sci.* **2022**, *23*, 14715. [CrossRef]
29. Cherny, I.; Shepshelovich, D.; Lahav, M.; Rabizadeh, E. The effect of infectious and autoimmune diseases on procoagulant activity of fibrinogen-like protein 2 in the peripheral blood. *Harefuah* **2019**, *158*, 180–183.
30. Li, Q.; Cong, R.; Kong, F.; Ma, J.; Wu, Q.; Ma, X. Fibrinogen Is A Coagulation Marker Associated With The Prognosis Of Endometrial Cancer. *Onco. Targets Ther.* **2019**, *12*, 9947–9956. [CrossRef]
31. Liu, Q.; Fang, S.; Liang, S.; Lv, J.; Wang, G.; Tang, R.; Ji, X.; Zhao, T.; Li, J.; Xu, L.; et al. The prognostic role of a combined fibrinogen and inflammation-based index in patients with metastatic breast cancer. *Transl. Cancer Res.* **2020**, *9*, 7065–7078. [CrossRef]
32. Mojzisch, A.; Brehm, M.A. The Manifold Cellular Functions of von Willebrand Factor. *Cells* **2021**, *10*, 2351. [CrossRef] [PubMed]
33. O'Donnell, J.S.; Byrne, C.; Preston, R.J.S. Von Willebrand factor-inflammation crosstalk in deep vein thrombosis. *J. Thromb. Haemost.* **2023**, *21*, 453–455. [CrossRef] [PubMed]
34. Tsyu, N.G.; Belyaev, A.V. Coarse-grained simulations of von Willebrand factor adsorption to collagen with consequent platelet recruitment. *Int. J. Numer. Meth. Biomed. Engng.* **2023**, *39*, e3747. [CrossRef] [PubMed]
35. Hoefer, T.; Rana, A.; Niego, B.; Jagdale, S.; Albers, H.J.; Gardiner, E.E.; Andrews, R.K.; Van der Meer, A.D.; Hagemeyer, C.E.; Westein, E. Targeting shear gradient activated von Willebrand factor by the novel single-chain antibody A1 reduces occlusive thrombus formation in vitro. *Haematologica* **2021**, *106*, 2874–2884. [CrossRef] [PubMed]
36. Westein, E.; van der Meer, A.D.; Kuijpers, M.J.; Frimat, J.P.; van den Berg, A.; Heemskerk, J.W. Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 1357–1362. [CrossRef]
37. Abdol Razak, N.B.; Jones, G.; Bhandari, M.; Berndt, M.C.; Metharom, P. Cancer-Associated Thrombosis: An Overview of Mechanisms, Risk Factors, and Treatment. *Cancers* **2018**, *10*, 380. [CrossRef]
38. Eichinger, S. Cancer associated thrombosis: Risk factors and outcomes. *Thromb. Res.* **2016**, *140*, S12–S17. [CrossRef]
39. Park, M.S.; Perkins, S.E.; Spears, G.M.; Ashrani, A.A.; Leibson, C.L.; Boos, C.M.; Harmsen, W.S.; Jenkins, D.H.; Bailey, K.R.; Ballman, K.V.; et al. Risk factors for venous thromboembolism after acute trauma: A population-based case-cohort study. *Thromb. Res.* **2016**, *144*, 40–45. [CrossRef]
40. Bahloul, M.; Dlela, M.; Bouchaala, K.; Kallel, H.; Ben Hamida, C.; Chelly, H.; Bouaziz, M. Post-traumatic pulmonary embolism: Incidence, physiopathology, risk factors of early occurrence, and impact outcome. A narrative review. *Am. J. Cardiovasc. Dis.* **2020**, *10*, 432–443.
41. Zeineddin, A.; Dong, J.F.; Wu, F.; Terse, P.; Kozar, R.A. Role of Von Willebrand Factor after Injury: It May Do More Than We Think. *Shock* **2021**, *55*, 717–722. [CrossRef]
42. Möller, C.; Schutte, S.W.; Botha-Le Roux, S. Von Willebrand factor, its cleaving protease (ADAMTS13), and inflammation in young adults: The African-PREDICT study. *Cytokine* **2020**, *136*, 155265. [CrossRef] [PubMed]
43. Kim, S.B.; Chi, H.S.; Park, J.S.; Hong, C.D.; Yang, W.S. Effect of increasing serum albumin on plasma D-dimer, von Willebrand factor, and platelet aggregation in CAPD patients. *Am. J. Kidney Dis.* **1999**, *2*, 312–317. [CrossRef] [PubMed]
44. Tarabishy, S.P.; Inglesby, D.; Tapp, M.; Corral, G.D.; Herrera, F.A. Thrombocytosis is associated with complications after microvascular surgery: An NSQIP data analysis. *Microsurgery* **2020**, *3*, 288–297. [CrossRef] [PubMed]
45. Simbrunner, B.; Villesen, I.F.; Scheiner, B.; Paternostro, R.; Schwabl, P.; Stättermayer, A.F.; Marculescu, R.; Pinter, M.; Quehenberger, P.; Trauner, M.; et al. Von Willebrand factor processing in patients with advanced chronic liver disease and its relation to portal hypertension and clinical outcome. *Hepatol. Int.* **2023**, *17*, 1532–1544. [CrossRef] [PubMed]
46. Navadurong, H.; Thanapirom, K.; Wejnaruemarn, S.; Prasoppokakorn, T.; Chaiteerakij, R.; Komolmit, P.; Treeprasertsuk, S. Validation of the albumin-bilirubin score for identifying decompensation risk in patients with compensated cirrhosis. *World J. Gastroenterol.* **2023**, *29*, 4873–4882. [CrossRef]
47. Brattinga, B.; Rutgers, A.; De Haan, J.J.; Absalom, A.R.; van der Wal-Huisman, H.; de Bock, G.H.; van Leeuwen, B.L. Preoperative Inflammatory Markers as a Predictor of Three-Year Overall Survival in Older Cancer Patients Undergoing Oncologic Surgery. *Cancers* **2021**, *8*, 1824. [CrossRef]
48. Matsui, R.; Inaki, N.; Tsuji, T.; Fukunaga, T. Preoperative Chronic Inflammation Is a Risk Factor for Postoperative Complications Independent of Body Composition in Gastric Cancer Patients Undergoing Radical Gastrectomy. *Cancers* **2024**, *4*, 833. [CrossRef]
49. Terenzi, V.; Della Monaca, M.; Raponi, I.; Battisti, A.; Priore, P.; Barbera, G.; Romeo, U.; Polimeni, A.; Valentini, V. MRONJ and ORNJ: When a single letter leads to substantial differences. *Oral Oncol.* **2020**, *110*, 104817. [CrossRef]

Fifth Publication

medicina

MDPI

Article

Early Postoperative Increase in Transforming Growth Factor Beta-1 Predicts Microvascular Flap Loss in Reconstructive Surgery: A Prospective Cohort Study

Rihards Peteris Rocans [1,2,*], Janis Zarins [3,4], Evita Bine [1], Insana Mahauri [2], Renars Deksnis [5], Margarita Citovica [6], Simona Donina [7,8], Sabine Gravelsina [7], Anda Vilmane [7], Santa Rasa-Dzelzkaleja [7], Olegs Sabelnikovs [2] and Biruta Mamaja [2]

1 Intensive Care Clinic, Riga East Clinical University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; evitabine@gmail.com
2 Department of Anaesthesia and Intensive Care, Riga Stradiņš University, Dzirciema Street 16, LV-1007 Riga, Latvia; insana.mahauri@rsu.lv (I.M.); olegs.sabelnikovs@rsu.lv (O.S.); biruta.mamaja@aslimnica.lv (B.M.)
3 Department of Hand and Plastic Surgery, Microsurgery Centre of Latvia, Brivibas Street 410, LV-1024 Riga, Latvia; janis.zarins@mcl.lv
4 Baltic Biomaterials Centre of Excellence, Riga Technical University, Pulka Street 3, LV-1007 Riga, Latvia
5 Surgical Oncology Clinic, Riga East Clinical University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia; renars.deksnis@gmail.com
6 Laboratory Department, Riga East Clinical University Hospital, Hipokrata Street 2, LV-1079 Riga, Latvia; margarita.citovica@aslimnica.lv
7 Institute of Microbiology and Virology, Rīga Stradiņš University, Ratsupites Street 5, LV-1067 Riga, Latvia; donsimon@inbox.lv (S.D.); sabine.gravelsina@rsu.lv (S.G.); santa.rasa-dzelzkaleja@rsu.lv (S.R.-D.)
8 Outpatient Department, Riga East Clinical University Hospital, Hipokrata Street 4, LV-1079 Riga, Latvia
* Correspondence: rihardspeteris.rocans@rsu.lv

check for updates

Academic Editors: Francesco De Francesco and Michele Riccio

Received: 10 February 2025
Revised: 21 April 2025
Accepted: 6 May 2025
Published: 8 May 2025

Citation: Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Deksnis, R.; Citovica, M.; Donina, S.; Gravelsina, S.; Vilmane, A.; Rasa-Dzelzkaleja, S.; et al. Early Postoperative Increase in Transforming Growth Factor Beta-1 Predicts Microvascular Flap Loss in Reconstructive Surgery: A Prospective Cohort Study. *Medicina* **2025**, *61*, 863. https://doi.org/10.3390/medicina61050863

Abstract: *Background and Objectives:* Microvascular flap surgery is a widely used reconstructive technique for the repair of various defects. Biomarkers have become an essential tool for monitoring flap viability, early detection of complications, and prediction of surgical outcomes. Studies focusing on immunomodulatory cytokines in the early prediction of microvascular flap complications are lacking. We aimed to investigate the predictive value of postoperative changes in transforming growth factor beta-1 (TGF-β1) for microvascular flap complications. *Materials and Methods:* This prospective observational study comprised 44 adults scheduled for elective microvascular flap surgery. Preoperative blood samples for analysis were obtained before surgery, prior to the administration of intravenous fluids. Postoperative blood draws were collected after surgery, before leaving the operating room. Preoperative and postoperative serum concentrations of TGF-β1, as well as preoperative plasma albumin, total protein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, full blood count, albumin, interleukin-6, C-reactive protein, and fibrinogen, were determined. *Results:* Postoperative changes in TGF-β1 were higher in cases with flap loss compared to patients with healthy recovery or patients with minor flap complications (0.403 log10 of ng/mL [0.024–0.782] vs. 0.157 [0.029–0.285] vs. −0.089 [−0.233–0.056], $p = 0.002$). Increased postoperative TGF-β1 was positively linked to preoperative C-reactive protein ($p = 0.021$), fibrinogen ($p = 0.020$), hematocrit ($p = 0.039$), and hemoglobin ($p = 0.009$). *Conclusions:* The postoperative increase in circulating TGF-β1 was associated with microvascular flap complications. Assessment of the postoperative changes in circulating TGF-β1 may be valuable for the early postoperative prediction of true flap loss.

Keywords: transforming growth factor beta-1 (TGF-β1); TGFB-1; true flap loss; microvascular flap complications; microvascular flap thrombosis; reconstructive surgery

1. Introduction

Microvascular flap reconstruction has become increasingly routine due to technological advancements [1], greater surgeon expertise [2], and advances in perioperative care [3]. Biomarkers have become an essential tool in microvascular flap surgery, where they help monitor the viability of tissue flaps, detect complications early, and predict surgical outcomes [3,4]. Recent studies have found multiple preoperative inflammatory [5–7] and hemostasis [4,8] biomarkers for the prediction of flap complications, as well as improving the understanding of flap complications' pathophysiology [3,4]. Microvascular flap surgery is an extensive surgical procedure, which elicits a generally pro-inflammatory immune response [9,10]. Interleukin-6 (IL-6), interleukin-8, and macrophage colony-stimulating factor are pro-inflammatory cytokines that have been linked to ischemia/reperfusion injury in microvascular flap surgery [10]. Pro-inflammatory states have been found to be linked to true flap loss through the modulation of von Willebrand factor [4,8], fibrinogen [8], and platelet function [11]. To date, studies focusing on the role of immunomodulatory cytokines in the pathophysiological patterns of different microvascular flap complications are lacking. Although a previous study used blood samples from microvascular flap blood vessels [10], to date, no studies have evaluated the postoperative changes in immunomodulatory cytokine concentrations in regular circulating blood samples. As an immunoregulatory cytokine, transforming growth factor beta (TGF-β) governs various cellular processes and biological functions, including immune regulation, inflammation, and wound healing [12]. The primary isoform, transforming growth factor beta-1 (TGF-β1) is a promising novel biomarker that influences several processes that can contribute to thrombus formation and stability [13]. TGF-β1 is secreted by platelets and becomes bioactive upon exposure to shear stress [14], which could have pathophysiological and predictive implications in microvascular flap surgery. This study aims to assess the prognostic significance of postoperative TGF-β1 changes in relation to various microvascular flap complications, and to examine the links between perioperative TGF-β1 levels and other biomarkers in microvascular flap surgery patients.

2. Materials and Methods

2.1. Patient Selection

This prospective observational cohort study included 173 individuals who underwent elective reconstructive microvascular flap surgery at Riga East University Hospital from 1 October 2022 to 31 March 2024. All patients who had true flap loss or secondary flap complications (N = 22) were included in the complications group. To ensure optimal internal validity and comparability between cases over the study period, simple randomization was used [15,16] to select an equal number of patients without complications (N = 22) from the overall cohort. This created a 44-patient cohort to match the available sample count for laboratory analysis. The Riga East University Hospital Science Department (Nr.AP/08-08/22/135) and the Riga Stradins University Ethics Committee (22-2/399/2021) approved the study protocol and the informed consent forms. Adult patients scheduled for elective microvascular flap surgery were included in the study. To avoid confounding factors, patients with coagulation abnormalities, history of smoking, recent blood clotting, or thromboembolic complications [17], along with patients taking hormonal contraceptives or estrogen therapy [18], were excluded, as these patient groups have been associated with

flap thrombosis. Patients on anticoagulants or antiplatelet medications were excluded from the study to avoid confounders related to flap bleeding [19]. Patients with active systemic infections [12], or autoimmune disorders [20] were excluded, as these patient groups may have abnormal TGF-β1 levels. Pregnant patients, lactating patients, and children under the age of 18 were also excluded, as they were outside the scope of our study design and were not included in the ethics approval. Patients with incomplete data were also excluded.

2.2. Outcome Definitions

True flap loss was defined as compromised blood flow resulting from anastomotic failure or thrombosis that leads to a complete loss of the transposed flap. Minor flap complications were defined as the occurrence of flap infection, delayed or incomplete wound closure, partial flap loss, or wound complications at the harvesting site. All instances of true flap loss required immediate surgical re-exploration.

2.3. Perioperative Considerations

The surgical team considered the type of defect, pedicle length, surgical positioning, body mass index, and patient body composition when selecting the flap type. The study included cases with the following flap types: anterolateral thigh, fibular, deep inferior epigastric artery perforator, gracilis muscle, radial forearm, serratus anterior, temporal artery, and latissimus dorsi. All patients received general anesthesia (GA). GA was induced with intravenous administration of fentanyl (1.5 μg/kg), propofol (1–2 mg/kg), and cisatracurium (0.15 mg/kg). All patients were subject to continuous monitoring of electrocardiogram, oxygen saturation, blood pressure (either invasive or noninvasive), body temperature, and end-tidal carbon dioxide levels, starting at the induction of anesthesia. Sevoflurane (Sevorane®, AbbVie S.r.l., Campoverde, Italy) at a 0.8–1.2 mean alveolar concentration was used to maintain GA, and fentanyl (1–1.5 μg/kg/h) was used to provide continuous analgesia during surgery. A continuous cisatracurium infusion of 1–2 μg/kg/min was used to achieve intraoperative myorelaxation. Crystalloid fluid administration (RiLac, B. Braun Melsungen AG, Melsungen, Germany) was provided intravenously at a rate of 3.5 to 5.5 mL/kg per hour throughout the surgical and early postoperative periods, with the goal of maintaining urine output at 1–1.5 mL/kg/h. Postoperative monitoring of vitals, temperature, urine output, and pain control was conducted in the recovery unit. All patients received anticoagulation with enoxaparin at a dose of 40 mg daily, commencing on the first postoperative day. The surgical team meticulously monitored the microvascular flap throughout the initial 7 days following surgery. Flap complications were monitored by the surgical team through clinical evaluation of flap perfusion, including assessment of tissue color, temperature, turgor, capillary refill, flap skin texture, the absence of edema, and pinprick tests.

2.4. Data Collection, Sample Handling, and Laboratory Analysis

Patient demographics, flap types, surgical indications, recipient sites, operative times, perioperative care, and clinical outcomes were collected from both written and electronic medical records, following a predefined protocol. The surgical outcomes were observed and documented directly by the surgical team. Preoperative blood samples for analysis were obtained immediately before surgery, prior to the administration of intravenous fluids. Postoperative blood draws were obtained after the end of surgery, before leaving the operating room. All blood draws were performed using gentle aspiration and careful handling of the blood collection tubes to avoid artificial stimulation of TGF-β1 release. Full blood count analysis of the preoperative samples was performed using the XN-1500 system (Sysmex Europe SE, Norderstedt, Germany). Preoperative albumin concentrations were obtained using the colorimetric method (Cobas C, Roche/Hitachi, Manheim, Germany). Preoperative IL-6

concentrations were obtained by electrochemiluminescence immunoassay (ECLIA) (Cobas e, Roche/Hitachi, Manheim, Germany). Preoperative C-reactive protein (CRP) concentrations were obtained using the method of immunoturbidimetry (Cobas C, Roche/Hitachi, Manheim, Germany). Preoperative fibrinogen concentrations were obtained using the CS 5100 system (Sysmex Corporation, Kobe, Japan). Preoperative total protein concentrations were analyzed using the colorimetric method (Cobas c, Roche/Hitachi, Manheim, Germany). Preoperative levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using the enzymatic colorimetric technique (Cobas c, Roche/Hitachi, Manheim, Germany). All full blood count and clinical chemistry analyses of the preoperative blood samples were processed and analyzed in a clinical laboratory within eight hours of collection. All blood samples designated for TGF-β1 evaluation were frozen within 6 h after collection. Before storage, blood samples for TGF-β1 analysis were centrifuged at 3500× *g* for 10 min. All samples were centrifuged within 2 h after collection. Sample handling was conducted with meticulous care, and light or heat exposure was strictly avoided. The serum samples were preserved at a consistent temperature of −80 °C in screw-cap tubes appropriate for long-term storage. TGF-β1 evaluation was conducted following a single thaw cycle with the TGF-β1 ELISA kit, according to the manufacturer's protocol (Merck, Darmstadt, Germany). All reagents, calibration standards, and samples were prepared following the instructions outlined in the product's protocol guide. The assay was conducted at ambient temperature (20–25 °C), in accordance with the manufacturer's protocol. The absorbance reading was performed on a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm, immediately after the stop solution was added. The acquired measurements were collected for subsequent statistical analysis.

2.5. Statistical Analysis

GraphPad Prism, version 5.03 (GraphPad Software Inc., San Diego, CA, USA) and SPSS Statistics for Windows, version 26.0. (IBM Corp., Armonk, NY, USA) were used to perform the statistical analysis. GraphPad Prism version 5.03 (GraphPad Software Inc., San Diego, CA, USA) was used to create graphic visualizations. The distribution of all variables was assessed for normality through visual examination of the quantile–quantile plot. The Kolmogorov–Smirnov test was employed to assess whether the data followed a normal distribution. Analysis of nominal variable datasets was performed using the chi-squared test. Pearson correlation was applied to assess the relationships between parametric datasets. Spearman's Rho was used to evaluate non-parametric correlations. Differences in data distribution between groups were assessed using the Mann–Whitney U test for non-parametric variables. Independent *t*-tests were conducted to compare the means of two groups with normally distributed data. The datasets for CRP, fibrinogen, hemoglobin, and hematocrit were divided into quartiles. The interquartile differences in mean TGF-β1 concentrations were further compared using the analysis of variance (ANOVA) test. ANOVA test comparisons were also performed for postoperative changes in the log10 of TGF-β1 in different surgical outcome groups. Diagnostic performance was evaluated using the Receiver Operating Characteristic (ROC) curve and the Area Under the Curve (AUC) of postoperative TGF-β1 changes in predicting microvascular flap complications. Cut-off values were determined using the concordance probability method [21]. Odds ratios (OR) for flap complications were calculated using binary logistic regression. Continuous variables with a normal distribution were expressed as the mean with a 95% confidence interval (CI95). Statistical significance was determined at a two-tailed *p*-value of less than 0.05.

3. Results

In total, 44 patients were included: 24 (59.1%) men and 20 (40.9%) women. Their mean age was 57.1 years (CI95 52.5–61.7). The complications group consisted of 22 patients, 12 of whom had minor flap complications, while 10 patients had true flap loss. Five of these cases had late flap loss (>72 h). All patients with early true flap loss underwent urgent and successful anastomosis revision. Four cases of late true flap loss were treated with repeated microvascular flap reconstruction, while one case required necrectomy followed by rotated flap reconstruction.

As indicated in Table 1, no significant differences in the rate of flap complications were observed with respect to age, gender, reconstruction sites, surgical indications, or flap types between the control and complications groups. Increased preoperative plasma fibrinogen was found to be associated with flap complications (4.04 [3.56–4.51] vs. 3.22 [2.69–3.75], $p = 0.044$).

Table 1. General characteristics, perioperative factors, comorbidities, and laboratory parameters: Data are presented as the mean (CI95) or count (percentage). Abbreviations—ENT (ear, nose, and throat surgery); ALT (anterolateral thigh); DIEP (deep inferior epigastric artery perforator); CRP (C-reactive protein); HDL-C (high-density lipoprotein cholesterol); LDL-C (low-density lipoprotein cholesterol); TGF-β1 (transforming growth factor beta-1).

Patient Group	Overall N = 44	Complications Group N = 22	Control Group N = 22	*p*-Value
Demographic data				
Mean age, years	57.9 (54.8–61.0)	59.5 (53.5–65.4)	54.4 (46.7–62.1)	0.389
Women, n (%)	20 (45.5%)	10 (45.5%)	10 (45.5%)	-
Location of reconstruction				
Extremity, n (%)	6 (13.6%)	4 (18.2%)	2 (9.1%)	0.248
ENT, n (%)	26 (59.1%)	14 (63.6%)	12 (54.5%)	0.539
Head and neck, n (%)	6 (13.6%)	2 (9.1%)	4 (18.2%)	0.248
Breast, n (%)	6 (13.6%)	2 (9.1%)	4 (18.2%)	0.248
Flap type				
ALT, n (%)	25 (56.8%)	12 (54.5%)	13 (59.1%)	0.773
Fibular flap, n (%)	5 (11.4%)	3 (13.6%)	2 (9.1%)	0.635
DIEP, n (%)	5 (11.4%)	1 (4.5%)	4 (18.2%)	0.154
Other, n (%)	9 (20.5%)	6 (27.3%)	3 (13.6%)	0.262
Indication				
Trauma, n (%)	5 (11.4%)	2 (9.1%)	3 (13.6%)	0.635
Oncology, n (%)	32 (72.7%)	14 (63.6%)	18 (81.8%)	0.517
Defect, n (%)	7 (15.9%)	6 (27.3%)	1 (4.5%)	0.099
Intraoperative and anesthesia considerations				
Duration of surgery, hours	6.03 (5.48–6.58)	5.93 (5.20–6.66)	6.18 (5.18–7.18)	0.739
Total intraoperative crystalloids, mL	2460.00 (2421.59–2498.41)	2480.00 (2434.76–2525.24)	2440.00 (2370.89–2509.11)	0.276
Total intraoperative colloids, mL	625.00 (521.04–728.94)	650.00 (477.22–822.78)	600.00 (449.19–750.81)	0.615
Intraoperative hematocrit, %	34.50 (33.30–35.70)	33.75 (31.76–35.74)	36.00 (34.16–37.84)	0.097
Use of vasopressors/sympathomimetics, n (%)	15 (34.1%)	10 (45.5%)	5 (22.7%)	0.112
Laboratory values				
Red blood cell count, 10^9/L	4.13 (3.97–4.28)	4.28 (4.06–4.49)	4.00 (3.77–4.23)	0.084
White blood cell count, 10^9/L	6.36 (5.57–7.17)	6.50 (5.62–7.38)	6.25 (4.91–7.59)	0.418
Lymphocyte count, 10^9/L	1.67 (1.48–1.86)	1.63 (1.31–1.93)	1.71 (1.45–1.96)	0.497
Neutrophil count, 10^9/L	3.90 (3.13–4.67)	4.06 (3.28–4.84)	3.76 (2.44–5.09)	0.162
Monocyte count, 10^9/L	0.56 (0.50–0.63)	0.56 (0.47–0.66)	0.57 (0.47–0.66)	0.958
Platelet count, 10^9/L	258.95 (230.52–287.38)	288.37 (238.33–338.40)	233.55 (202.96–264.14)	0.092
Hemoglobin, g/dL	12.43 (11.90–12.95)	12.87 (12.13–13.61)	12.05 (11.28–12.81)	0.087
Hematocrit, %	38.81 (37.41–40.22)	40.12 (38.22–42.01)	37.69 (35.64–39.74)	0.065
Total plasma protein, g/L	63.79 (61.94–65.94)	64.08 (61.32–66.83)	63.51 (60.79–66.23)	0.794
Plasma albumin, g/L	38.77 (37.58–39.95)	39.00 (37.05–40.96)	38.55 (36.98–40.11)	0.668
CRP, mg/L	8.40 (3.90–12.91)	6.93 (3.25–10.61)	9.87 (1.23–18.51)	0.718

Table 1. *Cont.*

Patient Group	Overall N = 44	Complications Group N = 22	Control Group N = 22	*p*-Value
Plasma fibrinogen, g/L	3.61 (3.24–3.98)	4.04 (3.56–4.51)	3.22 (2.69–3.75)	0.044
Interleukin-6, pg/mL	14.62 (10.32–18.92)	11.73 (6.32–17.13)	17.37 (10.51–24.24)	0.262
HDL-C, mmol/l	1.27 (1.16–1.39)	1.17 (1.01–1.32)	1.37 (1.20–1.54)	0.094
LDL-C, mmol/l	2.89 (2.57–3.21)	2.84 (2.49–3.19)	2.93 (2.38–3.49)	0.950
Preoperative TGF-β1, ng/ml	2.64 (2.25–3.03)	2.68 (2.13–3.24)	2.60 (2.01–3.20)	0.771
Postoperative TGF-β1, ng/ml	3.12 (2.71–3.53)	3.48 (2.90–4.06)	2.77 (2.20–3.35)	0.072

When evaluating different indications for surgery, defects had the highest postoperative TGF-β1 concentrations, followed by oncology, while patients with trauma had the lowest preoperative TGF-β1 concentrations (4.25 ng/mL [3.51–4.98] vs. 2.99 [2.51–3.48] vs. 2.33 [1.02–3.64], $p = 0.023$).

No significant differences were found when comparing preoperative TGF-β1, postoperative TGF-β1, or postoperative change in TGF-β1 between the different reconstruction sites or flap types used.

As illustrated in Figure 1, postoperative changes in TGF-β1 were positively correlated with preoperative fibrinogen (r = 0.369, $p = 0.021$) and preoperative CRP (r = 0.333, $p = 0.036$). Postoperative TGF-β1 levels were positively correlated with preoperative hemoglobin ($r = 0.328$, $p = 0.029$) and preoperative hematocrit (r = 0.341, $p = 0.031$). There were no significant links between preoperative TGF-β1 and any of the included preoperative biomarkers.

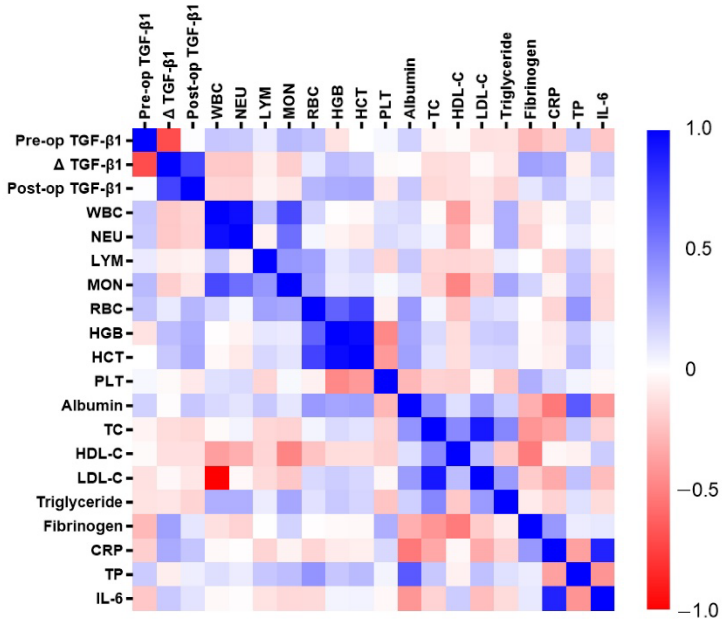

Figure 1. Correlation analysis between transforming growth factor beta-1 and selected biomarkers in microvascular flap surgery patients. Abbreviations—Pre-op (preoperative); TGF-β1 (transforming growth factor beta-1); Δ TGF-β1 (postoperative change in transforming growth factor beta-1); Post-op (postoperative); WBC (white blood cells); NEU (neutrophils); LYM (lymphocytes); MON (monocytes); RBC (red blood cells); HGB (hemoglobin); HCT (hematocrit); PLT (platelets); TC (total cholesterol); HDL-C (high-density lipoprotein cholesterol); LDL-C (low-density lipoprotein cholesterol); CRP (C-reactive protein); TP (total protein); IL-6 (interleukin-6).

As illustrated in Figure 2, postoperative changes in TGF-β1 concentrations were positively associated with preoperative plasma fibrinogen ($p = 0.020$) and plasma CRP ($p = 0.021$). Postoperative TGF-β1 concentrations were positively associated with preoperative hemoglobin ($p = 0.009$) and hematocrit ($p = 0.039$).

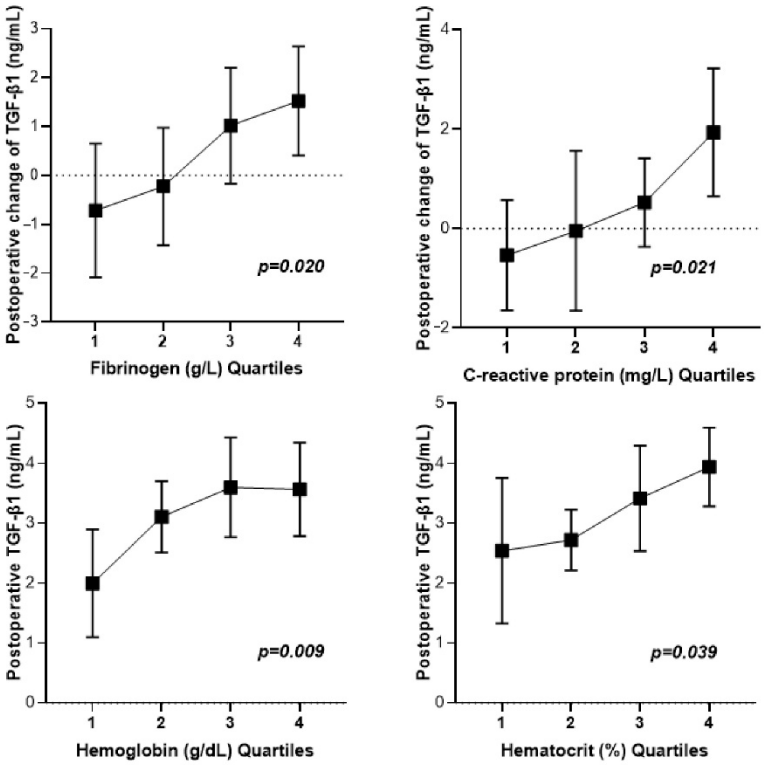

Figure 2. Association between perioperative TGF-β1 and preoperative biomarkers in microvascular flap surgery patients. Quartiles of fibrinogen (g/L): Q1 < 2.31; Q2 2.32–3.78; Q3 3.79–4.34; Q4 > 4.35. Quartiles of C-reactive protein (mg/L): Q1 < 1.93; Q2 1.94–3.86; Q3 3.87–14.00; Q4 > 14.01. Quartiles of hemoglobin (g/dL): Q1 < 11.6; Q2 11.7–12.6; Q3 12.7–13.7; Q4 > 13.8. Quartiles of hematocrit (%): Q1 < 36.6; Q2 36.7–39.0; Q3 39.1–42.2; Q4 > 42.3. Abbreviations—TGF-β1 (transforming growth factor beta-1). Data are presented as the mean (CI95).

As illustrated in Figure 3, the largest increase in the postoperative log10 of TGF-β1 (ng/mL) was found in cases with true flap loss (0.403 [0.024–0.782]), followed by minor flap complications (0.157 [0.029–0.285]). Patients without flap complications had the lowest postoperative change in the log10 of TGF-β1 (−0.089 [−0.233–0.056], $p = 0.002$). Analysis of the predictive accuracy of postoperative changes in TGF-β1 for true flap loss found that the AUC for log10 of TGF-β1 was 0.797 (0.588–0.997, $p = 0.005$). A postoperative change in TGF-β1 > 1.00 ng/mL was determined to be optimal based on the cut-off analysis (specificity 79.4%, sensitivity 80.0%, positive predictive value 53.3%, negative predictive value 93.1%). When adjusted for age, sex, and preoperative plasma fibrinogen, multivariate regression analysis revealed that an increase in the postoperative change in TGF-β1 increases the odds of true flap loss (OR 2.028, CI95 1.185–3.471, $p = 0.009$).

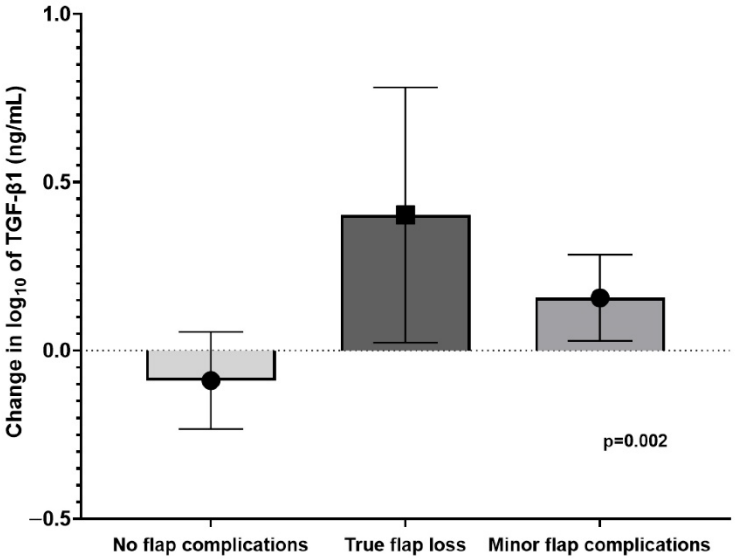

Figure 3. Postoperative changes in log10 of TGF-β1 for different surgical outcomes: ANOVA test comparisons of postoperative changes in log10 of transforming growth factor beta-1 in different surgical outcome groups. Abbreviations—TGF-β1 (transforming growth factor beta-1).

4. Discussion

The central finding of this study is that a postoperative increase in TGF-β1 is linked to true flap loss and, to a smaller extent, minor flap complications. Increased postoperative changes in TGF-β1 are linked to increased CRP and fibrinogen. Increased postoperative TGF-β1 concentrations are linked to increased preoperative hematocrit and hemoglobin.

While TGF-β is secreted by many cell types [22], platelets contribute approximately 45% of the total TGF-β present in plasma [12,23,24], and TGF-β1 is its main isoform, accounting for 95% of the total TGF-β [25]. Previous studies have shown that exposure to shear stress contributes to the release of TGF-β1 from platelets both in vitro [14] and in vivo [14,26]. We propose that the increase in shear forces in a dysfunctional vascular anastomosis site may partially contribute to the postoperative increase in TGF-β1. Prior research indicates that shear stress can efficiently activate latent TGF-β1 present in platelets and the extracellular matrix, highlighting the intricacies of flap survival under dynamic vascular settings [24,26]. In thrombosed arteries, particularly during flap surgery, the augmented shear stress might quadruple, resulting in a synergistic increase in TGF-β1 release [27]. The presence of co-secreted proteins, such as thrombospondin-1, may exacerbate this effect by facilitating the activation of latent TGF-β1 forms [28,29]. Moreover, inflammatory enzymes like matrix metalloproteinases may facilitate this activation process, establishing a feedback loop in which shear-induced TGF-β1 release enhances platelet activation and localized inflammation [28]. Interestingly, increases in TGF-β1 have been found to increase the shear stress exhibited on circulating platelets, and the presence of hypercholesterolemia has been found to exacerbate the release of TGF-β1 from platelets [26], although this was not supported by our findings. It must be noted that the shear stress interaction between the vessel wall and TGF-β1 has been previously studied only in the context of chronic vessel wall changes [26]. In addition to shear-stress-induced TGF-β1 release from platelets, the presence of thrombin during thrombosis of the anastomosis may activate platelets through protease-activated receptors 1/4 (PAR1/4), thereby promoting

further TGF-β1 secretion [30]. This process stimulates monocyte tissue factor expression and further amplifies thrombin generation, coagulation, and inflammation [31].

TGF-β1 has also been implicated in the regulation of thrombosis [32,33]. A study by Zhang et al. revealed that the presence of TGF-β1 facilitates the recruitment of neutrophils and monocytes into thrombi while also promoting the formation of neutrophil extracellular traps [13]; therefore, TGF-β1 positively modulates venous thrombus formation. In patients with portal venous thrombosis, increased levels of platelet-derived TGF-β1 have been linked to a hypercoagulable state and contribute to endothelial dysfunction [33]. Notably, the absence of TGF-β1 in mice did not impact the development of arterial thrombosis [13]. However, while our results showed that a postoperative increase in TGF-β1 was linked to true flap loss, we did not observe a link between increased preoperative TGF-β1 and the risk of true flap loss. Our findings corroborate recent evidence that platelet-derived TGF-β1 promotes the progression of venous thrombus development, rather than its initiation [32]. This may imply that the promotion of coagulation is a product of local TGF-β1 release at the site of thrombus formation, rather than its cause.

In venous flap thrombosis, pedicle kinking is a more prevalent cause of flap failure than anastomotic failure [34], and venous thrombosis in such conditions occurs under lower shear stress [35]. In pedicle kinking, blood stasis is considered to be the primary factor for the prothrombotic state at the kinked location [34]. Postoperative increases in TGF-β1 could be predictive of microvascular flap thrombosis progression and imminent flap failure, as the presence of thrombus formation increases the local TGF-β1 concentration [13,36]. This is further supported by our findings that postoperative increases in TGF-β1 were positively linked to increased preoperative plasma fibrinogen, which has also been previously linked to increased rates of flap thrombosis [4,8].

Our results revealed that increased preoperative CRP is positively linked to postoperative increases in TGF-β1. While increased baseline levels of TGF-β1 have been positively linked to CRP in kidney disease patients [37], no previous studies have evaluated the link between CRP and TGF-β1 in a surgical population. Increased preoperative CRP has previously been linked to flap complications [38], which may partially explain our findings.

Our findings indicate that postoperative TGF-β1 concentrations are linked to preoperative hemoglobin and hematocrit. Studies on systemic lupus erythematosus found TGF-β1 concentrations to be positively linked to hemoglobin, although these findings were likely due to lower disease activity [20]. Furthermore, our results did not show any significant link between preoperative hemoglobin and preoperative TGF-β1 concentrations in microvascular surgery patients. A potential explanation for the link between hematocrit and postoperative TGF-β1 is the effect of hematocrit on blood viscosity [39]. Increasing blood viscosity increases shear stress at a given blood flow [40]. As the stimulation of TGF-β1 secretion associated with platelet activation occurs under shear stress [26], increased blood viscosity might increase TGF-β1 secretion from platelets at different stenotic sites in the vasculature [26], and potentially at the site of microvascular anastomosis. It must be noted that increased blood viscosity due to increased hematocrit could also promote TGF-β1 release during the process of blood sample collection [23].

This study has several limitations, as well as several strengths. First, this study includes only a single-center experience and offers a limited patient population due to laboratory resource limitations. The exclusion criteria, which excluded patients with severe comorbidities, pre-existing vascular disorders, or anticoagulant use, may have created selection bias. This could have an impact on the external validity of this study, as the omitted individuals could exhibit substantially varying TGF-β1 concentrations [23]. Conversely, the reported concentrations of TGF-β in humans exhibit considerable variability across both pathological and physiological states, all of which could not be covered by the exclusion

criteria [23]. Tracking of TGF-β in plasma may be difficult due to rapid binding to target cells [23]. Therefore, verification with immunohistochemical analysis of SMAD protein expression in target cells would further improve the reliability of the results [23]. Our evaluation of the association between flap loss and TGF-β1 antigen concentrations did not distinguish between arterial and venous thrombotic events at the anastomotic site, despite the potential clinical relevance of this differentiation in relation to TGF-β1 secretion [13]. Given the use of multiple different flap types, the anastomosis site and subsequent vessel curvature were not individually evaluated, although they may influence blood flow and affect outcomes in certain cases. Postoperative TGF-β1 increases may have multiple implications in minor flap complications, such as tissue scarring of the transposed flap [41] or difficult wound healing [42], even after initial flap success. Intriguingly, the emerging technique of platelet-rich plasma injections in reconstructive surgery has demonstrated encouraging outcomes [43]. These benefits may be partially attributed to elevated local concentrations of TGF-β1 [44], although this potential mechanism warrants additional investigation. Further studies with longer postoperative TGF-β1 analysis periods may elucidate potential diagnostic and therapeutic applications of TGF-β1 for scarring and difficult wound healing in microvascular flap surgery.

5. Conclusions

The postoperative increase in circulating TGF-β1 is associated with microvascular flap complications. Assessment of the postoperative change in circulating TGF-β1 may be valuable for the prediction of true flap loss. While TGF-β1 has potential as a biomarker for flap viability, it requires improvement in measurement precision and consideration of other factors that impact its activity. Improved understanding of TGF-β1's dynamics and its clinical implications may lead to better outcomes for patients undergoing microvascular flap surgery.

Author Contributions: Conceptualization: R.P.R., B.M., S.D., O.S. and M.C. Methodology: J.Z., R.P.R., R.D., M.C., S.D., S.G., A.V., S.R.-D., O.S. and B.M. Validation: R.P.R., E.B., M.C., S.G., A.V. and S.R.-D. Formal analysis: E.B., I.M., S.G., A.V. and S.R.-D. Investigation: R.P.R., J.Z., R.D., M.C., S.G., A.V. and S.R.-D. Resources: J.Z., I.M., R.D., M.C., S.G., A.V. and S.R.-D. Data curation: R.P.R., E.B., I.M. and S.G. Writing—original draft: R.P.R., J.Z., R.D., M.C., B.M., S.D., I.M., O.S., S.G., A.V., S.R.-D. and E.B. Writing—review & editing: R.P.R., J.Z., R.D., M.C., S.D., O.S. and B.M. Visualization: R.P.R. Supervision: S.D., O.S. and B.M. Project administration: S.D., O.S. and B.M. Funding acquisition: O.S. and B.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors disclose that the publication fee for this article was funded by Riga Stradins University (Grant number: 6-DN-20/1/2024). The funder was not involved in the study design; the collection, analysis, or interpretation of the data; or the writing of this article.

Institutional Review Board Statement: The study protocol and the informed consent form were approved by the Ethics Committee of Riga Stradins University (Approval Number 22-2/399/2021; 8 July 2021) and by the Science Department of Riga East University Hospital (Approval Number Nr.AP/08-08/22/135; 8 November 2022).

Informed Consent Statement: Written informed consent was obtained from all individuals who participated in the study.

Data Availability Statement: The corresponding author will provide access to the analyzed datasets upon reasonable request. The corresponding author is responsible for securing the protection of individual privacy when transferring the datasets.

Acknowledgments: We would like to thank Vita Kalnberzina, from the Department of English Studies at the University of Latvia, for her valuable assistance in reviewing and improving the grammar and syntax of this manuscript. We also extend our gratitude to Irina Olehnovica (Riga East

University Hospital, Anesthesiology Clinic) and Anastasija Maksaja (Riga East University Hospital, Anesthesiology Clinic) for their support throughout the creation of this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Abdou, S.A.; Sharif-Askary, B.; Zolper, E.G.; Evans, K.K. Intraoperative Utility of the Implantable Doppler in Lower Extremity Reconstruction: A Matched Case-Control Study. *Plast. Reconstr. Surg. Glob. Open* **2020**, *8*, e3229. [CrossRef] [PubMed]
2. Min, K.; Hong, J.P.; Suh, H.P. Risk Factors for Partial Flap Loss in a Free Flap: A 12-Year Retrospective Study of Anterolateral Thigh Free Flaps in 303 Lower Extremity Cases. *Plast. Reconstr. Surg.* **2022**, *150*, 1071e–1081e. [CrossRef]
3. Ojuva, A.M.; Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Donina, S.; Mamaja, B.; Vanags, I. Novel Challenges and Opportunities for Anesthesia and Perioperative Care in Microvascular Flap Surgery: A Narrative Review. *Clin. Pract.* **2024**, *14*, 2187–2201. [CrossRef] [PubMed]
4. Rocans, R.P.; Zarins, J.; Bine, E.; Mahauri, I.; Deksnis, R.; Citovica, M.; Donina, S.; Vanags, I.; Gravelsina, S.; Vilmane, A.; et al. Von Willebrand Factor Antigen, Biomarkers of Inflammation, and Microvascular Flap Thrombosis in Reconstructive Surgery. *J. Clin. Med.* **2024**, *13*, 5411. [CrossRef]
5. da Silva, A.C.O.; Silva, G.B.; Cho, A.B.; Wei, T.H.; Mattar, R.; Iamaguchi, R.B. Hypoalbuminemia in microsurgical flaps of the musculoskeletal apparatus. *Acta Ortop. Bras.* **2020**, *28*, 168–171. [CrossRef]
6. Košec, A.; Solter, D.; Ribić, A.; Knežević, M.; Vagić, D.; Pegan, A. Systemic Inflammatory Markers as Predictors of Postoperative Complications and Survival in Patients with Advanced Head and Neck Squamous Cell Carcinoma Undergoing Free-Flap Reconstruction. *J. Oral Maxillofac. Surg.* **2022**, *80*, 744–755. [CrossRef]
7. Chargi, N.; Breik, O.; Forouzanfar, T.; Martin, T.; Praveen, P.; Idle, M.; Parmar, S.; de Bree, R. Association of low skeletal muscle mass and systemic inflammation with surgical complications and survival after microvascular flap reconstruction in patients with head and neck cancer. *Head Neck* **2022**, *44*, 2077–2094. [CrossRef] [PubMed]
8. Handschel, J.; Burghardt, S.; Naujoks, C.; Kübler, N.R.; Giers, G. Parameters predicting complications in flap surgery. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2013**, *115*, 589–594. [CrossRef]
9. Schmidt, A.; Bengtsson, A.; Tylman, M.; Blomqvist, L. Pro-inflammatory cytokines in elective flap surgery. *J. Surg. Res.* **2007**, *137*, 117–121. [CrossRef]
10. Finke, J.C.; Yang, J.; Bredell, M.; von Fritschen, U.; Glocker, M.O. Plasma Cytokine and Growth Factor Profiling during Free Flap Transplantation. In *Issues in Flap Surgery*; InTech: London, UK, 2018.
11. Rothweiler, R.M.; Metzger, M.C.; Zieger, B.; Huber-Schumacher, S.; Schmelzeisen, R.; Kalbhenn, J. The Role of von Willebrand Factor in Microvascular Surgery in Severely Injured Patients. *Plast. Reconstr. Surg. Glob. Open* **2021**, *9*, e3836. [CrossRef]
12. Deng, Z.; Fan, T.; Xiao, C.; Tian, H.; Zheng, Y.; Li, C.; He, J. TGF-β signaling in health, disease, and therapeutics. *Signal Transduct. Target. Ther.* **2024**, *9*, 61.
13. Zhang, S.; Li, Y.; Zhang, J.; Sun, Y.; Chu, X.; Gui, X.; Tong, H.; Ding, Y.; Ju, W.; Xu, M.; et al. Platelet-Derived TGF-β1 Promotes Deep Vein Thrombosis. *Thromb. Haemost.* **2024**, *124*, 641–648. [CrossRef]
14. Ahamed, J.; Burg, N.; Yoshinaga, K.; Janczak, C.A.; Rifkin, D.B.; Coller, B.S. In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor-beta1. *Blood* **2008**, *112*, 3650–3660. [CrossRef] [PubMed]
15. Mann, C.J. Observational research methods—Cohort studies, cross sectional studies, and case–control studies. *Afr. J. Emerg. Med.* **2012**, *2*, 38–46. [CrossRef]
16. Dettori, J.R.; Norvell, D.C.; Chapman, J.R. Grab Control! Choosing the Right Comparison Group in an Observational Study. *Glob. Spine J.* **2019**, *9*, 456–458. [CrossRef]
17. Lese, I.; Biedermann, R.; Constantinescu, M.; Grobbelaar, A.O.; Olariu, R. Predicting risk factors that lead to free flap failure and vascular compromise: A single unit experience with 565 free tissue transfers. *J. Plast. Reconstr. Aesthet. Surg.* **2021**, *74*, 512–522. [CrossRef]
18. Mahrhofer, M.; Reichert, R.; Siegwart, L.C.; Russe, E.; Schoeller, T.; Wechselberger, G.; Weitgasser, L. Risk of perioperative hormonal breast cancer therapy for microvascular flap complications in breast reconstruction. *J. Plast. Reconstr. Aesthet. Surg.* **2023**, *85*, 143–148. [CrossRef]
19. Chattha, A.S.; Ruan, Q.Z.; Bucknor, A.; Chen, A.D.; Lee, B.T.; Lin, S.J. Abstract: Impact of Blood Thinners on Flap Failure and Hematoma Rates in Patients Undergoing Non-Breast Flap Reconstruction: Analysis of 79,915 Patients. *Plast. Reconstr. Surg. Glob. Open* **2017**, *2*, 129–130. [CrossRef]
20. Gómez-Bernal, F.; Quevedo-Abeledo, J.C.; García-González, M.; Fernández-Cladera, Y.; González-Rivero, A.F.; de Vera-González, A.; Martín-González, C.; González-Gay, M.Á.; Ferraz-Amaro, I. Serum Levels of Transforming Growth Factor Beta 1 in Systemic Lupus Erythematosus Patients. *Biomolecules* **2022**, *13*, 73. [CrossRef]

21. Unal, I. Defining an Optimal Cut-Point Value in ROC Analysis: An Alternative Approach. *Comput. Math. Methods Med.* **2017**, *2017*, 3762651. [CrossRef]
22. Chen, P.Y.; Qin, L.; Simons, M. TGFβ signaling pathways in human health and disease. *Front. Mol. Biosci.* **2023**, *10*, 1113061. [CrossRef] [PubMed]
23. Karolczak, K.; Watala, C. Blood Platelets as an Important but Underrated Circulating Source of TGFβ. *Int. J. Mol. Sci.* **2021**, *22*, 4492. [CrossRef]
24. Ghafoory, S.; Varshney, R.; Robison, T.; Kouzbari, K.; Woolington, S.; Murphy, B.; Xia, L.; Ahamed, J. Platelet TGF-β1 deficiency decreases liver fibrosis in a mouse model of liver injury. *Blood Adv.* **2018**, *2*, 470–480. [CrossRef]
25. Grainger, D.J.; Wakefield, L.; Bethell, H.W.; Farndale, R.W.; Metcalfe, J.C. Release and activation of platelet latent TGF-beta in blood clots during dissolution with plasmin. *Nat. Med.* **1995**, *1*, 932–937. [CrossRef] [PubMed]
26. Wang, W.; Vootukuri, S.; Meyer, A.; Ahamed, J.; Coller, B.S. Association between shear stress and platelet-derived transforming growth factor-β1 release and activation in animal models of aortic valve stenosis. *Arterioscler Thromb. Vasc. Biol.* **2014**, *34*, 1924–1932. [CrossRef] [PubMed]
27. Walshe, T.E.; dela Paz, N.G.; D'Amore, P.A. The role of shear-induced transforming growth factor-β signaling in the endothelium. *Arterioscler Thromb Vasc Biol.* **2013**, *33*, 2608–2617. [CrossRef]
28. Murphy-Ullrich, J.E.; Suto, M.J. Thrombospondin-1 Regulation of Latent TGF-β Activation: A Therapeutic Target for Fibrotic Disease. *Matrix Biol.* **2018**, *68–69*, 28–43. [CrossRef]
29. Murphy-Ullrich, J.E.; Poczatek, M. Activation of latent TGF-beta by thrombospondin-1: Mechanisms and physiology. *Cytokine Growth Factor Rev.* **2000**, *11*, 59–69. [CrossRef]
30. Boknäs, N.; Faxälv, L.; Sanchez Centellas, D.; Wallstedt, M.; Ramström, S.; Grenegård, M.; Lindahl, T.L. Thrombin-induced platelet activation via PAR4: Pivotal role for exosite II. *Thromb Haemost.* **2014**, *2*, 58–65. [CrossRef]
31. Pihusch, V.; Pihusch, M.; Penovici, M.; Kolb, H.J.; Hiller, E.; Pihusch, R. Transforming growth factor beta-1 released from platelets contributes to hypercoagulability in veno-occlusive disease following hematopoetic stem cell transplantation. *Thromb. Res.* **2005**, *116*, 233–240. [CrossRef]
32. Słupski, W.; Jawień, P.; Nowak, B. Botanicals in Postmenopausal Osteoporosis. *Nutrients* **2021**, *13*, 1609. [CrossRef]
33. Jiang, S.; Ai, Y.; Ni, L.; Wu, L.; Huang, X.; Chen, S. Platelet-derived TGF-β1 is related to portal vein thrombosis in cirrhosis by promoting hypercoagulability and endothelial dysfunction. *Front. Cardiovasc. Med.* **2022**, *9*, 938397. [CrossRef] [PubMed]
34. Williams, J.G.; French, R.J.; Lalonde, D.H. Why do free flap vessels thrombose? Lessons learned from implantable Doppler monitoring. *Can. J. Plast. Surg.* **2004**, *12*, 23–26. [CrossRef] [PubMed]
35. Undas, A.; Ariëns, R.A. Fibrin clot structure and function: A role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, e88–e99. [CrossRef] [PubMed]
36. Smeda, M.; Maleki, E.H.; Jasztal, A. A possible role of plasmin-dependent activation of TGF-β in cancer-associated thrombosis: Implications for therapy. *Cancer Metastasis Rev.* **2024**, *44*, 2. [CrossRef]
37. Hassan, M.O.; Duarte, R.; Dix-Peek, T.; Dickens, C.; Naidoo, S.; Vachiat, A.; Grinter, S.; Manga, P.; Naicker, S. Transforming Growth Factor-β Protects against Inflammation-Related Atherosclerosis in South African CKD Patients. *Int. J. Nephrol.* **2018**, *2018*, 8702372. [CrossRef]
38. Schmidt, F.; Ward, M.; Repanos, C. Postoperative serum C-reactive protein dynamics after pharyngolaryngectomy with jejunal free-flap reconstruction. *Ann. R. Coll. Surg. Engl.* **2023**, *105*, 263–268. [CrossRef]
39. Ameenuddin, M.; Anand, M.; Massoudi, M. Effects of Shear-Dependent Viscosity and Hematocrit on Blood Flow. *Appl. Math. Comput.* **2019**, *356*, 299–311. [CrossRef]
40. Leo, J.A.; Simmonds, M.J.; Sabapathy, S. Shear-thinning behaviour of blood in response to active hyperaemia: Implications for the assessment of arterial shear stress-mediated dilatation. *Exp. Physiol.* **2020**, *105*, 244–257. [CrossRef]
41. Finnson, K.W.; McLean, S.; Di Guglielmo, G.M.; Philip, A. Dynamics of Transforming Growth Factor Beta Signaling in Wound Healing and Scarring. *Adv. Wound Care.* **2013**, *2*, 195–214. [CrossRef]
42. Tredget, E.B.; Demare, J.; Chandran, G.; Tredget, E.E.; Yang, L.; Ghahary, A. Transforming growth factor-beta and its effect on reepithelialization of partial-thickness ear wounds in transgenic mice. *Wound Repair Regen.* **2005**, *13*, 61–67. [CrossRef] [PubMed]
43. Gierek, M.; Klama-Baryła, A.; Łabuś, W.; Bergler-Czop, B.; Pietrauszka, K.; Niemiec, P. Platelet-Rich Plasma and Acellular Dermal Matrix in the Surgical Treatment of Hidradenitis Suppurativa: A Comparative Retrospective Study. *J. Clin. Med.* **2023**, *8*, 2112. [CrossRef] [PubMed]
44. Du, X.; Zhao, J.; Ren, Q.; Ma, Y.; Duan, P.; Huang, Y.; Wang, S. Clinical application of platelet rich plasma to promote healing of open hand injury with skin defect. *Regen. Ther.* **2024**, *26*, 308–314. [CrossRef] [PubMed]