Dissertation

Gender-related differences in blood parameters and their influence on the intraoperative blood loss in orthognathic surgery

submitted by

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Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Guidelines of the Medical University of Graz on Good Scientific Practice“.

Graz, April 2021 Dr. Dr. Michael Schwaiger

Disclosures

Parts of the data collated during the conduction of this dissertation were published within the clinical paper '***Is there a hidden blood loss in orthognathic surgery and should it be considered? Results of a prospective cohort study'***, authored by the doctoral candidate. The article is accessible as an 'open-access'-file, within the 'Journal of Cranio- and Maxillofacial Surgery' (JCMFS). https://doi.org/10.1016/j.jcms.2020.07.015

Title:

Is there a hidden blood loss in orthognathic surgery and should it be considered? Results of a prospective cohort study

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Abbreviations and Definitions

±	standard deviation
x	multiplication
2D	2-dimensional
3D	3-dimensional
Aptt	activated partial thromboplastin time
ASA	American Society of Anesthesiologists
AT-III	Antithrombin-III
BIMAX	Bimaxillary surgery
BMI	body mass index
BSSO	Bilateral sagittal split osteotomy
BV	blood volume
CBL	Calculated blood loss
EBV	estimated blood volume
EDTA	Ethylenediaminetetraacetic acid, blood tube
ETP	Endogenous Thrombin potential
ETP-auc	Endogenous Thrombin potential, area-under-the-curve
ETP-Cmax	Peak height of thrombin-generation
FVIII	Coagulation factor VIII
FIX	Coagulation factor IX
FX	Coagulation factor X
FXI	Coagulation factor XI
FXIII	Coagulation factor XIII
g/L	gram per litre
g/dl	gram per decilitre
H	bodyheight
Hb	Haemoglobin

Hct	Haematocrit
ICU	intensive care unit
INR	International Normalized Ratio
IOB	Intraoperative blood loss
IV	intravenous
kg	kilogram
min	minutes
ml	millilitres
mmHg	millilitres of mercury
MPV	mean platelet volume
NOAC’s	new oral anticoagulants
OT	operating time
PROMS	patient reported outcome measures
PT	prothrombin time
RBC	red blood cell
RBL	Relative blood loss
SARPE	Surgically assisted rapid palatal expansion
TEG	thromboelastography
TXA	tranexamic acid
vWF	Von Willebrand Factor
vWF-Ag	Von Willebrand Factor - Antigen
vWF-Akt.L	Von Willebrand Factor – activity
W	bodyweight

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ABSTRACT IN GERMAN

Einleitung

Orthognath-chirurgische Operationen zählen zu den häufigsten mund-, kiefer- und gesichtschirurgischen Eingriffen. Diese, als elektiv einzustufende, operative Verfahren dienen der Korrektur komplexer dento-fazialer Anomalien und damit einhergehender skelettaler Malokklusion. Trotz der Einstufung als sichere Verfahren, gehören der operative Blutverlust und damit assoziierte Komplikationen zu den größten Problematiken dieses Fachgebiets. In diesem Zusammenhang zeigte sich im direkten Geschlechtervergleich häufig ein signifikant erhöhter Blutverlust bei Männern. Mögliche Ursachen für signifikante geschlechterspezifische Unterschiede hinsichtlich des Blutverlusts in der orthognathen Chirurgie wurden allerdings bisher kaum erforscht.

Ziel dieser Studie war es daher den intra- und perioperativen Blutverlust bei Eingriffen der orthognathen Chirurgie und insbesondere gender-spezifische Unterschiede in diesem Zusammenhang zu untersuchen. Zusätzlich lag der wissenschaftliche Fokus auf der Identifikation signifikanter gender-spezifische Unterschiede des Gerinnungsprofils, um zu überprüfen, ob diese ursächlich auf die Höhe des Blutverlusts Einfluss nehmen.

Material und Methoden

Gesunde männliche und weibliche Proband*innen, die sich an unserer Abteilung einer bimaxillären Umstellungsosteomie oder Unterkieferosteotomie unterzogen, kamen für diese Studie in Betracht. Die Bestimmung des Blutverlusts erfolgte zu 3 standardisierten Zeitpunkten mithilfe von 2 etablierten Messmethoden: der intraoperative Blutverlust (IOB) wurde anhand der Subtraktionsmethode bestimmt; die Berechnung des perioperativen Blutverlusts, 24- und 48 Stunden postoperativ, basierte auf der ‚Haemoglobin-balance' Methode (CBL-24h, CBL-48h). Die Auswertung der Blutungsmengen wurde nach Operationsmethode und Geschlecht getrennt vorgenommen.

Vor dem Eingriff wurde eine detaillierte Gerinnungsanalyse durchgeführt um geschlechterspezifische Unterschiede hinsichtlich des Gerinnungsprofils zu identifizieren und in weiterer Folge relevante Gerinnungsparameter mit der Höhe des Blutverlusts zu korrelieren.

Ergebnisse

103 Patient*innen (38 männlich, 65 weiblich) wurden in die statistische Auswertung miteinbezogen. 54 Patient*innen unterzogen sich einer bimaxillären Umstellungsosteomie; bei 49 Patient*innen wurde eine Unterkieferosteotomie durchgeführt. In Bezug auf den

intraoperativen Blutverlust (IOB) zeigten sich keine geschlechterspezifischen Unterschiede, jedoch hinsichtlich des perioperativen Blutverlusts (CBL-48h) in der bimaxillären Gruppe. Hier wurde ein signifikant erhöhter perioperativer Blutverlust (CBL-48h) bei Männern festgestellt (männlich: 907.7 ml ± 246.1 vs weiblich: 730.8 ml ± 274.5; p=0.019).

Bei Analyse des Gerinnungsprofils zeigten sich nur wenige signifikante geschlechterspezifische Unterschiede. Diese beschränkten sich auf die Parameter aktivierte partielle Thromboplastinzeit (aPTT), Antithrombin III, Endogenes Thrombin Potential (ETP-auc) und Gerinnungsfaktor IX. In der Korrelationsanalyse mit IOB und CBL-48h zeigte sich ein signifikanter Zusammenhang zwischen CBL-48h und Antithrombin III in der bimaxillären Gruppe (r=0.474; p=0.001). Die Höhe des intraoperativen Blutverlusts (IOB) wurde maßgeblich von der Operationsdauer beeinflusst.

Schlussfolgerung

Signifikante Zusammenhänge zwischen untersuchten Parametern und der Höhe des Blutverlusts wurden nachgewiesen, wobei der Parameter ‚Gender' sich weniger auf den Blutverlust auswirkte als bisher suggeriert. In Bezug auf den intraoperativen Blutverlust (IOB), konnte gezeigt werden, dass vor allem die Operationsdauer und -methode, nicht aber das Geschlecht und gender-spezifische Unterschiede des Gerinnungsprofils ausschlaggebend die Höhe des Blutverlusts beeinflussen. Im Gegensatz dazu, zeigten unsere Resultate, dass gender-spezifische Unterschiede des Gerinnungsprofils vor allem unmittelbar postoperativ an Relevanz gewinnen und mit der Höhe des perioperativen Blutverlusts maßgeblich korrelieren.

ABSTRACT IN ENGLISH

Introduction

Orthognathic surgical interventions are common maxillofacial procedures that aim to correct complex dentofacial deformities and concomitant skeletal malocclusion. These elective procedures follow highly standardised intraoperative protocols, which optimise patient safety and help to accurately administer a surgical plan. However, owing to the well-vascularised anatomy of the midface, blood loss remains among the major issues associated with orthognathic surgery. In this context, patient gender has frequently been highlighted as a crucial factor, with males associated with a higher surgical blood loss. Specific underlying mechanisms leading to these gender-related differences in orthognathic bleeding volumes have scarcely been investigated. Hence, the aim of this study was to analyse blood loss related to standardised orthognathic procedures with a special emphasis on patient gender. An additional aim was to ascertain gender-based differences in blood and haemostatic parameters and subsequently correlate these parameters with orthognathic blood loss.

Material and Methods

Healthy male and female individuals, scheduled to undergo bimaxillary surgery or bilateral sagittal split osteotomy (BSSO), were considered eligible for this study. Blood loss was standardly measured at three time points by two well-established approaches in the field of orthognathic surgery. Intraoperative blood loss (IOB) was determined using the subtraction method; perioperative blood loss was calculated 24- and 48-hours after surgery (CBL-24h; CBL-48h) by means of the ‘haemoglobin balance method’. All bleeding volumes determined were analysed according to the treatment modality applied and patient gender. Prior to surgery, a detailed coagulation analysis was performed comprising routine coagulation assays, global coagulation assays (endogenous thrombin potential) and specific haemostatic parameters. Relevant parameters were correlated with the intra- and perioperative blood loss (IOB and CBL-48h) to investigate the effect of gender-related differences in the haemostatic profile on a patient’s blood loss.

Results

A total of 103 patients (38 male, 65 female) were included in the final analysis, 54 of whom underwent bimaxillary surgery and on 49 of whom BSSO was performed.

Regarding patient sex, no gender-specific differences in terms of the intraoperative blood loss (IOB) were detected. With reference to CBL-24h and CBL-48h, however, statistically significant differences in terms of the bleeding volumes were found, but, were confined to the bimaxillary-cohort. In more detail, CBL-48h in males was found to significantly outreach that associated with females (male 907.7ml ± 246.1 vs female: 730.8ml ± 274.5; p=0.019). Regarding the haemostatic parameters analysed, few significant gender-related differences were detected including the parameters 'activated partial thromboplastin time (aPTT)', Antithrombin III, endogenous thrombin potential (ETP-auc) and coagulation factor IX. A significant correlation between the level of Antithrombin III and CBL-48h in the bimaxillary-cohort (r=0.474; p=0.001) was found. Regarding the IOB, the length of the procedure was identified as most significantly affecting the amount of blood loss.

Conclusion

Several parameters were identified as responsible for affecting the amount of blood loss, whereby patient gender was found to be less of a contributing factor than previously suggested. While the amount of intraoperative blood loss (IOB) related to orthognathic surgical procedures appeared to be significantly affected by the treatment modality applied together with the length of the procedure, patient gender and associated differences in the haemostatic profile were irrelevant in this context. In contrast, our findings indicate that gender-related peculiarities in the haemostatic cascade are most visible in the immediate postoperative period, where bleeding into tissue spaces as well as the maxillary sinuses is likely to pertain.

1. INTRODUCTION

1.1 Orthognathic surgery – principles and treatment need

Orthognathic surgery aims at correcting skeletal dentofacial deformities which negatively affect the normal proportions of the maxilla-mandibular complex as well as the typical relationship of the dental arches (1, 2). Patients affected by dentofacial deformities and concomitant skeletal malocclusion frequently present with impaired functions of the head and neck area with respect to breathing, swallowing, speech articulation, lip posture and chewing. Furthermore, associated facial disproportions are likely to impair the aesthetic facial appearance of the individual and are known to have an unfavourable psychosocial effect (2).

To determine the incidence of dentofacial deformities and the treatment need, a number of surveys have been conducted (2-6). In this context, racial variations in terms of the overall incidence rate and the type of the prevalent skeletal malocclusion were observed. According to the U.S. National Health and Nutrition Examination survey (NHANES III), one third of the U.S. population aged between 8 and 50 years presented with severe sagittal jaw discrepancy (3). Another third of their study cohort was shown to have moderate malocclusion, leaving one third presenting with ideal sagittal dentofacial proportions. Significant reverse overjet was found to be more common among the black and Latin American community, whereas within the white community marked positive overjet was prevalent. Additional to sagittal malocclusion, vertical relationships were examined and showed that only 50% of the U.S. population had an ideal vertical relationship of the incisors. Frequently encountered forms of vertical discrepancies are associated with a severe deep bite together with maxillomandibular deficiency and a severe open bite in combination with bimaxillary dental protrusion (3).

Surgical treatment to address and correct the aforementioned dentofacial deformities is frequently advised. However, despite the presence of skeletal discrepancies, a number of patients will undergo compromised orthodontic treatment to eliminate dental malocclusion without moving the jaws into the correct anatomical, functional and aesthetical position. This approach has been shown to increase the risk of occlusal instability and periodontal problems, and has further been linked to impaired facial aesthetics (2). As a result, and in order to objectify the necessity for surgical correction, various indexes of treatment need have been established (4, 6-8). Within the U.K., Brook and Shaw introduced an index, defining 5 grades of treatment need (4). While grade 1 indicates no or very little treatment need, grades 4 and 5 implicate that urgent treatment is required to avoid severe compromise of maxillomandibular function and facial aesthetics. Findings relative to this treatment index

suggest that at least 5% of both, the U.K. and the U.S. general population have dentofacial deformities which require orthognathic surgery. Furthermore, it has been shown that approximately 19% of all individuals consulting an orthodontist would benefit from a combined orthognathic-orthodontic approach instead of undergoing orthodontic treatment-only (2-4).

1.2 Development and history of orthognathic surgery

Early orthognathic procedures primarily addressed the correction of mandibular prognathism (9). In 1849, the first report of a mandibular osteotomy was published (10). The operation performed equated a mandibular wedge ostectomy in the pre-molar region to position the mandible backward. In 1897, bilateral condylectomy for mandibular repositioning mandible using a pre-auricular approach was introduced (11). Since then, numerous mandibular orthognathic surgical techniques have you been introduced, contributing to the advancement of orthognathic surgery (9). Important milestones regarding the development of modern mandibular orthognathic procedures especially refer (1) to the establishment of the intraoral approach for mandibular ramus and body osteotomies (12, 13) and (2) the introduction of the bilateral sagittal split osteotomy (BSSO) (14). In 1955, Obwegeser and Trauner published their technique for the correction of skeletal mandibular malocclusion, which subsequently became the preferred procedure for mandibular orthognathic surgery worldwide, by virtue of its reliability, versatility, and functional and aesthetic results (14). Later on, however, the BSSO was subject to relevant modification. Dal-Pont suggested changes concerning the anterior aspect of the osteotomy, which allowed for greater advances of the mandible (15). Hunsuck and Epker added important refinements by limiting the extent of the medial horizontal osteotomy (16, 17). Also in 1955, Obwegeser and Trauner published their report on surgical chin advancement, labelled ‘genioplasty (14, 18).

In comparison to mandibular orthognathic surgery, maxillary orthognathic procedures have evolved at a much slower pace. While techniques to mobilise and reposition the maxilla were discovered at an early stage, maxillary osteotomies were rarely performed before the year 1958 (9). This was due to the fear of poor blood supply with respect to the osteotomised maxilla resulting in severe tissue necrosis. In 1927, Wassmund performed a maxillary osteotomy without releasing the maxilla from the pterygoid plates, which implicated limitations in terms of maxillary movement (19). Obwegeser was one of the first surgeons to add this important surgical step to the procedure (20). Bell studied the biological basis of orthognathic surgery, specifically focusing on vascularity and wound healing (21, 22). On the basis of his findings that there was a reliable blood supply in the osteotomised maxilla, maxillary orthognathic surgery became more and more widely spread. In 1970, it was

Obwegeser again who first published his clinical results regarding 'two-jaw surgery', referring to surgical repositioning of both, the mandible and the maxilla, during the same procedure (23). Nowadays, this 'bi-maxillary' approach represents the gold-standard with respect to surgical correction of combined maxillomandibular deformities (9). Despite major technical advances, predictability of surgical outcomes and occlusal stability with regard to orthognathic surgery, were severely restricted at this stage by the lack of adequate osteosynthesis (9). Advances in rigid internal fixation and the further refinement of orthodontic and orthognathic planning modalities in the 1980s and 1990s helped to find/provided a solution to this problem. As a result of stable osteosynthesis, predictability of surgical results and long-term stability drastically improved (9). While modern orthognathic surgery, for the most part, still relies on the surgical techniques discussed earlier in the manuscript, modern technology such as 3-D planning tools, the use of cutting guides and customised operation splints have been added to this surgical field (24-26). The advantages of these novel planning tools relate to an improved accuracy of surgical movements and improved predictability of surgical outcomes whilst enabling more attention to be focused on soft tissue changes and facial aesthetics (25, 27).

1.3 Common complications in orthognathic surgery

Orthognathic surgery can generally be considered as a safe and innocuous surgical field regarding predictability of outcomes, patient safety and risk of perioperative complications (28-31). Adequate preoperative surgical planning and careful tissue handling are key to ensure the safe conduction of multiple complex surgical steps in an operating field of limited vision and access. Continuous further development of surgical techniques, innovative scientific research and standardisation of orthognathic pathways have eminently contributed to the safety of orthognathic surgery (9, 25, 28, 31). These days, the vast majority of orthognathic surgical interventions follow well-established intraoperative protocols in terms of the surgical steps required (31). This allows for precise and safe realization of the surgical plan. Additional to well-defined intraoperative surgical protocols, optimised measures with respect to the perioperative management of patients undergoing orthognathic surgery, have been discussed (32-36). However, significant differences between centres regarding perioperative measures can still be observed as no consensus on the optimal perioperative care has been found. Common perioperative measures used to reduce the risk of complications in orthognathic surgery will be discussed later in the manuscript (see section 1.4.4).

The elective character of orthognathic surgery reinforces the importance of a high level of patient safety and a low rate of complications (37, 38). Despite all the advances made,

complication rates related to orthognathic surgery of up to 27% have recently been reported (30, 39-42). A multitude of complications occurring in the context of orthognathic surgery are relative to current literature. This includes excessive bleeding, scarring, malocclusion, bad split of the mandible, infection, nerve injury, and bony or dental relapse (30, 39-41, 43). When looking at orthognathic complication rates in more detail, percentages differ vastly between publications. Significant variation in terms of the complications included inevitably adds to the wide range of complication rates. A recent study published in 2019 by Zaroni et al., which retrospectively reviewed complications associated with orthognathic surgery in 485 cases, found a complication rate of 19.2% in their study cohort (30). Approximately two thirds of all complications were found to occur within the postoperative phase; one third of the complications reported was shown to occur intraoperatively. Their study identified haemorrhage (12.9%) and postoperative malocclusion (12.9%), followed by damage to the inferior alveolar nerve (9.6%), bad split (9.6%) and infection (8%) as the most common complications with regards to orthognathic surgery. In a review conducted by Sousa and Turrini, nerve injury was determined as the most common complication (12.13%), whereas excessive bleeding was only observed in 1.43% of cases (44). Another study, which assessed the incidence of severe haemorrhage in maxillofacial surgery, came to a similar conclusion, stating that excessive bleeding within the field of orthognathic surgery occurs in approximately 1% of cases (45). Lanigan et al. demonstrated in their survey that bleeding complications are not exclusively limited to the intraoperative phase but are also inclined to occur postoperatively, up to a period of 5 weeks after surgery (46).

In general, one can expect that the majority of patients undergoing orthognathic surgery are of a young age, generally fit and well, and present with few comorbidities. However, despite being relatively rare, excessive bleeding in orthognathic surgery poses a significant threat to a young and healthy study population (45, 47). With an increased vascularity of the midface and therewith, orthognathic surgery inevitably provokes bleeding. This is aggravated by multiple anastomoses across the facial midline in combination with limited access to the source of bleeding, which may further complicate bleeding control (45). The average amount of blood loss in orthognathic surgery was quoted to be 436.11 ml, yet, cases of up to 5000ml blood loss have been reported (47, 48). While minor bleeding does not usually require intervention, excessive haemorrhage frequently demands medical and surgical counter-measures to stop the bleeding. The vessels commonly injured in the context of orthognathic procedures include the sphenopalatine artery, the descending palatine artery, the pterygoid plexus, and the internal maxillary artery in the upper jaw and the inferior alveolar and the facial artery in the lower jaw (40, 45). As far as maxillary osteotomies are concerned, a review showed that excessive bleeding originated from an arterial source in more than two thirds of the cases (45). In this regard, the internal maxillary artery has been identified as the

most common source of massive haemorrhage. In 19% of cases, venous haemorrhage from the pterygoid plexus was observed. Down-fracture and concomitant detachment of the maxilla from the pterygoid have been identified as representing the most challenging surgical steps with respect to avoiding vascular lesion (45).

With respect to mandibular osteotomies, arterial haemorrhage was found to occur in 48.2% of cases; venous haemorrhage was detected in 20.6% of cases. In the remaining cases regarding maxillary and mandibular osteotomies, no obvious source of bleeding was identified (45).

Conservative measures used to manage excessive bleeding often fail in cases of limited visibility and access, wherefore posterior nasal packing, ligation of vessels or transcatheter arterial embolization have frequently been advocated (45). As far as the ligation of blood vessels goes, doing so at the origin of the external carotid artery is not to be recommended, due to the low success rate of this procedure secondary to collateral circulation (45-47, 49, 50). Ligation of the terminal branches of the posterior nasal, sphenopalatine and descending palatine arteries in combination with ligation of the superficial temporal artery have proven more effective in this regard (45). However, in order to gain adequate access to the source of the bleeding, re-down-fracture of the maxilla or re-split of the mandible might be indicated whereby the surgical results may deteriorate (46). In many centres transcatheter arterial embolization has been identified as the method of choice to control severe haemorrhage in orthognathic surgery with success rates as high as 96% (45, 49, 51). Advantages of this modality over ligation particularly include better access to surgically inaccessible intraosseous bleeding sources and a higher level of selectivity. Major complications associated with this technique are rare but can include cranial nerve injury, cerebrovascular incidents, bone or soft tissue necrosis and even death (45, 52). For that reason, indications for transcatheter arterial embolization in orthognathic surgery are still limited to blood loss over 1500ml and the presence of hypovolaemic shock (53, 54).

Uncontrolled haemorrhage as a result of orthognathic surgery can result in haemodynamic instability and compromise the airway, necessitating admission to the ICU, invasive airway management and the intravenous administration of red blood cell (RBC) transfusions (45, 47). Furthermore, excessive bleeding is frequently responsible for a prolonged postoperative recovery period, a reduced postoperative quality of life and impaired surgical results (55-57).

Blood transfusion is often linked to a number of risks for the patient, such as graft versus host reaction or the transmission of a disease and given the elective character of orthognathic interventions, additional risks should be minimised. What is more, reducing the number of blood transfusions is not only of medical interest, but also economically sound.

Equally, ICU admission should be kept to the bare minimum, from both, an economic and medical point of view. Rates of blood transfusion in orthognathic surgery were shown to range between 0% and 26.7% (48, 58-60). While the overall incidence of administering blood components in orthognathic surgery has generally decreased in recent years, a few research papers have reported relatively high rates of blood transfusion in the respective study cohorts analysed (55). Reduced rates of blood transfusion can primarily be explained by a change of blood transfusion criteria. Nowadays, haemoglobin values between 7 and 8 g/dl are widely accepted in healthy adult patients (61, 62) in contrast to earlier recommendations. In addition, a number of perioperative measures to reduce bleeding in orthognathic surgery have been established, which also decrease the need for a blood transfusion (32, 33, 38). Prophylactic autologous blood donation has been suggested on various occasions within the field of orthognathic surgery (63, 64). This is relative to homologous blood transfusions as fewer risks have been associated with this method (63). Some authors have gone a step further and proposed that autologous blood transfusions should be performed on every patient undergoing orthognathic surgery (63). This, however, has been considered unnecessary in more recent studies (60). Several studies analysed the relationship between blood components prepared and blood components administered in the context of orthognathic surgery (60, 65). Ash and Mercuri concluded that screening and typing should only be performed on patients undergoing bimaxillary surgery in combination with iliac crest bone grafting as the blood transfusion rate was 75% and blood transfusion in other orthognathic procedures was less likely (65). An Austrian study analysing the rate of actual use with regards to ordered blood components, showed that up to 60% of blood transfusions prepared are not administered (66). This greatly underlines the necessity to establish reliable predictors for blood loss to ensure maximum patient safety while minimising essential and scarce resource waste.

1.4 Blood loss in orthognathic surgery

Orthognathic surgery performed in close proximity to well-vascularised midfacial areas is frequently associated with extensive bleeding. Occasionally, and as previously mentioned, blood loss can be so great that admission to ICU and blood transfusions might be necessary (45, 47). For that reason, significant efforts to reduce bleeding complications have been made (32, 34, 35, 38). In this context, research into the average bleeding volume in orthognathic surgery and the identification of potential predictors and risk factors has been conducted (37, 47, 61, 62, 67-71).

With respect to the amount of blood loss in orthognathic surgery, a number of studies have researched this specific question. In 2011, Pineiro et al. conducted a review aiming to

summarise relevant findings with reference to blood loss in orthognathic surgery (47). Only 7 out of 90 papers met the quality requirements for inclusion in their review. Surgical techniques taken into consideration included BSSO and LeFort-I osteotomy as a stand-alone procedure, bimaxillary surgery, genioplasty and surgically assisted rapid palatal expansion. All the studies with the exception of one (72) used hypotensive anaesthesia as a standard measure to minimise bleeding, furthermore, antifibrinolytic or vasoconstricting substances were administered in 5/7 studies for respective intervention groups. A total of 827 patients were assessed within this review. The mean volume of intraoperative blood loss was 436.11ml (47). Panula et al., reported a mean bleeding volume of 451ml in their study where antifibrinolytic substances were not administered (41). A higher amount of blood loss in the bimaxillary group, which had undergone mandibular osteotomy, was detected in their study. In a recent paper, analysing blood loss in more than 900 patients, a mean perioperative bleeding volume of 421.5 ml was determined (68). As with Panula et al. (41), a higher blood loss in the bimaxillary-cohort (494.1ml) was observed in comparison with BSSO-only (291.6ml), BSSO and genioplasty (284.5ml) and LeFort-I osteotomy (378.7ml). In 2017, Secher et al., published their results of a randomised controlled clinical trial, which specifically focused on blood loss in the context of bimaxillary surgery. While the control group did not receive any antifibrinolytic medication, 1g of IV tranexamic acid was administered in the intervention cohort (38). Significant differences between the two groups were detected with a blood loss of 403ml in the control group and 275ml in the intervention group. Salma et al. indicated an even lower blood loss in their study without the use of antifibrinolytics (55). Approximately 62% of their patients underwent bimaxillary surgery. The bleeding volume averaged 345.2ml (+/- 149.7ml), however, up to 9% of patients required a blood transfusion. Other studies reported higher average bleeding volumes, such as 793ml (73). Assuming a patient's total blood volume ranged between 3500 and 6000ml, the mean bleeding volume detected within these studies indicated it should be easily tolerated by any patient. When looking at these numbers, however, it is important to highlight that several issues significantly reduce the validity and comparability of these findings. Limitations regarding current publications include the pooling of different surgical techniques (47), IV or topical administration of antifibrinolytics in some of the studies (38, 67, 69, 74, 75), and the use of different methods to determine the bleeding volume (58, 67, 68, 70). Additionally, vastly differing points in time were used to measure the blood loss (67, 68, 70). This is aggravated by the fact that certain methods used in these studies to determine the bleeding volume were found to be at risk of underestimating the actual blood loss (62, 76).

1.4.1 Factors influencing blood loss in orthognathic surgery

Specific factors affecting blood loss related to orthognathic procedures have been identified. Most studies agreed that blood loss increases with surgery duration (55, 58, 70, 77). In this regard, there is a positive correlation between a prolonged operating time, increased blood loss and length of hospitalisation (55, 57). The type of treatment represents another important factor affecting orthognathic blood loss. Bimaxillary surgery is frequently associated with a significantly higher blood loss relative to other orthognathic procedures, such as LeFort-I osteotomy and BSSO (37, 41, 68). Furthermore, and as a general rule, maxillary bleeding has been found to exceed that of mandibular osteotomies (45). Stehrer et al., using feature importance to predict blood loss, suggested that bimaxillary surgery is most strongly associated with an increased perioperative blood loss in comparison to other factors examined, such as operating time, BMI and gender (68). Numerous studies have investigated the effect of age, BMI gender and the surgeon's experience on orthognathic blood loss, and controversial findings have resulted (37, 62, 69, 70). With respect to the patient's age, no effect on blood loss has been determined, except in one study (61, 69, 78). Whereas Thastum et al. observed that the relative blood loss decreased with increasing BMI (70), no such correlation has been found in other studies (79). The surgeon's skills and experience have been subject to extensive discussion. The majority of studies have not included operations performed by surgical trainees due to an increased risk of bias, as a correlation between the length of the surgery and the volume of bleeding is suspected. Rummasak et al. confirmed this hypothesis (61). In contrast, Kretschmer et al. did not find any relevant differences between consultants and surgical trainees in terms of blood loss (62). This might be because a consultant surgeon, very experienced in orthognathic surgery was present during the procedure.

Preoperative thromboelastographic analysis (TEG-analysis) has been established as a reliable predictor for blood loss in orthognathic surgery (71). Significant associations between intraoperative blood loss and specific parameters such as clot formation time, the maximum clot firmness and the alpha angle have been found in this context. Moreover, intraoperative blood loss amounting to more or less than 400ml was predicted with 95% certainty by using preoperative TEG. In addition to the preoperative analysis of specific blood parameters, machine-learning algorithms, used to predict blood loss in orthognathic surgery, have recently been investigated. Findings of this study proposed excellent accuracy of the algorithm in predicting the actual blood loss (68).

1.4.2 The influence of gender on blood loss in orthognathic surgery

Gender has been associated orthognathic blood loss on numerous occasions. Most studies did not detect a correlation between gender and blood loss (47, 56). As opposed to this, few studies suggested an association of gender with the bleeding volume. In this regard, a significantly higher amount of perioperative blood loss in men in comparison to women undergoing the same orthognathic surgical procedure was observed (38, 61, 69, 78).

One study specifically focused on gender as the primary predictor variable (69). Olsen et al. (69) investigated the influence of gender-specific differences in haemostatic parameters on intraoperative blood loss within the context of bimaxillary surgery. Significant differences between male and female subjects were observed, with men bleeding twice as much as women (mean blood loss 400ml vs 200ml) (69). It is important to note that no antifibrinolytics were administered in their study. The increased blood loss in men was explained by gender-related differences related to TEG-results and specific markers for fibrin turnover. What is more, men were found to have higher preoperative levels of haemoglobin and haematocrit; women, on the other hand, presented with significantly higher levels of Fibrinogen, D-dimer and Prothrombin fragments 1+2. Furthermore, women showed higher values within the TEG-analysis including MCF, alpha angle and CFT.

In 2017, Secher et al. (38) conducted a randomized controlled clinical trial to assess the effect of tranexamic acid (TXA) on the orthognathic blood loss with reference to patient gender. 96 patients undergoing bimaxillary surgery were included in this study and were randomly allocated to either the TXA-group or the placebo-cohort. Within the statistical analysis, results were also assessed relative to patient gender. No statistically significant differences were observed between males and females regarding the blood loss within the placebo-group, however, men were found to bleed approximately 100ml more than women. With reference to the TXA-group, women undergoing bimaxillary surgery were shown to bleed significantly less in comparison with men (367ml vs. 153ml). The authors hypothesized that female sex hormones and oral contraception were among the underlying mechanisms, responsible for greater tranexamic acid effects in women. Moenning et al. (78) reviewed the estimated blood loss in 506 patients having undergone orthognathic surgery. A number of surgical techniques were included with reference to the respective study cohort. Statistical analysis of the data revealed statistically significantly higher blood loss in men compared with women, however, vastly differing treatment modalities were pooled for this purpose. Similarly, Rummasak et al. (61) found that the male gender was associated with significantly higher blood loss with respect to bimaxillary surgery. Further analysis of their study population calculated the relative blood loss with respect to acceptable blood loss. Here,

females were found to have significantly higher relative blood loss in comparison to acceptable blood loss than their male counterparts. Thastum et al. (70) evaluated the relative blood loss associated with bimaxillary surgery, BSSO and LeFort-I osteotomies and further analysed their results according to gender. While no gender-specific differences were established regarding relative blood loss in any of the procedures, significant gender-related differences were found in terms of the absolute blood loss with reference to bimaxillary surgery.

1.4.3 Methods to measure blood loss in orthognathic surgery

Overall, a multitude of methods and formulae to measure intra- and perioperative blood loss occurring in the context of surgical procedures are available (62, 70, 80-82). None of the methods to measure bleeding have proven superior to one another and, as a result, a gold standard is yet to be defined in this regard. All of the methods on hand were shown to implicate specific advantages in terms of measuring blood loss, however, similar limitations were reported (62, 76, 80). When looking at the estimated blood loss in a single patient calculated with different formulae, occasionally vastly differing results can be found (80). Most formulae designed to calculate the amount of blood loss include prior estimation of the patient's' total blood volume. Similarly, more than one method to estimate the patients' total blood volume can be used (83). One of the most popular formulae deployed for this purpose is 'Nadler's formula' (84). Furthermore, adequate approximation of the total blood volume is accomplished by considering the patient's bodyweight and assuming a blood volume of 65ml/kg in females and 70ml/kg in males (67).

Within the field of orthognathic surgery, various tools for estimation of intra- and perioperative blood loss have been used (58, 67, 68, 70). In addition to that, irregularities, in terms of the point in time to calculate the blood loss, were observed (67, 68, 70). These variations inevitably interfere with the validity and comparability of study results. In recent studies the following methods to measure blood loss in orthognathic surgery were deployed:

Most commonly, intraoperative blood loss was calculated by subtracting the amount of intraoperative irrigation fluid used from the total volume in the suction canister at the end of the operation (35, 57, 70, 71). In doing so, information can be gathered regarding intraoperative blood loss, occurring from skin incision to wound closure. Valid points of criticism, with reference to this method, refer to additional blood loss not being registered. This is especially true for blood swallowed or absorbed by surgical gauze material. To bypass these limitations, some studies added the weighing of the throat pack and the surgical gauze to the protocol (58, 62). Still, underestimation of the actual blood volume was

suggested as a certain amount of blood is likely to accumulate in the patient's sinuses, tissue spaces and stomach, and therefore, will not be included in the equation (62, 76).

Numerous formulae, based on the patient's pre- and postoperative levels of haemoglobin and haematocrit were applied. Stehrer et al. used the 'haemoglobin balance method' in order to retrospectively determine the patient's blood loss (68, 80). This formula was previously suggested to be the most reliable method to determine perioperative blood loss occurring in total knee arthroplasty (80). No such data for orthognathic surgery currently exists. Choi et al. applied a very similar formula, based on the patient's pre- and postoperative haemoglobin values (67). Additionally, a formula relying on the patient's haematocrit levels was used in this study (82). A number of studies calculated the relative blood loss from the patient's total estimated blood volume, based on either the subtraction method or a formula (37, 57, 70). Rummasak et al. specified blood loss in relation to acceptable blood loss with reference to gender (61). Further studies examined the drop in postoperative haemoglobin and haematocrit values, and compared these to respective preoperative parameters (55, 85).

Different timings to measure blood loss in orthognathic surgery can be found. Whereas most studies used the subtraction method and therefore, measured the amount of blood loss immediately after wound closure, blood samples used for calculation of the blood volume were taken at different points in time. Choi et al. calculated the amount of blood loss based on the haemoglobin and haematocrit values determined 48h postoperatively, assuming that the amount of blood would not be subject to further dilution (67). Ueki et al. measured the blood loss directly after wound closure; however, further blood samples one and two weeks after surgery were taken (58). Stehrer et al. did not provide any information on the timing of their measurements (68).

1.4.4 Measures to reduce blood loss in orthognathic surgery

Numerous measures have been introduced over the years to reduce the amount of intraoperative and perioperative blood loss and to reduce the risk of bleeding complications and minimize the need for blood transfusions in orthognathic surgery.

Measures most commonly applied in the context of orthognathic surgery range from the use of intravenously or topically administered antifibrinolytics in addition to procoagulant substances and hypotensive anaesthesia (32, 34, 36, 86). In terms of reducing blood loss and bleeding complications, the aforementioned measures have proven effective overall. However, their potential side effects need to be carefully considered and the pros and cons need to be weighed.

In the following section, the most common measures to reduce intraoperative and perioperative bleeding in the field of orthognathic surgery will be discussed.

1.4.4.1 Hypotensive Anaesthesia

The term “hypotensive anaesthesia” describes an anaesthesiologic procedure in the context of which the intraoperative blood pressure is purposely and predictably lowered. Hence, hypotensive anaesthesia is commonly referred to as “induced hypotension”. A decreased intraoperative blood pressure aims at reducing intraoperative blood loss (32, 76, 87).

When speaking of hypotensive anaesthesia, a range of mean arterial pressure between 50-65 mm Hg has frequently been discussed (32, 87). Certainly, the patients’ constitution and usual blood pressure levels need to be considered, before reducing their blood pressure to the aforementioned range of mean arterial pressure. A common recommendation is to set a safe target value regarding induced hypotension by reducing their blood pressure by 20 to 30% of the patient’s usual blood pressure levels (88-90). A mean arterial pressure of 50 mm Hg in healthy patients, rated class I, according to the American Society of Anesthesiologists classification has been defined as the acceptable minimum mean arterial pressure (32).

The concept of hypotensive anaesthesia was first introduced, over a hundred years ago, in 1917. Its first description delineates the use of reduced intraoperative blood pressure levels for intracranial procedures. Since then, various techniques have been proposed, to effectively and safely reduce intraoperative blood pressure during surgical procedures throughout varying surgical specialties (32). With regard to maxillofacial surgery, induced hypotension was first applied by Enderby in 1950 (91). The first clinical maxillofacial trial regarding the use of hypotensive anaesthesia was published in 1976 (76). By administering sodium nitroprusside in their study, the intraoperative blood pressure was significantly lowered so that a reduction of more than 40% in intraoperative blood loss was achieved. In relation to orthognathic surgery, various studies advocate the efficacy of induced hypotension to reduce intraoperative blood loss. Additionally, studies state a relatively low risk of adverse events associated with hypotensive anaesthesia. Both a reduction in intraoperative bleeding and low risk of complications have led to hypotensive anaesthesia being considered a routine procedure in orthognathic surgery (32). This is despite the fact that the effectiveness of hypotensive anaesthesia in terms of orthognathic surgery remains controversial.

In a recent meta-analysis assessing more than 180 articles on this topic, 10 high quality randomised clinical trials were identified which investigated the efficacy of hypotensive

anaesthesia in orthognathic surgery (32). All trials were evaluated according to the quality criteria introduced by Jadad and Delphi before being included in the review.

Among the studies included, various medications were used to induce hypotension. Most commonly sodium nitroprusside (SNP) was administered (in 4/10 trials) to lower intraoperative blood pressure (90, 92-94). Other medications used were Isoflurane (95), Propanolol and a combination of Nitrogylcerin and Remifenatil or Esmolol (96, 97).

Different target values in terms of induced hypotension were set. The overall range was found to be between 50 mm Hg and 72 mm Hg (32). In most studies a mean arterial pressure between 50 mm Hg and 65 mm Hg was tried to be maintained. One study referred to the patients' systolic blood pressure to calculate the target value in terms of induced hypotension (97).

Regarding the orthognathic surgical procedures performed in the context of these studies, most frequently correction of skeletal malocclusion involved re-positioning of the upper and lower jaw in terms of bimaxillary surgery (94, 95). Other techniques assessed were mandibular osteotomy, anterior maxillary osteotomy and LeFort-I osteotomy (32).

Different methods to measure and monitor the amount of intraoperative blood loss were applied. Most commonly, the amount of irrigation fluid used was subtracted from the total amount of fluid in the suction canister at the end of the operation to calculate the intraoperative blood loss (32). Additionally, gauzes and sponges were weighed before and after surgery. The postoperative difference in weight was considered to be equivalent to additional blood loss and hence, was added to the patient's total amount of blood loss (95, 98). In one study, a formula, based on pre- and postoperative Haematocrit values, was used to calculate intraoperative bleeding (97).

All studies combined, a total number of 178 patients were found to have undergone induced hypotension in the context of orthognathic surgical procedures. When figuring up control groups in all of which normotensive anaesthesia was performed, a number of 180 patients were identified. These numbers indicate rather small sample sizes for each clinical trial. In fact, sample sizes ranged from 11 to 25 patients for intervention groups and 10 to 29 patients for respective control groups (32).

When looking at the efficacy of hypotensive anaesthesia to reduce intraoperative blood loss the following findings were observed (32).

In general, the mean amount of blood loss among the studies varied widely, for both, intervention and control groups, equally. This could be attributed to the great variability with regards to surgical techniques, operation time, the mean arterial pressure target value, differing techniques to monitor blood loss and the surgeon's experience. The most significant mean blood loss amounted to 827.62 ml for the intervention group and 965.28 ml for the control group (94). Bimaxillary surgery was exclusively performed in this study. The lowest mean amount of blood loss was found to occur in a study including patients undergoing LeFort-I osteotomy, with a blood loss of 120 ml in the intervention group and 270 ml in the control group (98). In the aforementioned studies the mean arterial pressure was found to range between 55 to 65 mm Hg and 50 to 60 mm Hg, respectively.

Despite small sample sizes and variations in study parameters and outcome measures, differences regarding intraoperative blood loss between hypotensive groups and normotensive groups were observed. By calculating the pooled estimate of effect size for the 10 randomized clinical trials reporting on this topic, a statistically significant difference favouring hypotensive anaesthesia was found. In more detail, hypotensive anaesthesia was found to reduce the amount of intraoperative blood loss by approximately 170 ml (32). On a related note, moderate heterogeneity was found among the trials. In a subgroup analysis, which considered the type of surgical procedure, interesting findings were reported. When combining three studies, including only those patients undergoing bimaxillary surgery, a total number of 101 patients were found for the intervention and control groups. Hypotensive anaesthesia was found to statistically significantly reduce the intraoperative blood loss by 175ml in comparison to normotensive anaesthesia (32). Looking at anterior maxillary osteotomies, no statistically significant differences were observed regarding intraoperative blood loss among hypotensive and normotensive anaesthesiologic protocols (90). However, a reduction in blood loss in the context of hypotensive anaesthesia was stated. 4/10 studies (188 patients) provided data on the use of intraoperative local anaesthesia (87, 92, 95, 96). Statistically significant differences between normotensive and hypotensive protocols were observed in this regard, also favouring induced hypotension by 254.93 ml (32).

In terms of the operation time needed to complete the surgical procedure, no statistically significant differences between normotensive and hypotensive anaesthesiologic protocols were found (32). In addition to the operation time and the amount of intraoperative blood loss, information on the quality of the visibility in the surgical field was provided in 6/10 trials (32). 238 patients were assessed in terms of the intraoperative visibility in the surgical field. In 4/6 trials, the scale established by Fromme et al. (94) was used to analyse the quality of the surgical field. Statistically significant advantages with regards to induced hypotensive were reported.

1.4.4.2 Antifibrinolytics

Antifibrinolytics are a class of drugs that inhibit fibrinolysis on different levels (99). Over the years, a variety of drugs have been introduced to the market, that can be categorised as being antifibrinolytic. Since their introduction, antifibrinolytics have been used throughout a wide spectrum of surgical specialties (36, 86, 99-101). At first, these medications were mainly administered to counteract and prevent bleeding complications in patients suffering from coagulopathies, such as haemophilia. Nowadays, however, antifibrinolytics are administered in various indications to minimise the amount of intraoperative and perioperative bleeding (36, 86, 99-101).

To better understand antifibrinolytics, a brief summary regarding fibrinolysis will be given in the following paragraph.

1.4.4.2.1 Fibrinolysis

During any surgical intervention blood vessels and tissues are damaged. This in turn, triggers the activation of the haemostatic cascade: contraction of the damaged blood vessels, platelet aggregation and the process of coagulation eventually lead to the formation of fibrin clots. The physiological process of fibrinolysis starts simultaneously. In the context of fibrinolysis fibrin clots are degraded. This ensures a balance between clot formation and the patency of blood vessels (99).

Plasmin has been identified as the crucial factor in the cascade of fibrinolysis and is formed through the activation of Plasminogen (99).

The Plasminogen molecule has various lysine binding sites (LBS) which enable it bind to fibrin. Activation of Plasminogen is triggered by the tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA); tPA playing the most important role in this regard. tPA is formed by endothelial cells and its secretion is increased in case of tissue damage, such as surgery. tPA binds to fibrin in a similar way to that of Plasminogen. When bound to fibrin, tPA converts Plasminogen to Plasmin and fibrinolysis subsequently commences (99).

A number of molecules are known to counteract fibrinolysis: Plasminogen activator inhibitors, α_2-macroglobulin, Plasmin inhibitor (PI) and Thrombin activatable fibrinolysis inhibitor (TAFI). Plasminogen activator inhibitors 1 and 2 (PAI-1; PAI-2) have been shown to incapacitate tPA and uPA. As a consequence, Plasminogen is not converted into Plasmin. The direct inhibitor of Plasmin is called Plasmin inhibitor. This molecule intervenes on a number of levels: 1. PI

forms a complex with Plasmin and ensures the inactivation of fibrinolysis; 2. PI prevents further Plasmin generation; 3. PI crosslinks to fibrin via Factor XIII. This prevents fibrin clots from degrading prematurely. Activated TAFI (TAFIa) has been shown to remove lysine from the fibrin clot. This leads to a reduction of Plasmin activity (99).

1.4.4.2.2 Development of antifibrinolytics

During the early 1950s, the amino acid lysine was found to stop Plasminogen activation. However, the impact of lysine on Plasminogen turned out to be too weak to allow for the desired haemostatic effects (99). Further research into lysine and lysine derivates eventually led to the discovery of more effectual substances to prevent Plasminogen activation. In the first step, Epsilon-aminocaproic acid (EACA), a synthetic derivate of lysine, was successfully introduced (99). EACA was found to be highly effective, however, large doses had to be administered. Furthermore, gastrointestinal side effects were reported on a regular basis. Consequently, an alternative drug, named trans-4-aminomethyl-cyclohexanecarboxylic acid (tranexamic acid), was introduced to the market in 1962 (99). Tranexamic acid (TXA) was found to be much more effective than EACA with fewer side effects reported. Aprotinin, a direct Plasmin inhibitor, was introduced in the 1980s and since then has courted controversy in terms of its safety (102, 103).

1.4.4.2.3 Tranexamic acid (TXA) and orthognathic surgery

Tranexamic acid (TXA) is a synthetic lysine derivate, reversibly and competitively binding to lysine binding sites on Plasminogen molecules. As a result, Plasminogen molecules are unable to bind to fibrin clots and subsequently cannot be converted to Plasmin, which is essential factor within for fibrinolysis. Hence, fibrin clots are stopped from being degraded and bleeding is decreased. Pharmacokinetic studies determined the highest plasma concentration to be approximately one hour after intravenous administration of TXA 10mg/kg bodyweight. The biological half-time of TXA was found to be around 80 minutes. TXA is metabolised renally (99).

Regarding TXA dosage, various indications for its application have to be considered. In order to reduce local fibrinolytic bleedings, an IV dose of 10mg/kg bodyweight, three to four times daily has proven sufficient. For systemic fibrinolytic bleedings an IV dose of 10 mg/kg bodyweight, six to eight times a day has been recommended. However, in massive bleedings, i.e. in the context of severe trauma a loading dose of 1g IV, independent from the patient's bodyweight has been advocated. Despite these recommendations, uncertainty remains in terms of IV TXA (99).

The clinical effectiveness of TXA has been confirmed numerous clinical studies (34-36, 101). In a recent review of 104 clinical trials, it was shown that TXA reduced intraoperative bleeding by about one third throughout various surgical specialties and interventions (101). Additionally, it was found that TXA decreased the number of blood transfusions in surgery(101). Most commonly, TXA has been applied in the context of cardiac surgery, orthopaedic surgery, obstetrics and gynaecology, urologic surgery and head and neck surgery (101). Moreover, it has been introduced to the field of orthognathic surgery. Comparable results, in terms of reducing intraoperative blood loss, were reported. However, the number of high-quality randomised clinical trials was fairly small (34-36). To date only a small number of clinical trials with a reasonably low risk of bias have reported on the application of TXA in orthognathic surgery (34-36).

A recent meta-analysis reviewing bleeding in orthognathic surgery included six randomised clinical trials on TXA (35). Another topical meta-analysis, published in 2019, specifically reviewed the effect of TXA on blood loss in orthognathic surgery (34). The same six TXA studies, conducted between 2009 and 2015, were included in this meta-analysis (34). Overall, 288 patients were treated in these studies: 146 were assigned to the intervention groups; 142 were assigned to respective control groups. In four out of the six trials focusing on TXA in the field of orthognathic surgery, TXA was applied intravenously. Regarding intravenous dosage protocols, noticeable differences were observed: in two of the studies TXA 10mg/ kg bodyweight were administered preoperatively (104, 105); compared to TXA 20mg/ kg bodyweight in the remaining two studies (67, 106).

In two of the trials TXA was applied topically (74, 75). This was done by mixing TXA with the irrigation fluid used during surgery. One trial used a 1% TXA irrigation fluid (74); the other used a 0.05% TXA irrigation fluid (75). In terms of the surgical techniques examined, bimaxillary osteotomies were the preferred procedure in 4/6 studies (67, 74, 75, 106); one study focused on LeFort-I osteotomies solely (104); another study, treating 50 consecutive patients undergoing orthognathic surgery, included a wide variety of orthognathic surgical techniques (105).

With regards to intraoperative bleeding (IOB) significant reduction in blood loss was observed in the TXA group compared to the control group (34).

Looking at the IV TXA studies included in the meta-analysis in more detail, reduction of blood loss between 26% and 45% was observed in comparison to the control groups (67, 104-106). The examined study cohort treated with IV TXA consisted of 196 patients in total (98 in the intervention group and 94 in the control group). When using the random-effects model for the aforementioned studies, a reduction in blood loss of 168ml was found (34).

Two more recent randomised controlled clinical trial were added in a review, published in 2019 (36). In 2017, Secher et al. (38) published a trial examining the effect of preoperatively intravenously administered 1g of TXA on intraoperative blood loss. Moreover, the study group analysed gender-specific outcomes in terms of blood loss and TXA. 96 patients male and female patients, diagnosed with skeletal malocclusion were included in the study. 51 patients (26 males; 25 females) were assigned to the intervention group; 45 patients (22 males; 23 females) were allocated to the control group. All patients underwent bimaxillary surgery for the correction of the skeletal malocclusion. A significant reduction of 128 m in intraoperative blood loss was found, when comparing the intervention to the control group. With regards to gender-specific differences, the following findings were reported: 1. In the TXA group significant differences between male and female participants were found, with female patients bleeding significantly less; 2. Regarding female patients, statistically significant differences between the TXA group and the control group were reported. TXA was found to significantly reduce intraoperative bleeding in women compared to the female control group; 3. No statistically significant difference among men in the intervention and the control group were found.

Apiban et al. (107) analysed three different dosage regimens regarding the intravenous application of TXA in terms of its efficacy to reduce the amount of intraoperative bleeding. 80 patients underwent bimaxillary surgery. The subjects included were randomly assigned to one of four groups. Group 1 received a placebo; Group 2 was administered 10mg/kg TXA IV; Group 3 was administered 15mg/kg TXA IV and Group 4 was administered 20mg/kg TXA IV. Statistically significant differences between the groups, using the one-way ANOVA analysis were determined. Statistically significant differences were found among Group 0 and the interventions groups. However, no statistically significant differences were noted among any of the intervention groups.

Two studies reported topical application of TXA during bimaxillary surgery. Reduction in blood loss of 198 ml was found. Whereas Kaewpradub et al. (75) did not find any statistically significant effect of topically applied TXA on blood loss, Eftekharian et al. (74) concluded that topically administered TXA as 1% irrigation fluid statistically significantly reduced the intraoperative blood loss by 29%.

1.4.4.2.4 ε-aminocaproic acid (EACA)

The lysine derivate ε-aminocaproic acid has not been used in the context of orthognathic surgery in recent years. However, its administration within the related field of craniofacial surgery has been reported on a regular basis. Hsu et al. retrospectively reported on the

efficacy of EACA in craniofacial surgical procedures to correct craniosynostosis (108). Thompson and Reddy examined the administration of aminocaproic acid in the context of reconstructive craniofacial procedures (86, 109).

1.4.4.2.5 Aprotinin

Aprotinin is a serine protease inhibitor, directly inhibiting Plasmin. In a study conducted in 2008, the use of Aprotinin in paediatric cardiac surgery turned out to be problematic, hence, its administration has, since then, been considered dangerous for various reasons (102). One randomised controlled clinical trial investigated the use of aprotinin in the context of bimaxillary surgery (72). The study cohort consisted of 29 patients, randomly allocated to either the intervention or control group. In the intervention group, 70mg Aprotinin was administered intravenously before surgery; the control group received IV saline as the placebo. Intraoperative blood loss was reduced by 52%.

1.4.4.2.6 Yunnan Baiyao

Yunnan Baiyao is a Chinese herbal medicine. Tang et al. published a randomised controlled clinical trial in 2009, reporting the use of Yunnan Baiyao capsules for the prevention of blood loss in orthognathic surgery (110). 87 patients underwent bimaxillary surgery in the study. It was shown that preoperative administration of Yunnan Baiyao capsules reduced intraoperative bleeding by 21%. However, it has to be highlighted that Yunnan Baiyao is – similar to other herbal medicines – used off-label and its dose and active ingredients cannot be determined accurately when administered.

1.5 Justification of the research question

Excessive blood loss and associated sequelae remain major concerns within the field of orthognathic surgery. Commonly encountered side-effects with reference to major bleeding in orthognathic surgery are admission to ICU, compromise of the airway, frequent need for blood transfusion, prolonged inpatient stay and postoperative quality of life impairment (28, 45, 111). Orthognathic surgery is mainly categorised as being an elective intervention. This implies that high treatment standards including minimal blood loss are considered indispensable.

A number of studies focused on the average blood loss expected in orthognathic surgery (35, 47). In this context, largely differing results in terms of the bleeding volumes detected were observed. This can partly be explained by different treatment modalities deployed within study populations and the pooling of varying surgical techniques for statistical analysis. In addition, a wide variety of methods were used to determine the bleeding volume (62, 67, 68, 70). This was aggravated by the fact that blood loss was calculated at different points in time (67, 68, 70). All of the aforementioned irregularities in terms of the study design inevitably led to a drastic impairment of the comparability of study results. The method most commonly applied to measure blood loss in orthognathic surgery is based on the subtraction of the irrigation fluid from the total amount of fluid in the suction canister (35, 57, 70, 71). This method has frequently been subject to criticism due to an elevated risk of underestimation with respect to the bleeding volume (62, 76). Numerous formulae exist to calculate blood loss on the basis of pre- and postoperative haemoglobin levels (80, 81). These formulae are thought to better incorporate undetected blood loss, however, they are based on the estimated patient blood volume. The ‘haemoglobin balance method’ has recently been established as a reliable formula to determine blood loss (80). Standardised comparisons between the subtraction method and the ‘haemoglobin balance method’ on the basis of a homogenous study cohort would prove beneficial to gather relevant information on the amount of ‘hidden blood loss’ (112).

Perioperative measures, such as the use of antifibrinolytics, have been introduced to reduce blood loss in orthognathic surgery (34, 36, 86). These measures have proven effective in reducing bleeding, yet, they involve additional risks for the patient which have to be considered. In recent years, bleeding reductive measures have been promoted vigorously. However, a well-targeted use in (1) patients undergoing orthognathic surgical procedures

associated with high blood loss and (2) patients with preoperatively identified predictive factors indicating a higher bleeding volume, would be more appropriate. This would allow to decrease the bleeding volume where needed while simultaneously minimising the risk of adverse events related to the application of bleeding reductive measures. A number of patient specific parameters were shown to affect the amount of blood loss in orthognathic surgery (37, 69, 71). Males undergoing orthognathic surgery have more frequently been associated with a higher bleeding volume than females undergoing the same orthognathic surgical procedure (61, 69). Underlying mechanisms accountable for these differences remain vastly elusive. Research into predictive factors and algorithms in terms of blood loss in orthognathic surgery have been conducted to help identify patients at risk (68, 69, 71). However, more well-structured and prospectively conducted studies are needed to better understand causalities leading to significant differences in terms of the bleeding volume.

1.6 Aims of the dissertation

The primary aim of this prospective observational study is to monitor intra- and perioperative bleeding related to standardised orthognathic procedures in a homogeneous study population with particular emphasis on analysing gender-specific differences.

The secondary aims of this study are as follows: to identify significant differences in terms of the haemostatic profile among males and females and to further correlate relevant haemostatic parameters with the amount of blood loss determined. Hence, gender-related peculiarities in a patient's haemostatic profile and associated underlying mechanisms, which significantly affect the bleeding volume in orthognathic surgery, may be identified and may further be used as predictive factors for the amount of blood loss to be expected.

Furthermore, information will be gathered on the amount of 'hidden blood loss' with respect to orthognathic surgery by calculating the bleeding volume immediately after surgery, as well as 24h and 48h postoperatively. Differences relating to 'hidden blood loss' in terms of gender, will be assessed in detail.

For this purpose, the following null hypothesis and respective alternative hypotheses have been identified:

1.7 Hypotheses

Null-hypothesis (H_0)

There is **no** statistically significant gender-specific difference regarding the bleeding volume in male and female patients undergoing orthognathic surgical procedures.

Alternative hypotheses

H_1

There is a statistically significant gender-specific difference regarding the bleeding volume in male and female patients, undergoing orthognathic surgical procedures.

H_2

There is a statistically significant difference between bimaxillary surgery and bilateral sagittal split osteotomy, regarding intraoperative and perioperative bleeding.

H_3

There is a statistically significant gender-specific difference regarding preoperatively determined levels of endogenous thrombin potential (ETP).

H_4

Preoperatively measured endogenous thrombin potential (ETP) is a reliable predictor for the amount of intraoperative and perioperative blood loss in male and female patients undergoing orthognathic surgery.

H_5

There is a statistically significant gender-specific difference regarding the preoperative coagulation profile.

H_6

Gender-related differences in terms of the coagulation profile affect the amount of intraoperative and perioperative blood loss in male and female patients undergoing orthognathic surgery.

H_7

There is a statistically significant gender-specific difference regarding the ‘hidden blood loss’ in male and female patients undergoing orthognathic surgical procedures (HBL-48h).

H_8

There is a statistically significant gender-specific difference regarding relative blood loss in male and female patients, undergoing orthognathic surgical procedures (RBL-IOB; RBL-24h; RBL-48h).

H_9

The length of the operation correlates with the amount of blood loss (IOB; CBL-48h).

H_{10}

Specific patient characteristics such as BMI and age do not correlate with the bleeding volume.

1.8 Novelty value

Novelty values with regards to this prospective observational study refer to:

(1) *standardised monitoring of the bleeding volume in orthognathic surgery, focusing on gender related differences, on the one hand, and on determining the hidden blood loss, on the other hand.*

The bleeding volume in male and female patients undergoing bimaxillary surgery or bilateral sagittal split osteotomy will be assessed. Two state-of-the-art methods to measure blood loss will be deployed to gather relevant information about the intra- and perioperative blood loss, occurring up to 48-hours postoperatively (68, 70). This will allow to highlight gender-specific differences in terms of the bleeding volume and to point out differences with regards to the amount of blood loss occurring after wound closure. This specific study design and respective outcome measures are novel and unprecedented in the context of orthognathic surgery. The hidden blood loss in orthognathic surgery and related gender-specific aspects have not been investigated in orthognathic surgery, so far.

(2) *preoperative analysis of the endogenous thrombin potential and the specific coagulation profile.*

Within the field of orthognathic surgery, specific factors and patient characteristics have been shown to have an effect on the amount of blood loss (37, 69, 71). In this regard, male gender was frequently associated with a higher bleeding volume relative to female gender (35, 38). However, underlying mechanisms remain largely unclear. We suggest that by identifying significant gender-related differences regarding the 'endogenous thrombin potential', Fibrinogen, Antithrombin III, von Willebrand-factor, as well as the specific coagulation factors VIII, IX, XI and XIII', causalities and reliable predictive factors in terms of the blood loss in orthognathic surgery can be established. These parameters have not been investigated in the context of orthognathic surgery.

(3) *comparison of the total amount of blood loss with the relative blood loss with special attention on gender-specific differences.*

Studies have focused either on the total amount of blood loss or the relative blood loss. Comparison of these two modalities with reference to gender has not been performed.

1.9 Limitations of the research topic

Limitations of this research topic primarily concern the chance of inaccuracies in terms of blood loss calculation. Blood loss calculation by means of a formula is based on the patients' estimated total blood volume as well as pre- and postoperative values of haemoglobin or haematocrit (80, 84).

Preoperative dehydration due to fasting may potentially falsify preoperative haemoglobin and haematocrit values. In addition to that, temporary blood dilution triggered by intravenous administration of large amounts of fluids may alter relevant blood parameters postoperatively.

However, we believe that this risk can be considered very low in our study. This is as: (1) preoperative blood samples were taken on the day before surgery, at which point patients did not need to be fasted; (2) IV fluids were mainly administered intraoperatively and within the immediate postoperative period (6-8 hours postoperatively); and, most importantly, (3) appropriate time points were chosen (24-hours and 48-hours postoperatively) to calculate the bleeding volumes, where normalised blood volumes are already to be expected.

Additional points of criticism related to this study may refer to calculated blood loss relying on estimation of the patient's total blood volume. No gold standard in terms of the formula to be used to most accurately estimate the patient's total blood volume exists (83). In this study, Nadler's formula was applied (84). This approach has already been used in other studies assessing blood loss in orthognathic surgery and takes the patient's body weight, height and gender into account (68). To investigate the influence of differing formulae on the patient's estimated blood volume and the blood loss determined, Lopez-Picado et al. compared three commonly used approaches (83). Moore's formula, Nadler's formula and ICSH-formula were set in comparison for this purpose. No significant differences with reference to the estimated total blood volumes and more importantly the blood loss determined were detected in this regard. These findings suggest that the choice of method to estimate the patient's total blood volume does not alter overall results and that Nadler's formula represents a valid option in this context.

2. MATERIAL AND METHODS

2.1 Subjects

2.1.1 Inclusion criteria

Gender

Male and female patients diagnosed with skeletal malocclusion that required surgical correction in terms of orthognathic surgery, were found eligible for inclusion in this prospective observational study.

Age

Patients, aged between 18 and 60 years, were considered.

Skeletal malocclusion

Skeletal class II malocclusion, skeletal class III malocclusion, anterior open bite malocclusion as well as cases of facial asymmetry qualified for this study.

Surgical procedures

As orthognathic procedures of interest, mono-maxillary and bi-maxillary surgical techniques were defined. In terms of the mono-maxillary approach, only mandibular osteotomies were considered. In this regard, solely bilateral sagittal split osteotomies (BSSO), according to the technique introduced by Hunsuck and Epker were included in the study (16, 17). Regarding bimaxillary surgical techniques, the combination of LeFort-I osteotomy, as decribed by Bell et al. (113) in the upper jaw and BSSO in the lower jaw were considered relevant. Additionally, genioplasties in combination with the aforementioned techniques were included. Patients needed to be scheduled for orthognathic surgery, including a detailed treatment plan, in order to be screened for the study.

ASA status

Patients, with a defined ASA status of ASA 1 or ASA 2, determined one week before surgery, were enrolled in the study.

Informed Consent

Written informed consent needed to be given by the patient as an obligatory requirement, in order to be enrolled in the study.

2.1.2 Exclusion criteria

Simultaneous surgical procedures

Patients, having more than one surgical procedure done in the same operative session, were not included in the study. This especially concerned patients, undergoing rhinoplasty surgery at the same time as mandibular orthognathic surgery or patients undergoing simultaneous dental implant surgery. In addition to that, patients undergoing bimaxillary surgery, who required sectioned LeFort-I osteotomy to match the mandible, were excluded. This is because (1) sectioning of the maxilla is rarely needed and (2) is likely to increase the length of the procedure and associated blood loss and, hence, would have interfered with the homogeneity of our study population.

Complex orthognathic cases

Patients with severe craniofacial deformities, requiring complex correction of the facial skeleton, such as LeFort-II or LeFort-III osteotomies were excluded from the study cohort. Moreover, subjects with a history of cleft lip and palate were not considered eligible for the current study, due to excessive scarring, increased difficulty to mobilise the upper jaw and reduced blood supply in the area of the cleft.

Medical conditions

Patients suffering from the following medical conditions were excluded from the study:

- Diabetes mellitus type I and II
- Coagulopathies, including thrombophilia and haemophilia
- Connective tissue disorders (i.e. scleroderma)
- Pregnancy
- Malignant tumour diseases

Intake of medications

Patients taking anticoagulant medication were not enrolled in this study.

This included the following groups of medication:

- Vitamin K antagonists (i.e. Marcoumar)
- Antiplatelet drugs (i.e. Clopidogrel, Acetyl salicylic acid)
- NOACs (Apixaban, Dabigatran, Edoxaban, Rivaroxaban)

- Heparinoids

Intake of substances with pro- or anticoagulant side-effects

The oral intake of the following substances of up to 10 days before surgery were considered an exclusion criterion.

- Omega 3 fatty acids
- Ginseng
- Garlic
- Ginkgo biloba

Surgical procedures

Patients undergoing

- Segmental osteotomies as a stand-alone procedure or in combination with other orthognathic procedures, such as bimaxillary surgery or mandibular osteotomy in terms of a BSSO,
- LeFort-I osteotomy, as a stand-alone procedure,
- LeFort II osteotomy,
- LeFort III osteotomy
- Surgically assisted rapid palatal expansion (SARPE)
- or Quadrangular Lefort I osteotomy, were not found eligible for inclusion in this study.

ASA status

Patients with a defined ASA status of ASA 3 or ASA 4 were not considered suitable for inclusion in this study.

Informed Consent

Patients who were unable to give consent were excluded from the study cohort.

2.2 Interdisciplinary orthognathic pathway

Correction of skeletal malocclusion involved an interdisciplinary approach, consisting of (1) orthodontic pre-treatment for a period of 12 to 18 months; (2) the orthognathic surgical intervention and (3) post-operative orthodontic treatment for fine adjustment of dental relationships. Before the interdisciplinary treatment was started, patients were referred to the outpatient clinic for dysgnathia and skeletal deformity to be clinically examined by a senior orthognathic surgeon. The decision that orthognathic surgery was required, was based on orthodontic, aesthetic and functional issues in conjunction with the patients' wishes and expectations. The decision which orthognathic surgical procedure to be applied to best address skeletal malocclusion in each individual case was made by a senior orthognathic surgeon. When teeth and dental arches were aligned accurately to allow for surgical correction of the skeletal malocclusion patients were scheduled for surgery. One week before the planned surgery, patients were asked to attend a mandatory preliminary anaesthesiologic examination, in which context the patients' ASA status was determined.

On the same day, a clinical orthognathic examination was performed and dental impressions, checkbites and x-rays were taken. Detailed pre-surgical planning was performed, including model surgery and 2d-analysis of lateral cephalograms, using the analysis by Schwarz (114). In cases of severe asymmetry, 3d-planning with the aid of the IPS case designer (KLS Martin) was performed.

Patients undergoing orthognathic surgery were admitted to hospital on the day before the surgical intervention (admission time: 07.30; 24 hours before surgery).

2.3 Perioperative surgical and anaesthesiolgic protocol

All surgical interventions followed a standardised surgical and anaesthesiologic protocol.

2.3.1 Anaesthesiologic protocol

Patients needed to be fasted for a minimum of 8 hours before surgery. 60 minutes before the start of the surgical procedure 7.5mg of Midazolam were applied orally. At induction, antibiotics were administered intravenously, in terms of a single shot antibiosis. Routinely, Amoxicillin/clavulanic acid 2.2g IV were given. In cases of an established allergy to penicillin, Clindamycin 600mg IV was used.

A total intravenous anaesthesia (TIVA) was performed, including the following medications:

- Rocuronium bromide IV; 0.5 mg per kg bodyweight at induction
- Fentanyl IV or Remifentanil IV
- Propofol IV

All patients underwent nasal intubation. A throat pack was inserted after successful intubation was performed. Patients were positioned supine with no head-up tilt.

Throughout the whole procedure, controlled hypotensive anaesthesia, was maintained. A mean blood pressure of 60 mm/Hg was set as target value.

During the procedure the following medications were routinely administered:

- Fortecortin 0.5 – 1.0 mg per kg bodyweight IV
- Piritramid 7.5 mg IV
- Neodolpasse 250ml (Diclofenac and Ophenadrine) IV
- Metamizole 1g IV
- Elomel 500ml – to maintain adequate blood pressure levels

2.3.2 Surgical Protocol

Within this prospective observational study, the following orthognathic surgical interventions were performed:

1. Bilateral sagittal split osteotomy, according to the technique modified by Hunsuck and Epker (BSSO) (16, 17).

2. Bimaxillary surgery, comprising of a LeFort-I osteotomy according to Bell-technique (113) in combination with a BSSO.

At the beginning of each surgical intervention, chlorhexidine mouthwash (CHX 0.2%) was applied for 30 seconds to reduce the number of intraoral bacteria. Consecutively, local anaesthesia was administered to the operation field. For this purpose, 10 to 15ml of Xylocaine 2% with 1:200.000 adrenaline, were used. Three to five minutes after the application of local anaesthesia, mucosal incisions were performed.

2.3.2.1 Bilateral sagittal split osteotomy (BSSO)

With regard to the BSSO, two intraoral vestibular mucosal incisions were made in the retromolar area, in order to gain access to the underlying bone. Subperiosteal buccal mobilisation extending from the premolars to the ascendant mandibular ramus was performed. Lingually, subperiosteal mobilisation was continued towards the distal edge of the ascendant mandibular ramus. Subsequently, designated orthognathic retractors were inserted buccally and lingually to protect surrounding tissues including the mandibular nerve bundle. Osteotomies were performed, in accordance with the osteotomy lines, described by Hunsuck and Epker (16, 17). A jigsaw, an oscillating saw and a pear-shaped bur were used for this purpose.

First, a vertical mono-cortical osteotomy line on the buccal side of mandible in the area of the last molar was performed. Osteotomies proceeded with a crestal sagittal incision towards the ascendant mandibular ramus. Next, a horizontal mono-cortical osteotomy on the lingual aspect of the ascendant mandibular ramus was made, using bur and jigsaw. Finally, lines were joined up. Mandibular splitting was implemented using osteotomes. After successful bilateral mandibular split, the operation splint defining the new position of the mandible was inserted.

Consecutively, mandibulomaxillary fixation with the aid of dental wires was performed, to secure the new mandibular position. Once, mandibulomaxillary was implemented a 3mm-long extraoral incision near the mandibular angle was performed, to allow for insertion of the trans-buccal retractor. When the osteotomised mandibular segments where aligned in the correct position, osteosynthesis, using bi-cortical screws was performed. On each side of the mandible three screws were inserted. After adequate osteosynthesis, the mandibulomaxillary fixation was removed and the occlusion was checked.

Before wound closure, two pieces of oxygenated regenerated cellulose were (Tabotamp) inserted subperiosteally into the operation field. Wound closure was performed using Vicryl 4.0.

2.3.2.2 Bimaxillary osteotomy

For bimaxillary orthognathic surgery, LeFort-I osteotomy and BSSO were combined.

In a first step, BSSO was realised. This was done in accordance with the aforementioned technique (see section “BSSO”) (16, 17). Osteotomies were prepared; however, mandibular splitting was postponed until after the LeFort-I osteotomy was completed. This was done in order to use the mandible as a reference to define the new position of the maxilla.

In a second step, the LeFort-I osteotomy was initiated (113). At the beginning of this process, midfacial measures, using a caliper were performed to define the vertical position of the maxilla. Vestibular mucosal incision extending from left first molar to right first molar was performed. Careful subperiosteal mobilisation of surrounding tissues and the nasal mucosa ensured access to the maxillary region of interest. Two retractors were put next to the pterygomaxillary junction to protect the soft tissues; a raspatory was used to preserve the nasal mucosa. Osteotomies were conducted, using an oscillating saw, approximately 10mm above the roots of the teeth. A jigsaw was used to detach the maxilla posteriorly, in vicinity of the pterygoid. Nasal and pterygoid osteotomes ensured completion of the bony maxillary detachment. Now, maxillary down fracture was performed. Subsequently, the maxilla was adequately mobilised. The first operation splint, defining the new position of the maxilla was inserted and mandibulomaxillary fixation was realised by using dental wires. Bone irregularities and discrepancies, which complicated positioning of the maxilla in the planned position were removed. Four L-shaped miniplates (orthognathics, Medartis AG), were used for midfacial osteosynthesis. After osteosynthesis had been performed, the mandibulo-maxillary fixation, as well as the first operation splint were removed.

Now, the mandibular split was completed and the second operation splint, defining the new position of the mandible was inserted. Mandibular positioning and osteosynthesis were continued according to the protocol for BSSO. Before wound closure, four pieces of oxygenated regenerated cellulose were inserted into maxillary and mandibular operation areas. Wound closure was performed, using Vicryl 4.0.

2.3.3 Postoperative protocol

Immediately postoperatively a head bandage was applied to gently compress the cheeks and prevent swelling.

After the procedure the patients were brought to recovery. In recovery, patients were nursed at 30° degrees.

Postoperatively, pain was standardly controlled using Ibuprofen 600mg 2x1 IV and Metamizole 1g 3x1 IV. Paracetamol 1g up to 4x1 IV and Piritramid 7.5mg up to 4x1 IV were additionally administered, if required. On the second postoperative day all IV medications were stopped. Pain control was continued using oral pain medication.

2.4 Study design and sample

2.4.1 Study design

The current study was conducted prospectively, as a gender-related observational cohort study. Male and female patients with skeletal malocclusion, aged between 18 and 60 years and scheduled for orthognathic surgery, were found eligible for inclusion in this study.

The orthognathic procedures performed were bimaxillary surgery (113) and mono-maxillary surgery in terms of a bilateral sagittal split osteotomy (BSSO) (16, 17).

Subjects were split into two groups, according to the surgical procedure applied. The first group comprised of male and female patients undergoing bimaxillary surgery. The second group consisted of male and female patients in whom mandibular orthognathic surgery (BSSO) was performed.

2.4.2 Sample Size

Before conducting the study, a sample size calculation was performed. Within the sample size calculation, a number of 80 patients was defined as the required sample size, in order to allow for statistically profound statements. For each group (Group 1, bimaxillary surgery; Group 2, BSSO), 40 patients were envisaged. The sample size calculation was based on the following statistical assumptions:

A sample size of 37 in each group will have 80% power to detect a difference in means of -100,000 (the difference between a Group 1 mean, m1, of 400ml and a Group 2 mean, m2, of 500ml) assuming that the common standard deviation is 150 using a two-group t-test with a 0,05 two-sided significance level. (Alpha: 5%; Power: 80; Drop-out rate <5%.) Sample size calculation was performed by Ms Irene Mischak Dipl. Ing., employed as a statistician by the University Clinic of Dentistry and Oral Health.

2.4.3 Ethical approval

The outlined project was submitted to the ethical committee at the Medical University of Graz and was approved in terms of study design, sample size calculation and required resources (EK 31-161 ex18/19).

2.4.4 Methodology

2.4.4.1 Admission to the hospital

Patients undergoing orthognathic surgery were admitted to hospital on the day before the surgical intervention (admission time: 07.30 a.m.).

2.4.4.2 Screening

On the day before surgery, screening with regards to this prospective observational study was performed. This was solely done by the PhD student (MS). For this purpose, the patients' medical notes were screened, to check for eligibility for inclusion in this study, according to the set inclusion and exclusion criteria (see section inclusion and exclusion criteria). Additionally, a preoperative medical discussion, taking up to 20 minutes, was conducted reviewing results of the preliminary anaesthesiologic examination and specifically asking about medical conditions, such as coagulopathies, and intake of medications and oral contraceptives.

Detailed information about the purpose and the schedule of the planned observational study were given. Written informed consent was taken, if the patient agreed to participate in this observational study.

2.4.4.3 Active study phase

The study was conducted within the daily clinical routine. No additional medical interventions besides the routine orthognathic protocol were performed.

The active study phase extended over a period of 4 days.

2.4.4.3.1 Day 1

Preoperative blood sample

A preoperative blood sample was taken on the day before surgery. Two sodium citrate tubes, one EDTA tube and one lithium citrate tube blood were used. The blood sample amounted to 14.5mls of blood taken.

The blood tubes were sent to the medical laboratory for analysis of relevant blood parameters. One of the natrium citrate tubes was put aside and stored in the freezer for later analysis of the endogenous thrombin potential (ETP).

The following relevant blood parameters were analysed (Figure 1):

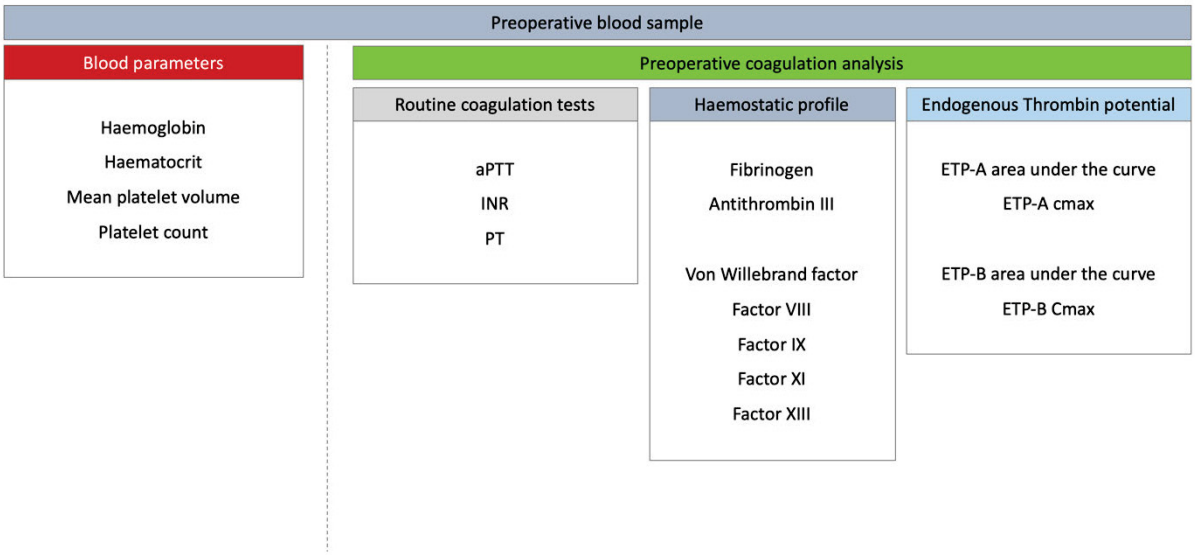

Figure 1: Figure to show relevant blood and haemostatic parameters determined on the day prior to surgery (Preoperative blood sample)

BMI

The patient's height, weight and body mass index were recorded on the day before surgery.

2.4.4.3.2 Day 2:

Surgical Intervention

The surgical intervention consisted of either a bimaxillary surgery or a BSSO (see section Surgical protocol). The intraoperatively occurring amount of blood loss was monitored throughout the procedure by subtracting the amount of irrigation fluid used from the total amount of fluid in the suction canister, at the end of the operation. Furthermore, the pre- to postoperatively measured weight differences of the surgical gauze and throat pack used were added to the amount of blood loss determined.

Operation time and the need for blood transfusion were also recorded.

2.4.4.3.3 Day 3:

Postoperative Blood sample – taken 24-hours postoperatively

On the first postoperative day, the first postoperative blood sample was taken. Three blood tubes were used (Sodium citrate tube, EDTA tube and Lithium citrate tube). A total amount of 11.5ml were taken.

Amid routinely measured blood parameters haemoglobin levels were recorded. Haemoglobin was used to calculate the amount of the perioperative blood loss (CBL-24h)

2.4.4.3.4 Day 4:

Postoperative Blood sample – taken 48-hours postoperatively

On the second postoperative day, another blood sample was taken from the patients, similar to the previous day (Three blood tubes, 11.5ml).

Haemoglobin levels determined 48-hours postoperatively were used to calculate the amount of the perioperative blood loss occurring up to 48-hours postoperatively (CBL-48h).

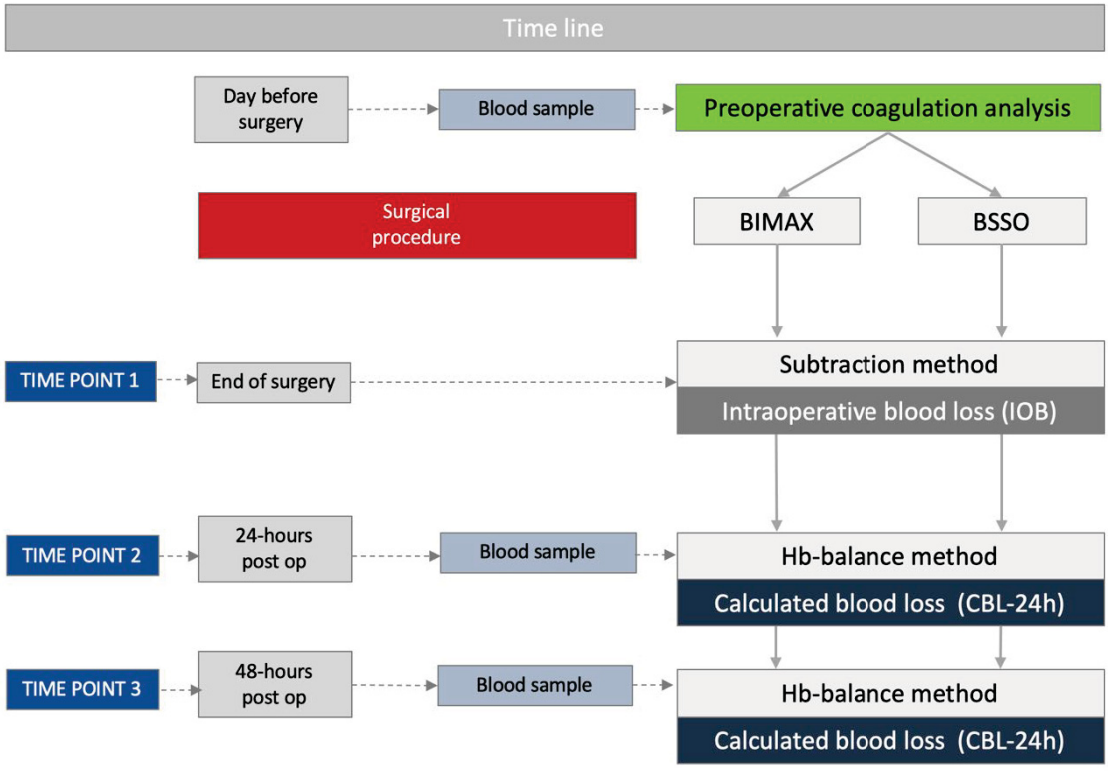

Figure 2: Analysis of blood loss respective to the three time points and the two formulae used in the current study (58, 80).

2.5 Study variables

2.5.1 Primary outcome measure

2.5.1.1 Intraoperative blood loss (IOB) in ml

The intraoperatively occurring blood loss, measured in ml, was defined as the primary outcome measure. In order to determine the intraoperative blood loss (IOB), the blood loss from mucosal incision to last suture was monitored (Figure 2).

For this purpose, the total amount of fluid in the suction canister at the end of the operation was measured. By subtracting the amount of irrigation fluid used within the operation, the amount of fluid remaining in the suction canister was assumed to be equivalent to the patient's blood loss. Additionally, the weight difference of the throat pack and the surgical gauze used were added to the blood loss determined. This was done in accordance with the measuring protocol as described by Ueki and Kretschmer (58, 62).

2.5.2 Secondary outcome measures

2.5.2.1 Calculated blood loss (CBL)

The perioperative blood loss, incorporating visible intraoperative bleeding volumes (IOB) as well as the blood occurring after wound closure, was calculated based on pre- and postoperatively measured haemoglobin levels. This was done by means of the 'haemoglobin balance method' (Equation 1), (80). With the aid of this formula, information about the peri-operative blood loss, measured in ml, was gathered.

The aforementioned formula was used for both, 24-hours and 48-hours postoperatively measured haemoglobin values to calculate the amount of the peri-operative blood loss 24-hours and 48-hours after surgery (Figure 2).

Calculated blood loss (ml)		
Haemoglobin balance method (80)	Index	
$Hb_{loss\ total} = EBV \times (Hb_{pre} - Hb_{post}) \times 0.001 + Hb_t$	EBV – patient's estimated blood volume	ml
	Hb_{pre}- levels of haemoglobin before surgery	g/L
$CBL = 1000 \times (Hb_{loss\ total}/Hb_{pre})$	Hb_{post} – levels of haemoglobin after surgery	g/L
	Hb_t – total volume of blood transfusion	g/L
	Hb_t – 1 Unit banked blood	52g (±5.4)
	$Hb_{loss\ total}$ – loss volume of haemoglobin	g
	CBL – calculated blood loss	ml

Equation 1: The 'haemoglobin balance method' was used to calculate perioperative blood loss 24- and 48-hours postoperatively (80)

The following calculations regarding the 'CBL' were performed:

- **CBL 24h** – based on Haemoglobin values, measured 24-hours postoperatively
- **CBL 48h** – based on Haemoglobin levels, determined 48-hours postoperatively

The 'haemoglobin balance method' requires estimation of the patient's total blood volume prior to surgery (EBV) as to reliably and accurately calculate bleeding volumes. In order to determine EBV Nadler's formula was applied in the present study (84).
Nadler's formula takes into account the patient's height, weight and gender and has been recently determined a valid and accurate option to estimate the patient's total blood volume (Equation 2) (83). In addition to that it has already been used to assess blood loss associated with orthognathic surgery (68).

Estimation of the patient's blood volume	
Nadler's formula (84)	Index
Male $EBV = 0.3669 \times H^3 + 0.03219 \times W + 0.6041$ Female $EBV = 0.3561 \times H^3 + 0.03308 \times W + 0.183$	EBV – estimated blood volume ml H – bodyheight m W- bodyweight kg

Equation 2: Nadler's formula, used to estimate a patient's total blood volume prior to surgery (84)

2.5.2.2 Hidden blood loss (HBL)

The parameter "hidden blood loss" incorporates the blood loss remaining undetected during the procedure, as well as the blood loss occurring after wound closure has been performed (115-117).

In patients undergoing orthognathic surgery in our unit, routinely no postoperative surgical drains are inserted, however, a certain amount of bleeding into surrounding tissues is likely to occur. For this purpose, haemoglobin-values and haematocrit -values are routinely being monitored for 48-hours postoperatively.

In order to determine the amount of the hidden blood loss associated with the orthognathic surgical interventions performed in our study the following calculations were made:

1. The intraoperative blood loss was determined (IOB); 2. the perioperative blood loss was calculated 48h after surgery (see previous section 'calculated blood loss' CBL-48h) and 3. the visible intraoperative bleeding volume (IOB) was subtracted from the calculated blood loss (CBL-48h) allowing for estimation of the hidden blood loss (Equation 3) (115).

Hidden blood loss (ml)		
	Index	
HBL= CBL-48h – IOB	HBL – hidden blood loss	ml
	CBL-48h – calculated blood loss, 48h-postoperatively	ml
	IOB – intraoperative blood loss	ml

Equation 3: Hidden blood loss, defined as the amount of blood loss remaining undetected together with the perioperative blood loss occurring after wound closure (115)

2.5.2.3 Relative blood loss (RBL)

The 'relative blood loss' indicates the percentage of blood loss occurring, relatively to the patients' total estimated blood volume (EBV). This is especially relevant as significant differences between the EBV in male and female gender are to be expected.
The 'relative blood loss' (RBL) was calculated for all three time points measuring the patients' blood loss (IOB; CBL-24h; CBL-48h).

The following formula was used (Equation 4) (57, 70):

Relative blood loss (%)		
	Index	
RBL= (Blood loss*/EBV) x 100	RBL – relative blood loss	%
	EBV – Estimated blood volume	ml
*(IOB, CBL-24h; CBL-48h)	CBL – calculated blood loss	ml
	IOB – intraoperative blood loss	ml

Equation 4: Relative blood loss (%), (57, 70)

2.5.2.4 Haemoglobin-drop and Haematocrit-drop

Pre- and postoperative levels of haemoglobin and haematocrit were monitored and assessed according to the time-point of the blood sample. The amount of decrease between pre- and postoperative levels of aforementioned parameters was measured and statistically analysed. Respective parameters related to haemoglobin were referred to as Hb-drop-24h and Hb-drop-48h; for analysis of changes in haematocrit levels the terms ‘Hct-drop-24h’ and ‘Hct-drop-48h’ were used.

2.5.2.5 Blood parameters and coagulation profile

The following blood parameters were assessed within this observational study:

2.5.2.5.1 Routine blood parameters

2.5.2.5.1.1 Haemoglobin and Haematocrit

Pre- and postoperative haemoglobin and haematocrit levels were monitored.
Haemoglobin was used to calculate the perioperative blood loss 24-hours and 48-hours postoperatively, according to the abovementioned formula ('haemoglobin-balance method'; (80)).
Additionally, these parameters were analysed separately, to statistically assess postoperative drops in haemoglobin and haematocrit levels in relation to gender, blood loss, surgical technique and operation time.

2.5.2.5.1.2 Platelet count

Platelets are anucleate cells involved in numerous haemostatic processes. The platelet count, assessed in conjunction with the mean platelet volume allows for basic information on platelet function. While decreased levels generally trigger bleeding, elevated levels were found to be associated with thrombotic disorders. With reference to gender, females have frequently been shown to present with a higher platelet count, in comparison with men (118).

The platelet count was recorded on the day prior to surgery.

2.5.2.5.1.3 Mean platelet volume (MPV)

The mean platelet volume was recorded preoperatively. This parameter describes the average size of platelets in the blood and provides basic information on platelet function (119). It is a widely accepted fact that larger platelets are linked to a more efficient haemostasis due to the release of more vasoactive and prothrombotic substances in the process of coagulation (120). Increased levels of MPV have regularly been associated with thrombotic events, whereas decreased levels of MPV have been found to be linked to bleeding (119).

2.5.2.5.2 Coagulation profile

2.5.2.5.2.1 Routine coagulation parameters

2.5.2.5.2.1.1 Activated partial thromboplastin time (APTT), Prothrombin time (PT) and International Normalised Ratio (INR)

These classic coagulation tests allow for information about the patients' haemostatic status. These parameters were assessed before surgery, as part of the preoperatively taken blood sample. All of these parameters were analysed according to the patients' gender.

The activated partial thromboplastin time is a commonly used, inexpensive coagulation test, with the of aid which the status and function of the intrinsic coagulation pathway can be assessed. Severe to moderate deficiencies in terms of the coagulation factors II, V, VIII, IX and XI can be identified. In addition to that it also allows for information about coagulation factors X and XII, prekallikrein and high molecular weight kininogen. All of these deficiencies generally entail a prolongation of aPTT (121, 122).

The prothrombin time (PT) is used to monitor the extrinsic coagulation pathway and helps to detect deficiencies in terms of the coagulation factors II, V, VII and X (122).
The international normalised ratio (INR) was developed to ensure comparability of the PT measured in different laboratories to counteract the enormous variability of determined results in this context (122, 123).

2.5.2.5.2.2 Specific coagulation parameters

Plasma concentrations of specific coagulation parameters were measured on the day before surgery and were further correlated with gender and blood loss.

2.5.2.5.2.2.1 Fibrinogen

Fibrinogen is an abundant plasma protein, being considered both a coagulation factor and a structural protein. It provides the main building block of the blood clot, once converted to fibrin by thrombin during the final stages of the coagulation cascade. Normal plasma concentrations have been shown to be of utmost importance to ensure adequate clot

formation and structural stability of the blood clot (124). Deficiencies with regards to Fibrinogen relate to quantitative or qualitative shortcomings of the protein, which have been associated with different phenotypes: whereas many patients deficient in Fibrinogen are symptomless, increased bleeding as well as a higher risk of thrombosis have also been observed in this patient cohort. Reasons for this wide range of symptoms remain uncertain, but may relate to differences in clot strength and clot structure. Increased levels of Fibrinogen, being referred to as hyperfibrinogenaemia, have been linked to a higher risk of thrombosis (124).

2.5.2.5.2.2.2 Antithrombin-III (AT-III)

Antithrombin-III is a natural anticoagulant, inhibiting serine proteases such as thrombin, plasmin, kallikrein and coagulation factors IX, X, XI and XII. Its anticoagulant function is known to be accelerated by the use of heparin (125, 126).
AT-III-deficiency is often associated with a significantly increased risk of thrombosis. While the risk of venous thromboembolism amounts to 5% for factor V Leiden, it has been described to be as high as 50% for AT-III-deficient patients (125).

2.5.2.5.2.2.3 Von Willebrand Factor (vWF)

Von Willebrand factor is produced within endothelial cells and serves as a crucial contributor to platelet adhesion, platelet aggregation as well as the secondary haemostatic cascade through preventing factor VIII from premature proteolysis (127).
Deficient or defective plasma-vWF trigger a severe haemostatic disorder, referred to as von-Willebrand disease (vWD). It can either be inherited or acquired, whereas the hereditary form is much more common. Three different types of vWD are known: Type I represents the most common form, making up for 75% of vWD. Quantitative deficiency of vWF has been identified as the issue in this regard. Type II amounts to 25% of vWD and is characterised by qualitative issues in terms of vWF. With respect to type III, almost complete absence of vWF has been described (127).
Typical symptoms which relate to vWD may range from mucocutaneous bleeding to late-onset haemorrhage, depending on the severity and the type of the vWF-deficiency or malfunction(127).

Two parameters reliably assessing the quantity and the function of vWF were evaluated in the present study:

- *vWF: Ag* – for quantitative analysis of vWF
- *vWF activity* – for qualitative analysis of vWF

2.5.2.5.2.2.4 Coagulation Factor VIII

Coagulation factor VIII is an important parameter within the intrinsic coagulation cascade. It is closely linked to von Willebrand-factor, the two of them forming a non-covalent complex in the blood. This complex protects factor VIII from premature proteolysis. Additionally, von Willebrand-factor ensures transportation of factor VIII to the site of the endothelial injury. Activation of factor VIII is established by thrombin. Once activated, its purpose is to accelerate activation of factor X (128).
Decreased activity of factor VIII is associated with an elevated risk of bleeding.
Very commonly, mutations in the gene encoding factor VIII can be held responsible for a reduced activity of factor VIII, causing a congenital disorder, better known as haemophilia A. This disease is much more prevalent in men, while women stand out as disease-carriers. Carriers usually do not show any symptoms, as plasma concentrations of factor VIII amount to approximately 50% of the normal range.
Whereas the primary haemostasis works normally in haemophilia A, issues arise within the secondary haemostatic cascade as the formed blood clot cannot be stabilised adequately. As a result of that, formed blood clots dissolve easily, which triggers excessive bleeding. The intensity of the bleeding relies on the severity of factor-VIII deficiency (128).

2.5.2.5.2.2.5 Coagulation Factor IX

The role of coagulation factor IX within the coagulation cascade is to activate factor X, by means of factor VIII as a contributing parameter. It is a vitamin-k dependant coagulation factor, involved in the intrinsic pathway. Reduced levels or absence of factor IX lead to a common bleeding disorder, known as haemophilia B. This congenital disorder is much more common in males, with females being carriers of the disease. Symptoms and their severity are similar to those in haemophilia A: prolonged bleeding and bruising are common, as well as bleeding from the nose and into muscles and joints. Spontaneous bleeding has only been found to occur in moderately to severely affected patients, whereas mild haemophilia B remains often undiagnosed, until surgical procedures or trauma (129).

2.5.2.5.2.2.6 Coagulation Factor XI

Numerous functions and effects of factor XI have been described with regards to intrinsic and extrinsic haemostasis (130). Most importantly, it triggers activation of factor IX, however, has also been shown to promote factor X activation as well as thrombin generation.
Factor XI deficiency is generally associated with mild bleeding; however, cases of severe intraoperative haemorrhage have been found to occur in this connection. Increased activity of factor XI, on the other hand, has been found to enhance the risk of thrombosis (130).

2.5.2.5.2.2.7 Coagulation Factor XIII (FXIII)

Factor XIII has been shown to play an important role in the haemostatic cascade (131). Several forms of Factor XIII are known, one of which is the activated Factor XIII (FXIIIa). FXIIIa is of utmost relevance, as it has been shown to stabilise the blood clot, by making it stiffer and subsequently, more resistant to fibrinolysis (132). With regards to patients deficient in FXIII, bleeding complications are common, due to reduced stability of the blood clot (133). This has especially been found true for the immediate postoperative period (134-136).

In the current study FXIIIa is measured, based on the preoperatively taken blood sample. The level of FXIIIa was set in comparison to the intraoperatively and perioperatively occurring amount of blood loss as to identify relevant correlations between gender, blood loss and this specific haemostatic parameter.

2.5.2.5.2.3 Endogenous Thrombin Potential (ETP) and peak-thrombin formation

Adequate thrombin formation has been shown to play an important role in the context of haemostasis. The main task of thrombin is to convert Fibrinogen to Fibrin, which has proven essential in order for the blood clot to form (137, 138). Deficient thrombin formation is therefore associated with excessive bleeding and haemorrhage. In contrast, increased levels of thrombin are linked to thrombosis (137). By means of a specific coagulation test introduced by Hemker et al., thrombin generation can be monitored in detail (139). The parameters ‘lag time’, ‘thrombin-peak height’ and the ‘endogenous thrombin potential’ defining the so-called thrombogram, can be determined in this regard (138).

The ‘endogenous thrombin potential’ (ETP) describes the net amount of thrombin generated by test plasma under experimental conditions (137, 138). It is defined as the area under the thrombin generation curve within the thrombogram and has been shown to represent a

useful marker in various haematological indications. The level of ETP is heavily reliant on the balance between procoagulant and anticoagulant parameters. Procoagulants are linked to thrombin formation, whereas anticoagulants inhibit the generation of thrombin (138).The 'lag time' refers to the time needed for the first amount of thrombin to form. The thrombin-peak height marks the peak of the thrombin formation within the thrombogram (138).

Numerous studies have investigated the thrombin generation potential with regards to gender (137, 140-142). On various occasions, statistically significant differences have been identified, with the female gender showing greater thrombin generation potential, a shorter lag time as well as greater peak-thrombin formation. Among potential influencing factors leading to these differences, oral contraception and the menstrual cycle were entrenched (137, 140, 143).

In this study, the ETP was recorded pre-operatively in each subject to assess potential gender-specific differences and to subsequently correlate it with the intraoperatively and peri-operatively occurring amount of blood loss.

Four parameters were assessed in the context of ETP-analysis:

- ETP – A-area under the curve (%) = total thrombin formation (**ETP-A-auc**)
- ETP – A Cmax. (%) = measurement of the thrombin-peak height (**ETP-A-Cmax**).

- ETP – B-area under the curve (%) = total thrombin formation (**ETP-B-auc**)
- ETP – B Cmax. (%) = measurement of the thrombin-peak height (**ETP-B-Cmax**).

2.5.2.6 Operation time

The operation time, defined as the time from mucosal incision to last suture, was recorded. Correlations between the intraoperatively measured blood loss, the perioperative blood loss, the surgical procedure and the operation time were calculated.

2.5.2.7 BMI

The patients' body mass index was recorded before surgery was performed. Correlations between the patients' body mass index and the intraoperatively and perioperatively occurring amount of blood loss were calculated.

$$BMI = \text{weight (kg)}/\text{height (m}^2\text{)}$$

2.6 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 26 (IBM Corp., Armonk, N.Y.). To statistically assess primary and secondary measures, descriptive statistics together with the following statistical tests were applied: the independent Student's t-test was used for continuous nonparametric data analysis. Correlation between variables was calculated by means of the Pearson Correlation Coefficient. Linear Regression Model and General Linear Model with Repeated Measurements were applied to quantify the effect of independent variables on blood loss according to the different time points used, the treatment modality applied and patient gender. A p-value of $p<0.05$ was defined as the cutoff for statistical significance.

2.7 Data management

Patient data collated in the present study was safely kept in a folder, stored in a locker only accessible by the PhD student (MS). For every patient included there was a designated section in folder, where the following study documents were classified: signed informed consent, printed blood results (prior to surgery, 24-hours and 48-hours postoperatively), and the intraoperative bleeding volumes recorded. Microsoft Excel was used to electronically classify patient data. A password protected spreadsheet was designed incorporating relevant sections with regards to respective study parameters. The excel spreadsheet was saved on an external hard disk device that was kept in the same locker as the study folder.

Patient data collated in the present study was anonymised prior to statistical analysis. For this purpose, a study number was assigned to each patient included. For statistical analysis anonymised patient data was transferred to SPSS Version 26.

3. RESULTS

3.1 Patients

A total of 103 patients (male 38, female 65) were included in the final analysis of this study. 54/103 patients underwent correction of skeletal malocclusion in terms of a bimaxillary osteotomy; 49/103 patients were treated by means of a BSSO. Of the 54 patients undergoing bimaxillary skeletal correction, 22 were males and 32 patients were females. This accounted for a male-to-female ratio of 1: 1.45 (male 40.7%; female 59.3%). The BSSO-cohort comprised 16 male patients and 33 female patients. This equated to a male-to-female ratio of 1: 2.06 (male 32.7%; female 67.3%).

3.1.1 ASA Status

With reference to the ASA status determined, 76/103 patients (73.8%) were categorised as ASA grade 1. The remaining 27/103 patients were classified as ASA grade 2 (26,2%).
50/65 female patients (76.9%) were graded as ASA grade 1; 26/38 male patients (68.4%) fulfilled the criteria for ASA grade 1. Within the bimaxillary-cohort 34/54 patients (63%) were attributed ASA grade 1; in the BSSO-cohort 42/49 were graded ASA grade 1 (85.7%).

3.1.2 Age

Overall, a mean age of 27.1 years (± 8.8) was determined. With regards to gender, a mean age of 28.3 years (± 9.1) in men and of 26.4 years (± 8.7) in women was observed.
In the bimaxillary-cohort a mean age of 27.0 years (± 8.6) was found; in the BSSO group the mean age amounted to 27.2 years (± 8.0). No differences between men and women in the bimaxillary-cohort in terms of age were found, however, men undergoing BSSO were found to be on average 4 years older (25.9 years vs. 29.9 years) than their female counterparts.

3.1.3 Body Mass Index (BMI)

The mean BMI determined in men differed statistically significantly from the BMI calculated in women, with men showing higher levels of BMI (male 24.56 ± 3.77; female 22.87 ± 3.38; p=0.025). Within the bimaxillary-cohort, men showed a higher mean BMI (25.8 ± 4.1) in comparison with women having undergone the same surgical procedure (23.3 ± 3.3).

In the BSSO-cohort, no gender-specific differences with regards to BMI were detected.

3.2 Operating time (OT)

Average operating times were shown to differ significantly with regards to the treatment modality applied. Bimaxillary surgery was found to be associated with a significantly longer operating time (131.2 min ± 52.3) than BSSO (68.1 min ± 40.5), (p<0.001), (Figure 3).

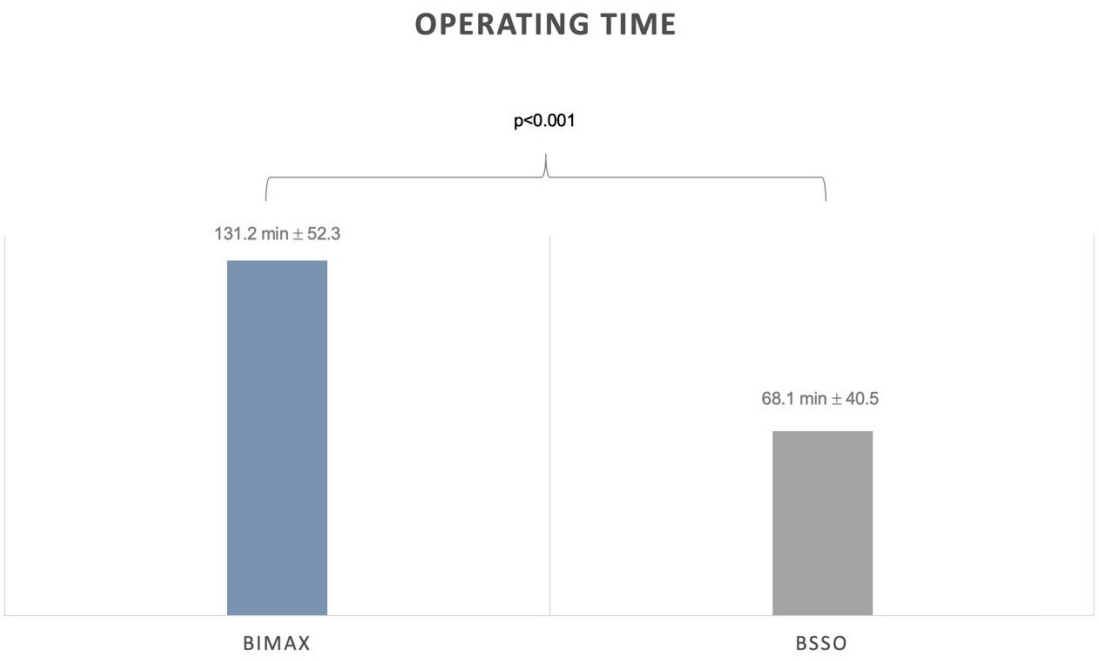

Figure 3: Figure to show the operating time needed with reference to the two treatment modalities applied (min), (BIMAX vs. BSSO)

When looking at gender-specific differences in terms of the length of the procedure, bimaxillary surgery in men was found to averagely take 25.9 min longer than in women. However, no statistically significant differences were found to occur in this regard, allowing to assume similar starting conditions for further statistical analysis (p= 0.076).
In the BSSO-cohort, average operating times in men were shown to equal those in women (p= 0.875), (Figure 4).

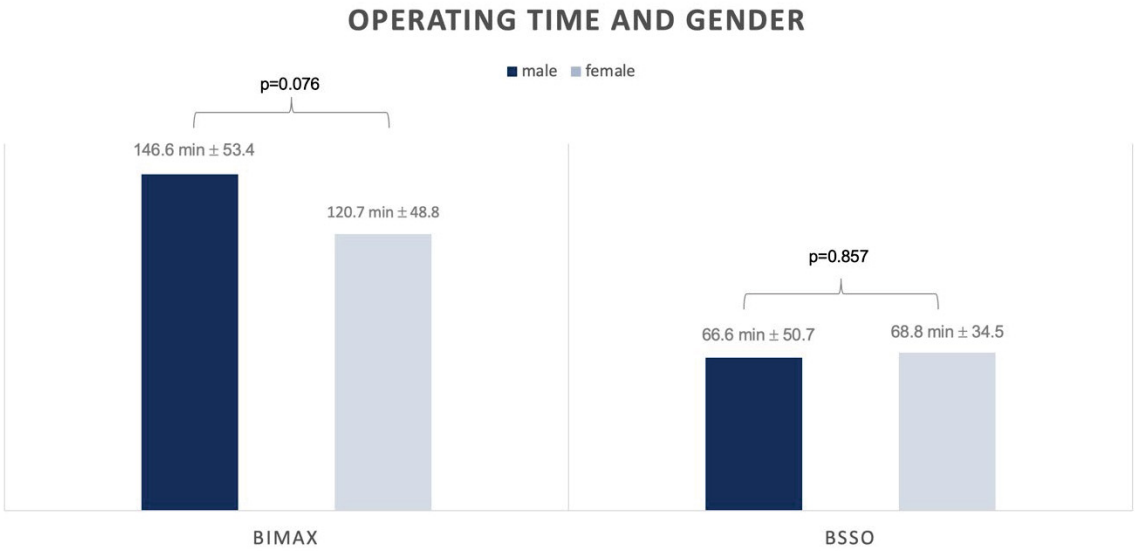

Figure 4: Figure to show the operating time (min) according to the treatment modality and patient gender. No statistically significant differences in terms of patient gender were determined.

3.3 Analysis of blood loss

In a first step, blood loss was assessed according to the treatment modality applied. Secondly, a gender-specific subgroup analysis of the data, focusing on sex-related differences in terms of intra- and perioperative bleeding volumes was performed.

3.3.1 Bimaxillary Surgery vs. Bilateral sagittal split osteotomy (BSSO)

3.3.1.1 Estimated total blood volume (TBV)

No statistically significant differences between the bimaxillary-cohort and the BSSO-group, in terms of the patients' estimated total blood volume applying Nadler's formula were observed (p=0.128) (84). In the bimaxillary-cohort the mean estimated blood volume amounted to 4593.5 ml (±932.8 ml); in the BSSO-cohort a mean estimated blood volume of 4348.4 ml (±645.7 ml) was calculated.

3.3.1.2 Intraoperative blood loss (IOB)

The parameter 'intraoperative blood loss' (IOB), incorporating surgical bleeding from mucosal incision to wound closure amounted to 520.5 ml (± 266.7 ml) in the bimaxillary-cohort and to 140.8 ml (± 96.7 ml) in the BSSO-cohort. In this regard, statistically significant differences between the two surgical modalities were found to occur with bimaxillary surgery being associated with a significantly higher intraoperative blood loss (p<0.001), (Figure 5).

3.3.1.3 Calculated blood loss: CBL 24h and CBL 48h

Perioperative blood loss calculated on the first and second postoperative day based on the levels of haemoglobin recorded 24-hours and 48-hours postoperatively confirmed statistically significant differences in terms of surgical bleeding with reference to the two orthognathic surgical modalities applied (CBL-24h p<0.001; CBL-48h p<0.001), (Figure 5).

24-hours postoperatively, a bleeding volume of 667.5 ml (± 296.5 ml) was calculated for the bimaxillary-cohort; calculation of blood loss 48-hours postoperatively revealed a bleeding volume of 802,9 ml (± 275.3ml) in this patient cohort.

In patients undergoing mandibular osteotomy-only (BSSO) a significantly lower blood loss of was recorded for both measurements (CBL-24h and CBL-48h).

24-hours postoperatively, a perioperative blood loss of 298 ml (± 235.9 ml) was detected (CBL-24h); 48-hours postoperatively, blood loss calculated amounted to 495.2 ml (± 267.6 ml) in the BSSO-cohort (CBL-48h).

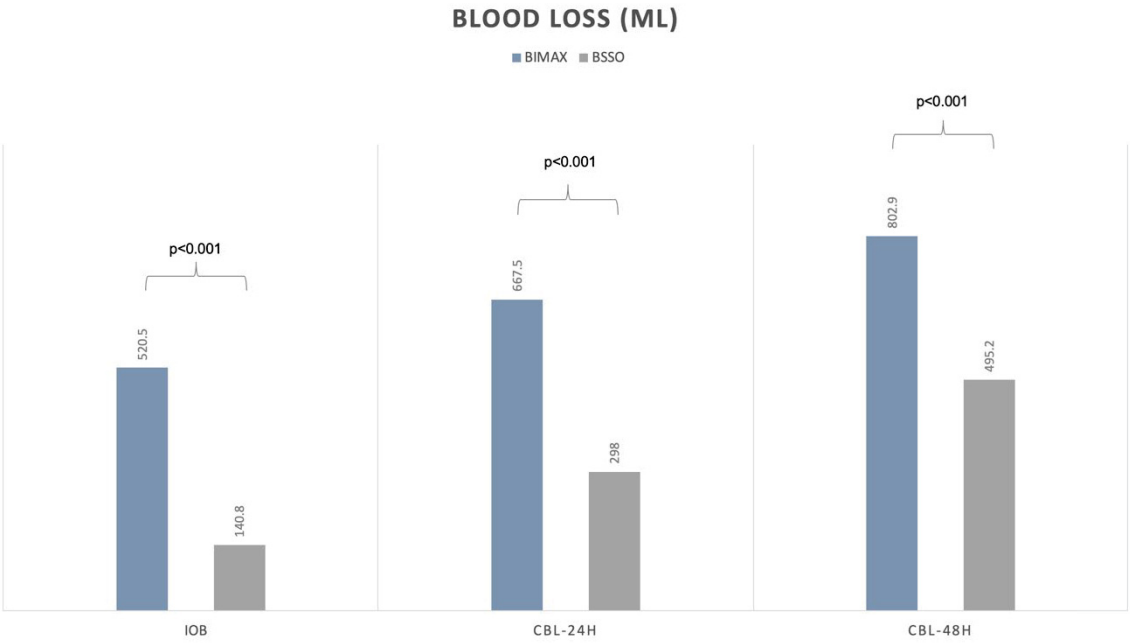

Figure 5: Figure to display blood loss with reference to the treatment modality applied at three different time points (ml); (BIMAX vs BSSO). Statistically significant differences between bimaxillary surgery and BSSO were detected, indicating a significantly higher blood loss in bimaxillary surgery.

3.3.1.4 Analysis of IOB, CBL-24h and CBL-48h (General Linear Model with Repeated Measurements)

Blood loss was shown to increase significantly in the first 48-hours postoperatively with reference to both treatment modalities (bimaxillary surgery p<0.001; BSSO p<0.001). Statistically significant differences in terms of the amount of blood loss regarding bimaxillary surgery and BSSO were found (p<0.001). This goes in conformity with the results determined within individual statistical analysis of the parameters IOB, CBL-24h and CBL-48h. In terms of the increase of blood loss between measurements, no statistically significant differences between the bimaxillary-cohort and the BSSO-group were observed (p=0.150), stating similar increase of blood loss in both groups.

3.3.1.5 Relative blood loss (RBL%)

The parameter ‘relative blood loss’ indicates the percentage of blood loss occurring in relation to the patient’s estimated total blood volume. In this study, IOB, as well as CBL 24h and CBL 48h were set in proportion to the patient’s estimated total blood volume resulting in the parameters RBL- IOB, RBL-24h and RBL-48h.

In the bimaxillary-cohort, relative blood loss amounted to 11.5% (± 5.9%), 14.8% (± 6.5%) and 17,7% (5,9%) with regard to respective bleeding parameters RBL- IOB, RBL-24h and RBL-48h. Within the BSSO-cohort, an RBL- IOB of 3.3% (± 2.2%), RBL 24h of 7% (± 5.5%) and an RBL 48h of 11.6% (± 6.2%) were recorded.
When comparing the two surgical techniques in terms of the relative blood loss detected, statistically significant differences in all of the parameters indicating relative bleeding volumes were found to occur. In more detail, bimaxillary surgery was linked to a significantly higher percentage of blood loss in relation to the patient’s estimated total blood volume (RBL- IOB p<0.001; RBL-24h p<0.001, RBL-48h p<0.001), (Figure 6).

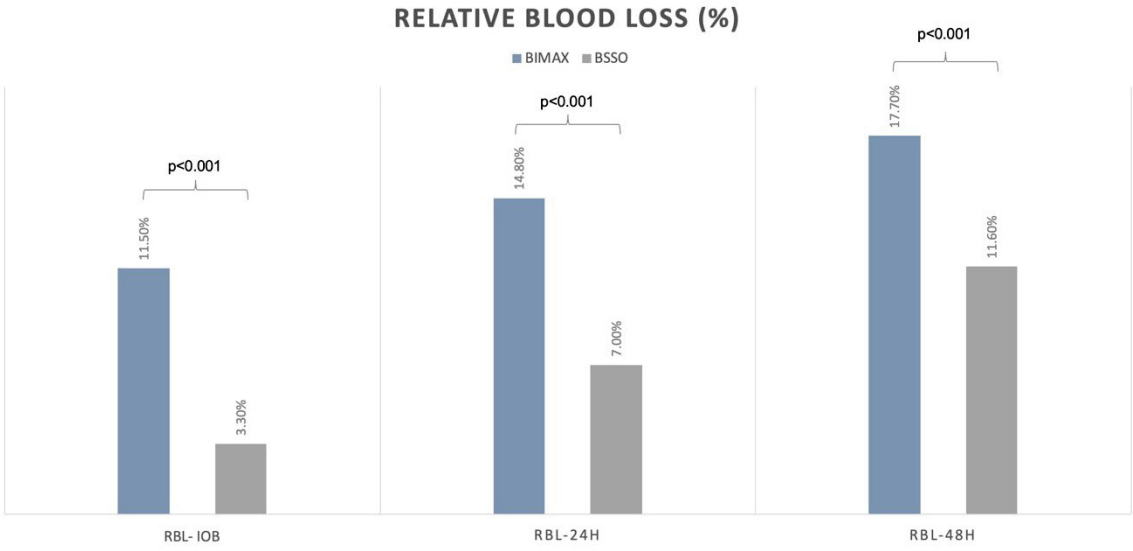

Figure 6: Relative blood loss (%), (BIMAX vs. BSSO)

3.3.1.6 Hidden blood loss (HBL)

The analysis of the parameter ‘hidden blood loss’ (HBL), representing the difference between the intraoperative blood loss (IOB) and the calculated blood loss 48-hours postoperatively, equated to a considerable amount of undetected blood loss in both groups (Figure 7). With

reference to the bimaxillary-cohort a mean HBL of 282.4 ml (± 244.8 ml) was found. An even higher average hidden bleeding volume for the BSSO group, with a mean HBL of 354.3 ml (±258.6 ml) was reported. No statistically significant differences were found to occur when comparing the two treatment modalities (p= 0.150).

Figure 7: Hidden blood loss (ml), (BIMAX vs. BSSO)

3.3.1.7 Haemoglobin-drop

Differences regarding pre- and postoperative levels of haemoglobin were analysed and set in relation to the surgical technique applied (Hb-drop-24h, Hb-drop-48h).

Blood samples taken 24-hours postoperatively indicated a decrease in haemoglobin levels of 2.10 g/dl (± 0.95) with reference to the bimaxillary-cohort. 48-hours postoperatively, haemoglobin levels were found to have decreased by 2.51 g/dl (± 0.89) in comparison to preoperatively recorded haemoglobin levels.
Within the BSSO-cohort, less substantial alterations regarding haemoglobin levels were noted. 24-hours postoperatively, a haemoglobin drop of 0.9 g/dl (± 0.82) was observed; 48-hours postoperatively a decrease of 1.58 (± 0.86) in relation to preoperative haemoglobin levels were found.

Statistically significant differences occurred when comparing bimaxillary surgery and BSSO in this context, with bimaxillary surgery being associated with more substantial haemoglobin decrease in both measurements. (24h $p<0.001$; 48h $p<0.001$).

3.3.1.8 Haematocrit-drop

With regards to bimaxillary surgery, substantial difference between pre- and 24-hours postoperatively recorded haematocrit levels was noted (6.34% (± 2.81)); 48-hours postoperatively a difference of 7.06% (± 2.67) was found.
BSSO was found to be associated with less substantial decrease of postoperative haematocrit levels. 24-hours postoperatively a mean haematocrit drop of 2.70% (± 2.68) was determined; 48-hours postoperatively a mean decrease in terms of haematocrit of 4.27% (± 2.80) was detected.

Levels of haematocrit were shown to decrease statistically significantly more in the bimaxillary-cohort, than the BSSO- group (24h $p<0.001$; 48h $p<0.001$).

3.3.2 Blood loss and patient gender

3.3.2.1 Bimaxillary surgery and gender-specific blood loss

3.3.2.1.1 Intraoperative blood loss (IOB)

In terms of the intraoperative blood loss determined during bimaxillary surgery a clear statistical tendency towards higher intraoperative blood loss associated with male gender was observed (p=0.056). A mean IOB of 603.9 ml (± 254.8 ml) in male patients was found. In females undergoing the same operation a considerably lower IOB of 463.1 ml (± 263.2 ml) was detected (Figure 8).

3.3.2.1.2 Calculated blood loss: CBL-24h and CBL-48h

Gender-specific analysis of the perioperative blood loss (CBL 24h and CBL 48h) associated with bimaxillary surgery revealed substantial differences between male and female gender. In males, CBL 24h and CBL 48h amounted to 748.7 ml (± 320.2 ml) and 907.7 ml (± 246.1 ml), respectively. In females, a CBL-24h of 611.6ml (± 270.0ml) and a CBL-48h of 730.8 ml (± 274.5ml) were determined.

Whereas no statistically significant differences with reference to CBL-24h were found (p=0.095), male gender was linked to a statistically significantly higher perioperative bleeding volume in terms of the parameter CBL-48h (p=0.019), (Figure 8).

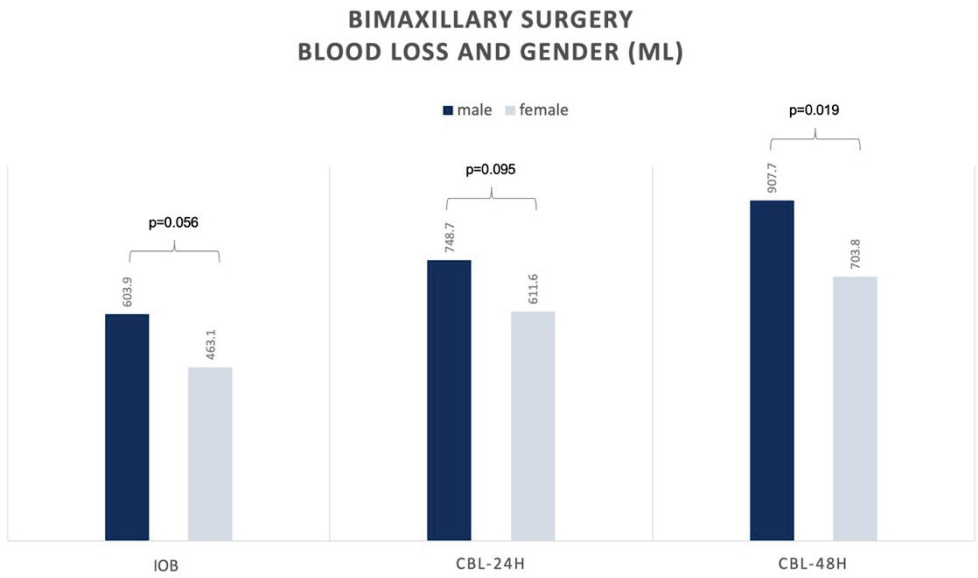

Figure 8: Blood loss (ml) determined in bimaxillary surgery with respect to the male and female gender

3.3.2.1.3 Analysis of IOB, CBL-24h and CBL-48h (General Linear Model with Repeated Measurements)

As far as bimaxillary surgery goes, significant increase of blood loss for both the male and female gender in the first 48-hours after surgery was observed (male $p<0.001$; female $p<0.001$). No gender-specific differences in terms of the amount of increase were found ($p=0.599$), however, statistically significant differences between male and female patients with regards to the bleeding volumes determined were detected ($p=0.021$).

3.3.2.1.4 Relative blood loss (RBL %)

Analysis of the relative blood loss did not show any statistically significant differences within the bimaxillary-cohort in any of the parameters assessed (RBL-IOB, RBL-24h, RBL-48h). RBL- IOB amounted to 11.3% (± 5.1%) in males and 11.7% (± 6.6%) in female patients ($p=0.814$); RBL-24h showed a relative blood loss of 14% (± 6.2%) in males and 15.3% (± 6.7%) in females ($p=0.477$); and RBL-48h revealed a relative blood loss of 17.1% (± 5.3%) in males and of 18.2% (±6.2%) in females ($p=0.492$), (Figure 9).

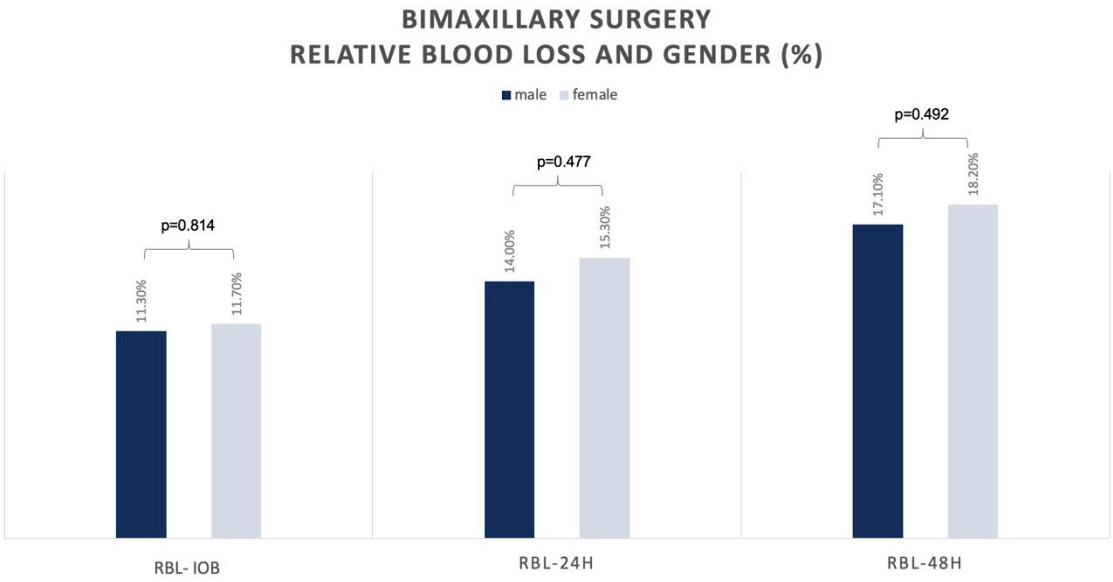

Figure 9: Relative blood loss in bimaxillary surgery according to the male and female gender (%)

3.3.2.1.5 Hidden blood loss (HBL)

The parameter ‘hidden blood loss’, calculated on the basis of IOB and CBL-48h, showed a mean amount of hidden blood loss of 303.9 ml (± 218.3 ml) related to male gender and of 267.7 ml (± 263.9 ml) linked to female gender. No statistically significant gender-specific differences with reference to HBL were detected (p=0.599).

3.3.2.1.6 Haemoglobin-drop

Differences regarding pre- and postoperative levels of haemoglobin were analysed and set in relation to the patient’s gender. In male patients, a mean decrease of haemoglobin levels of 2.16 g/dl (± 0.96) measured on the first postoperative day were found. 48-hours postoperatively taken blood samples revealed a haemoglobin-drop of 2.62 g/dl (± 0.82).

In female patients, a mean decrease of haemoglobin levels of 2.05 g/dl (± 0.96) and 2.44 g/dl (± 0.93) determined 24- and 48-hours after surgery was observed.

No statistically significant differences between the male and female gender resulted in any of the analyses made.

3.3.2.1.7 Haematocrit-drop

No statistically significant gender-related differences were observed, regarding the decrease of haematocrit levels measured 24- and 48-hours postoperatively.

3.3.2.2 Bilateral sagittal split osteotomy (BSSO) and gender-specific blood loss

3.3.2.2.1 Intraoperative blood loss (IOB)

IOB averaged 122.2 ml (± 69.1 ml) in male patients, undergoing mandibular osteotomy. In females, treated with the same surgical technique, an average intraoperative bleeding volume of 149.9 ml (± 107.4 ml) was detected.
No statistically significant differences were found when comparing IOB in males and females in this context (p=0.353).

3.3.2.2.2 Calculated blood loss: CBL-24h and CBL-48h

Analysis of CBL-24h and CBL-48h showed that blood loss increased substantially within the first 48-hours postoperatively. This was true for both male and female gender.
In a bit more detail, a CBL-24h of 279.6ml (± 242.2 ml) in males and 306.9 ml (± 236.1 ml) in females was observed. CBL-48h amounted to 483.6 ml (± 263.9 ml) in men and 500,8 ml (± 273.3 ml) in women. No statistically significant gender-specific differences in terms of CBL-24h (p=0.709) and CBL-48h (p=0.836) were found to occur (Figure 10).

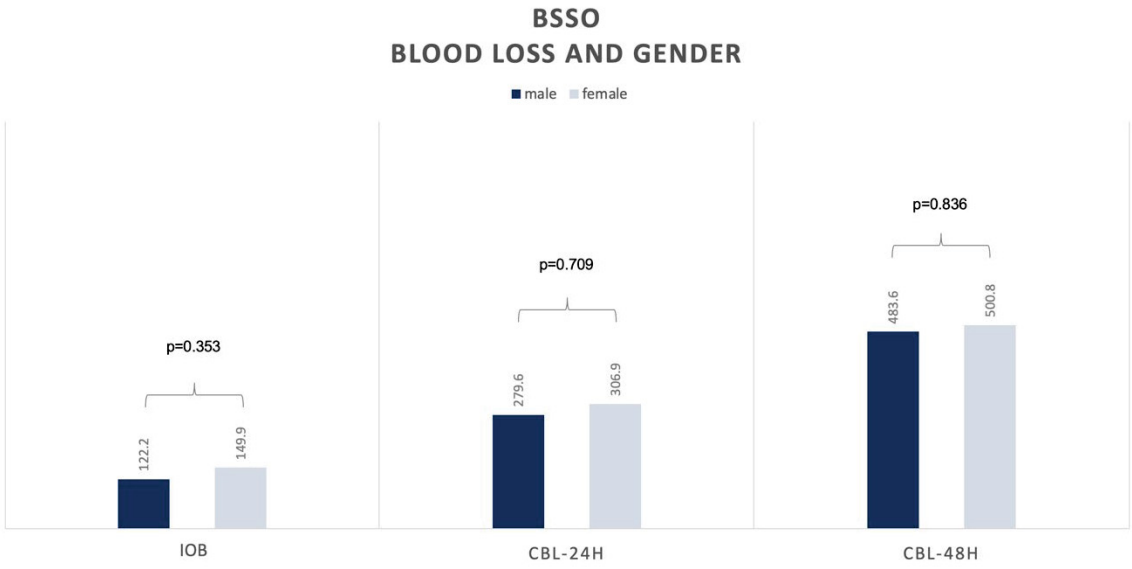

Figure 10: BSSO and Gender-specific blood loss (ml)

3.3.2.2.3 Analysis of IOB, CBL-24h and CBL-48h (General Linear Model with Repeated Measurements)

Blood loss was shown to increase significantly with time in male and female patients undergoing BSSO ($p<0.001$). No relevant differences in terms of the bleeding volumes determined ($p=0.629$) and the amount of increase between measurements ($p= 0.895$) were found to occur, when focusing on the patient's gender.

3.3.2.2.4 Relative blood loss (RBL %)

Analysis of bleeding volumes relative to the patient's estimated total blood volume did not result in any statistically significant differences between male and female participants (RBL-IOB $p=0.092$; RBL-24h $p=0.180$; RBL-48h $p=0.110$). However, substantial increase of relative blood loss in the first 48-hours after surgery was shown.

Regarding males, the following percentages were determined: RBL- IOB 2.5% (± 1.4%); RBL-24h 5.4% (± 4.2%) and RBL-48h 9.5% (± 4.7%).

With reference to female gender relative bleeding volumes of 3.8% (± 2.9) (RBL- IOB), 7.7% (± 6.6) (RBL-24h), and 12.6% (± 6.2%) (RBL-48h) were determined (Figure 11).

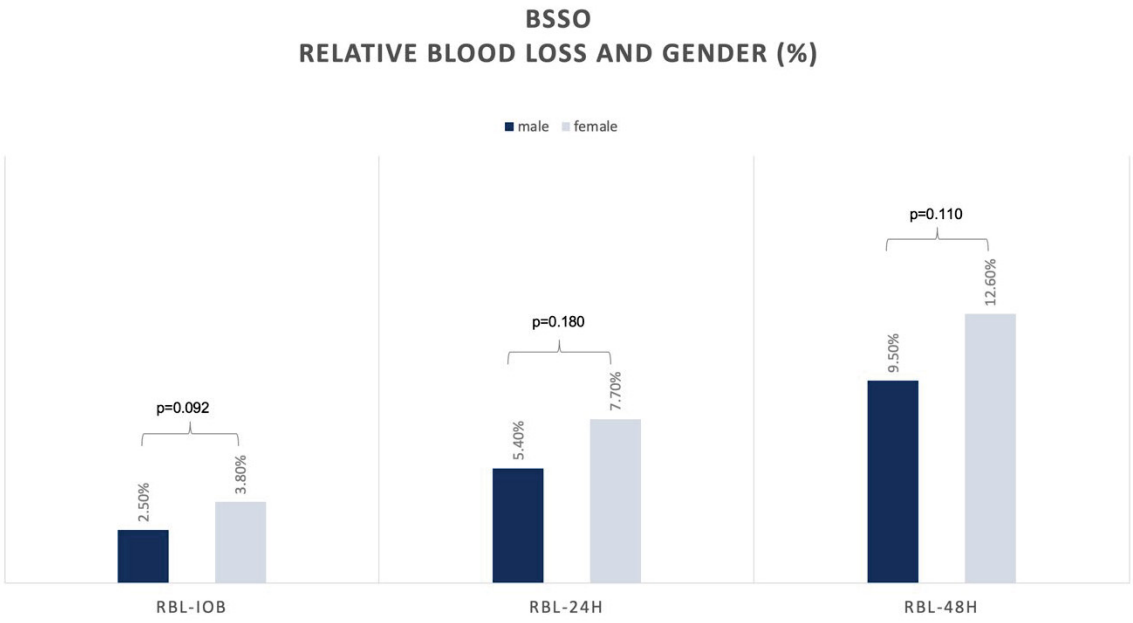

Figure 11: Relative blood loss related to BSSO displayed according to the male and female gender

3.3.2.2.5 Hidden blood loss (HBL)

When focusing on HBL, composed of the difference of IOB and CBL-48h, mean hidden bleeding volumes of 361.4 ml (± 282.9 ml) in males and of 350.9 ml (± 250.4 ml) in females were found. No statistically significant differences were found to occur with reference to male and female gender (p=0.895).

3.3.2.2.6 Haemoglobin-drop

With reference to the decrease in haemoglobin levels observed in the first 48-hours after surgery, no statistically significant gender-related differences resulted.

3.3.2.2.7 Haematocrit-drop

No differences between the male and female gender resulted, when analysing the decrease of haematocrit levels this patient cohort.

3.4 Gender-specific analysis of blood and haemostatic parameters

3.4.1 Blood parameters (male vs. female)

3.4.1.1 Haemoglobin

Gender-specific analysis of preoperative haemoglobin levels indicated statistically significant differences among males and female females (Figure 12). Men were shown to have significantly higher preoperative haemoglobin levels compared with women (p< 0.001; male 15.11 g/dl ± 1.03 vs. female 13.18 g/dl ± 0.89).

Within the bimaxillary-cohort mean levels of haemoglobin of 13.24 g/dl in women (± 0.92) and 15.37 (± 1.14) in men were determined (p<0.001).

In the BSSO-cohort similar conclusions were drawn (p<0.001).

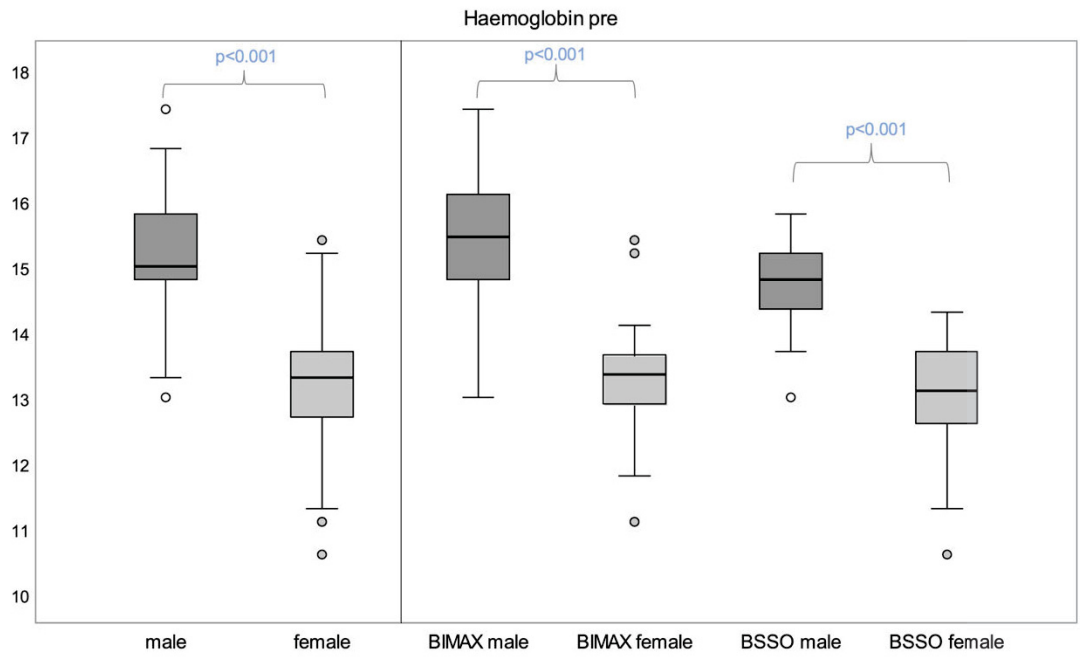

Figure 12: preoperative levels of haemoglobin (g/dL), displayed according to gender

3.4.1.2 Haematocrit

With regards to the haematocrit, male patients were found to present with significantly higher preoperative baseline values (p<0.001), (Figure 13). In men, a mean preoperative haematocrit of 44.5% (± 2.9) was found; in women this value amounted to 39.8% (± 2.5).

Similarly, statistically significant differences occurred, when analysing gender-related differences in relation to the surgical technique (BIMAX p<0.001; BSSO p<0.001).

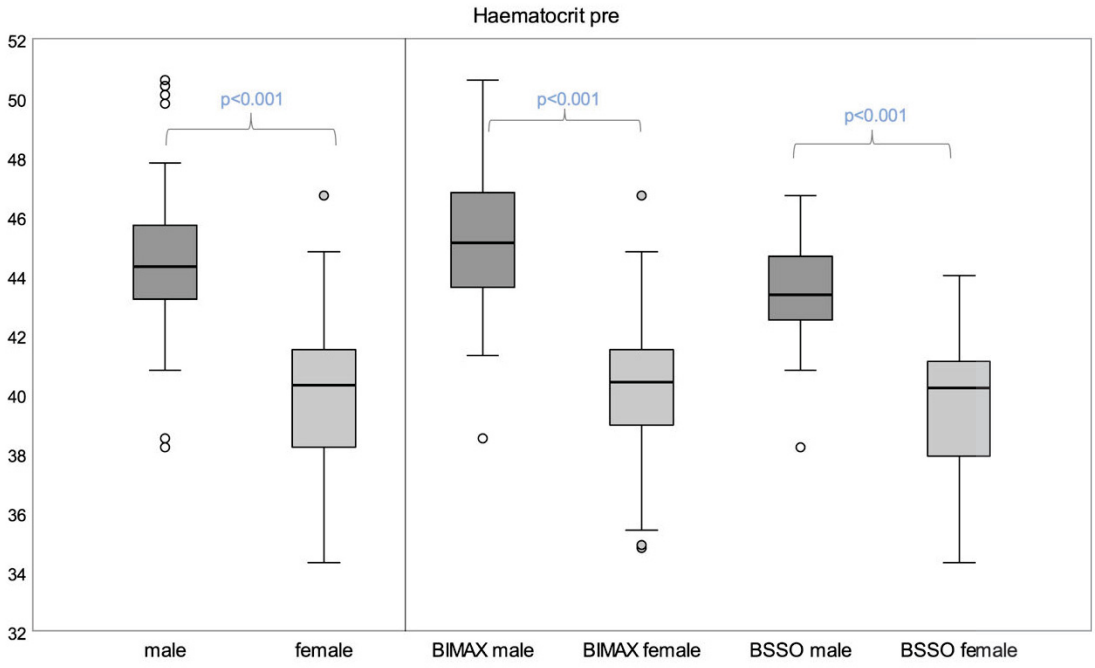

Figure 13: preoperative levels of haematocrit (%), assessed according to gender

3.4.1.3 Platelet count

Preoperative values of thrombocytes in women outweighed those in men (female 265.2 ± 56.4 vs. male 243.5 ± 58.2), (Figure 14). A clear statistical tendency towards a significantly increased number of thrombocytes in women in comparison with men was found (p=0.065).

Interestingly, no statistically significant differences of preoperative thrombocyte levels between men and women were found when also taking into account the treatment modality applied.

Within the bimaxillary-group and the BSSO-cohort p-values amounted to p=0.374 and p=0.105, respectively.

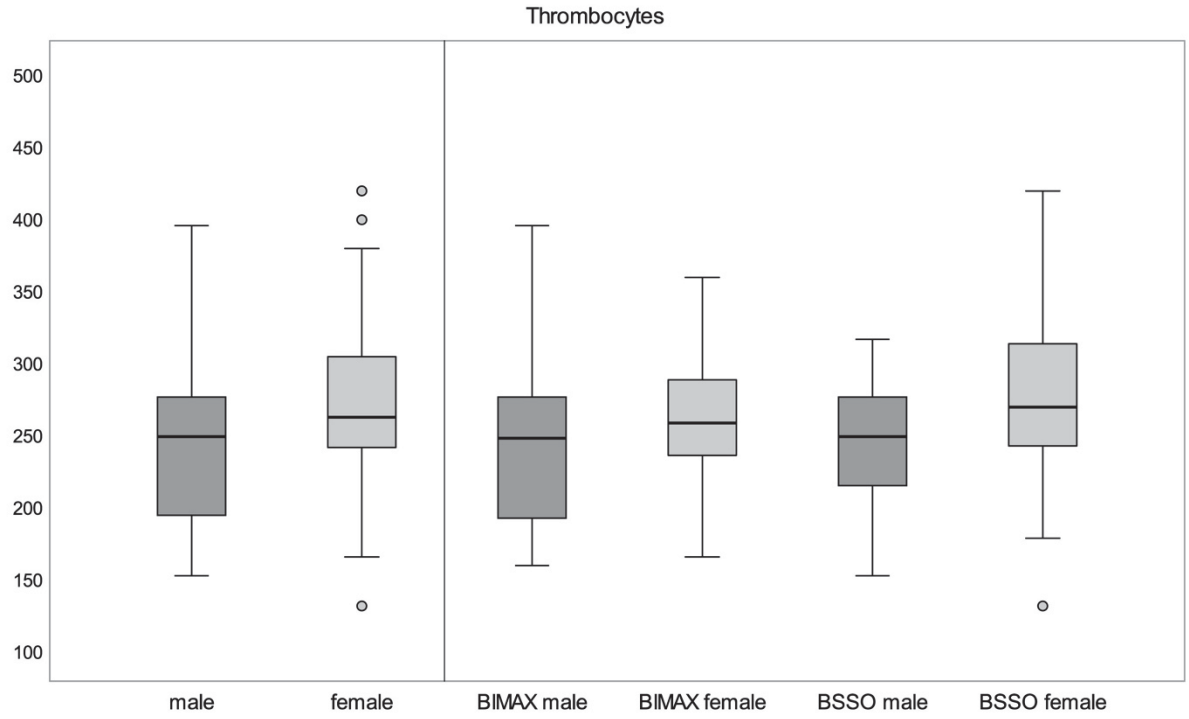

Figure 14: Platelet count, displayed according to patient gender

3.4.1.4 Mean platelet volume (MPV)

With reference to the mean platelet volume (MPV) determined preoperatively, no statistically significant differences among males and females were found to occur (p=0.777), (Figure 15). MPV amounted to 10.33 (± 1.48) in males and 10.41 (± 0.82) in females.

In a more detailed analysis, additionally considering the surgical method, also no statistically significant differences were detected (BIMAX, p= 0.755; BSSO, p=0.979).

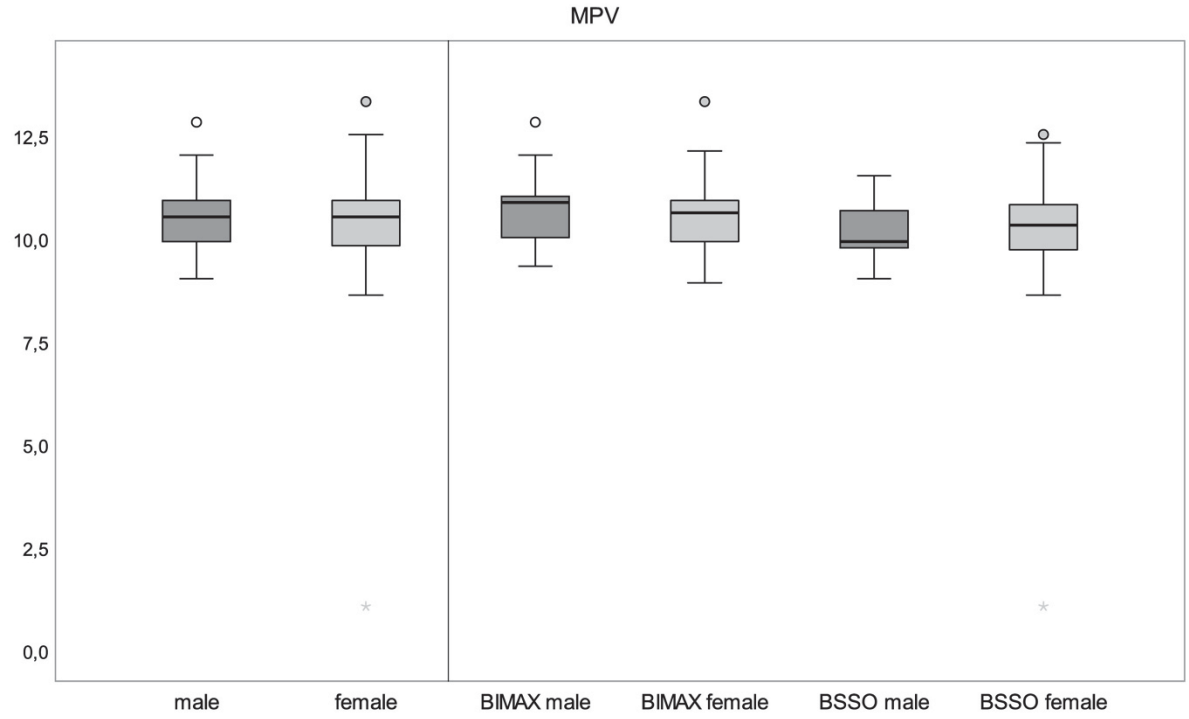

Figure 15: mean platelet volume and patient gender

3.4.2 Preoperative coagulation analysis

3.4.2.1 Activated partial thromboplastin time (aPTT)

Preoperatively, levels within the normal range with regards to the activated partial thromboplastin time (aPTT) were observed. In terms of the male and female gender statistically significant differences were observed, with males showing higher levels of aPTT (male 29.5 ± 2.7; female 27.6 ± 3.1; p=0.002), (Figure 16).

Within a more detailed analysis considering the treatment modality applied, statistically significant differences among men and women were found to occur with reference to the BSSO-cohort, (p=0.002). In this subgroup, the male gender was associated with a significantly increased aPTT relative to the female gender (male 29.95 ± 2.12; female 27.42 ± 2.62).

No such preoperative gender-specific differences in the bimaxillary-cohort were detected in this regard (male 29.25 ± 3.03; female 27.69 ± 3.58; p=0.102).

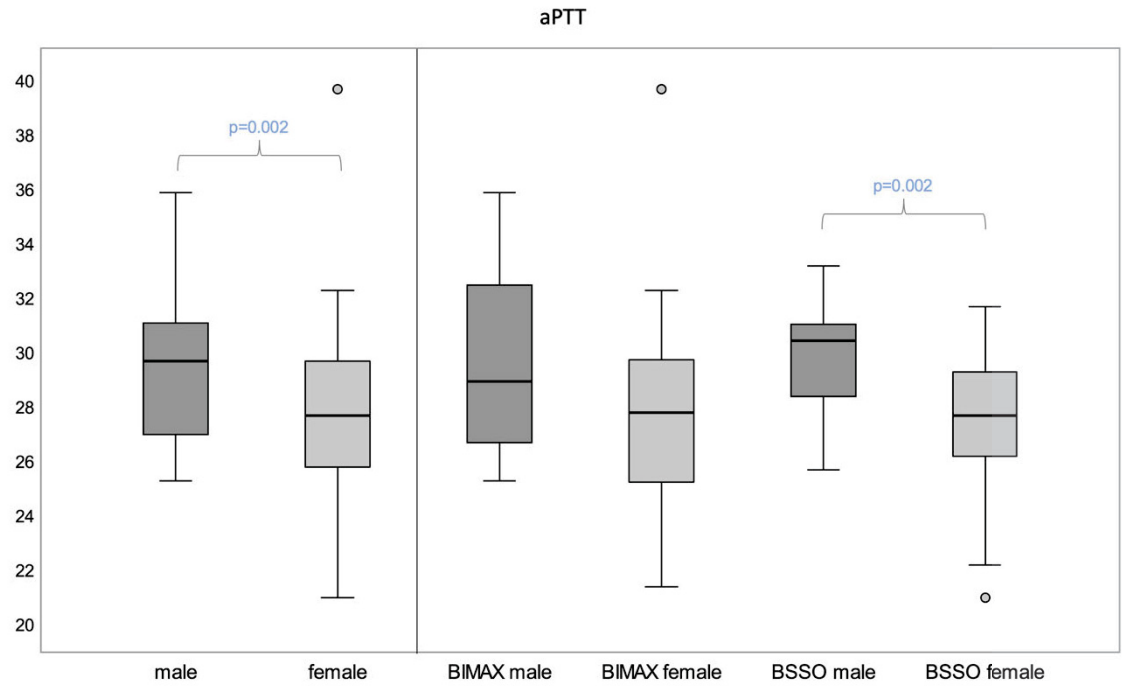

Figure 16: Figure displaying statistically significant gender-specific differences in terms of the parameter aPTT

3.4.2.2 International normalised ratio (INR)

With reference to the INR determined preoperatively, all patients were found to be within the normal range (range: 0.82 – 1.22). With respect to gender no statistically significant differences were observed (male 0.99; female 0.97; p=0.111), (Figure 17).

This was also found true for gender-specific analysis in terms of the treatment modality applied (BIMAX: male 0.97, female 0.99; BSSO: male 1.00, female 0.97).

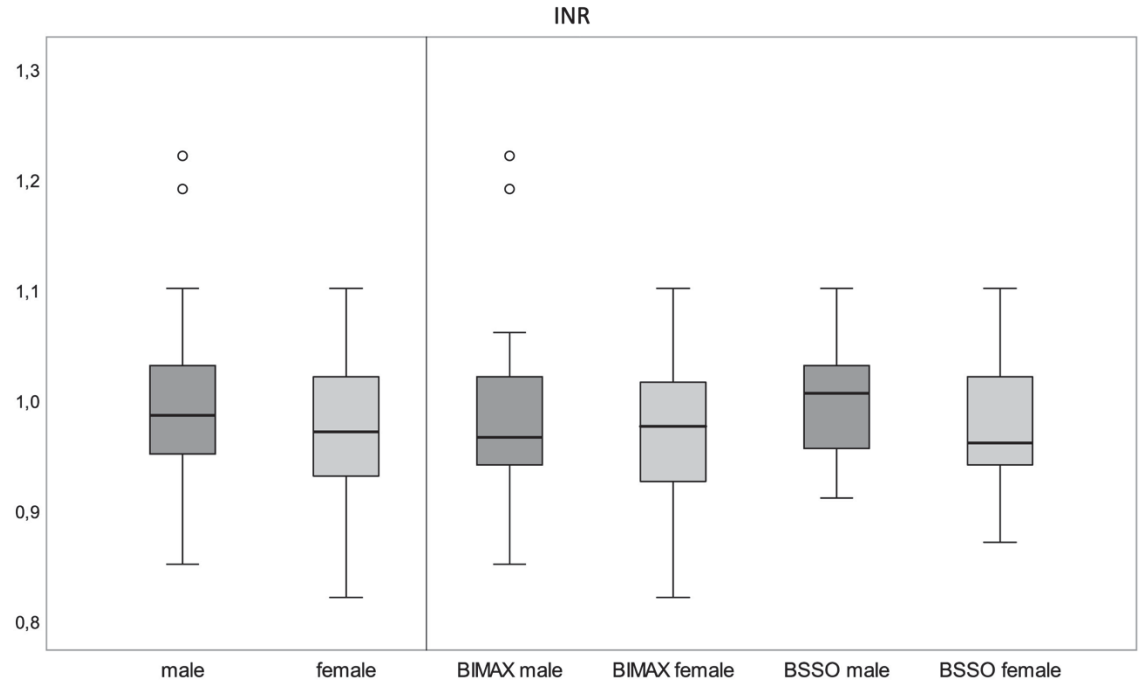

Figure 17: INR, displayed according to patient gender

3.4.2.3 Prothrombin time (PT)

The parameter 'Prothrombin time (PT)' showed normal preoperative levels in all of the patients included (range 69 – 120). No gender-related differences were detected when comparing male with female patients in this context (male 100.5; female 102.8; p=0.413).

Analysis of patient gender according to the treatment modality applied did not show any statistically significant differences either (BIMAX p=0.453; BSSO p=0.151), (Figure 18).

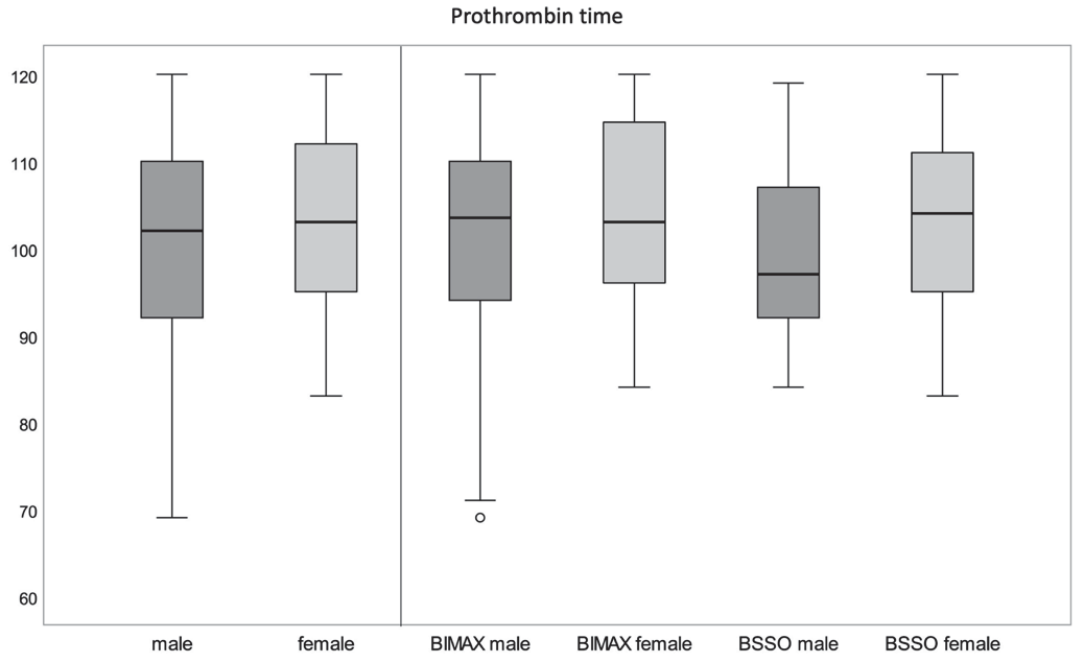

Figure 18: Prothrombin time (PT), showing no differences between the male and female gender

3.4.2.4 Fibrinogen

In terms of Fibrinogen, which plays an important role during the final stages of the coagulation cascade, no relevant differences between the male and female gender were observed (p=0.513), (Figure 19). Mean values amounted to 230.0 (± 72.31) in men and to 239.69 (± 67.92) in women.

This was also true when analysing the data according to the underlying surgical technique applied (BIMAX: p=0.573; BSSO: p=0.725).

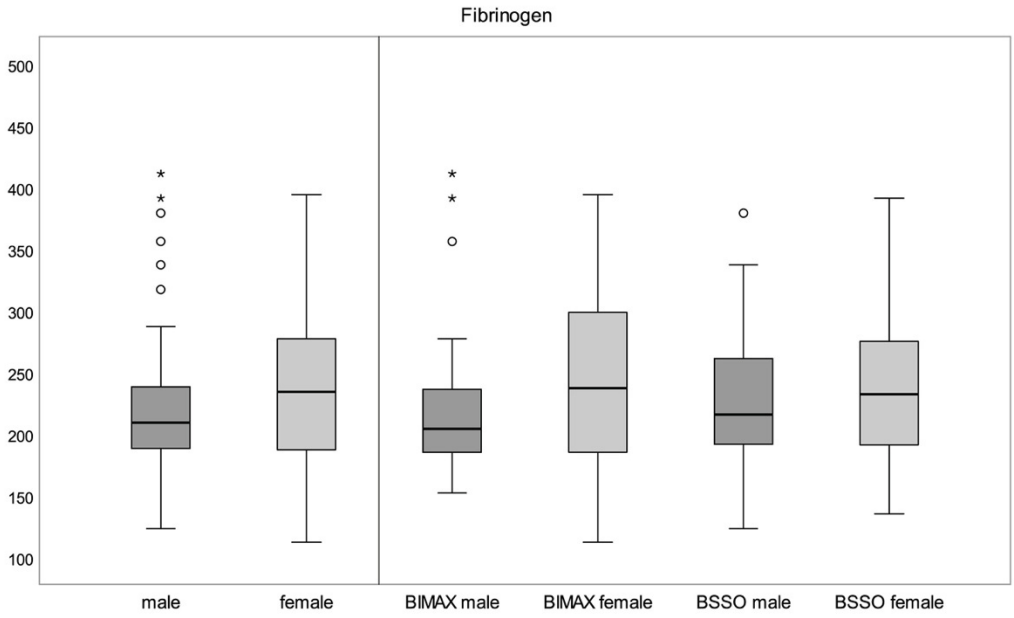

Figure 19: Levels of Fibrinogen, analysed according to gender. No statistically significant differences were found to occur with regards to the male and female gender.

3.4.2.5 Antithrombin-III (AT-III)

Looking at the analysis of the parameter 'Antithrombin-III', results indicated a clear tendency towards relevant statistical differences between men and women (p=0.057), with men showing elevated levels of AT-III in comparison with women (male 103.14 ± 11.96; female 97.9 ± 13.28).

Within the bimaxillary-cohort these statistical findings were found to be reinforced, with a p-value of 0.015 determined (Figure 20).

In contrast, no such differences within the BSSO-cohort were found to occur (p=0.991).

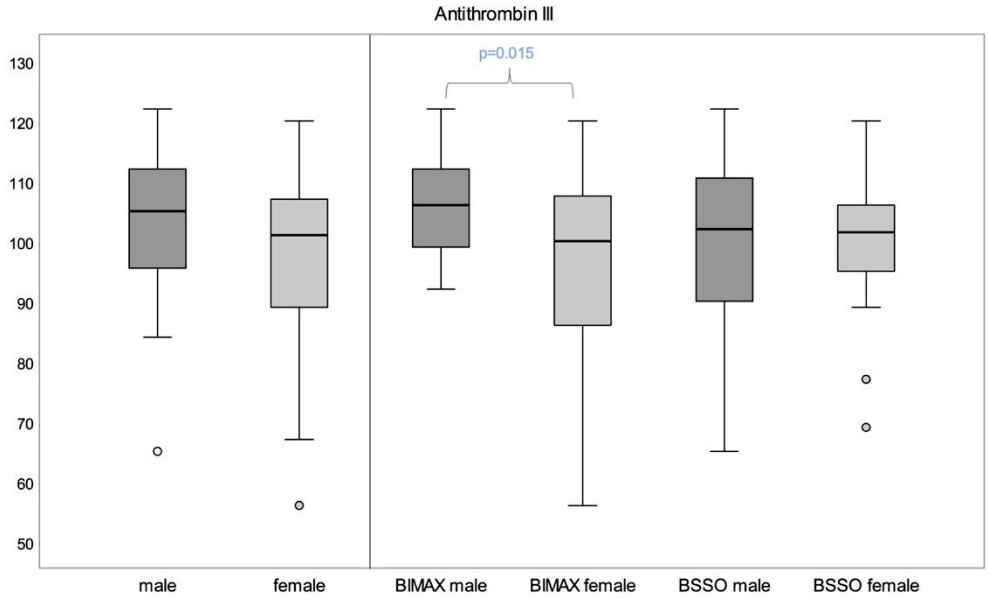

Figure 20: In terms of Antithrombin-III (AT-III), relevant differences between the male and female gender in the bimaxillary-cohort resulted.

3.4.2.6 Von Willebrand factor (vWF)

3.4.2.6.1 vWF: Ag – quantitative analysis of von Willebrand factor (vWF)

In terms of the quantitative analysis of vWF, no differences between male and female gender were revealed (p=0.148). In women vWF: Ag amounted to 89.41 (± 31.04); in men this parameter came to 99.77 (± 37.44), (Figure 21).

Similar results were shown when additionally considering the surgical method (BIMAX: p=0.198; BSSO: p=0.496).

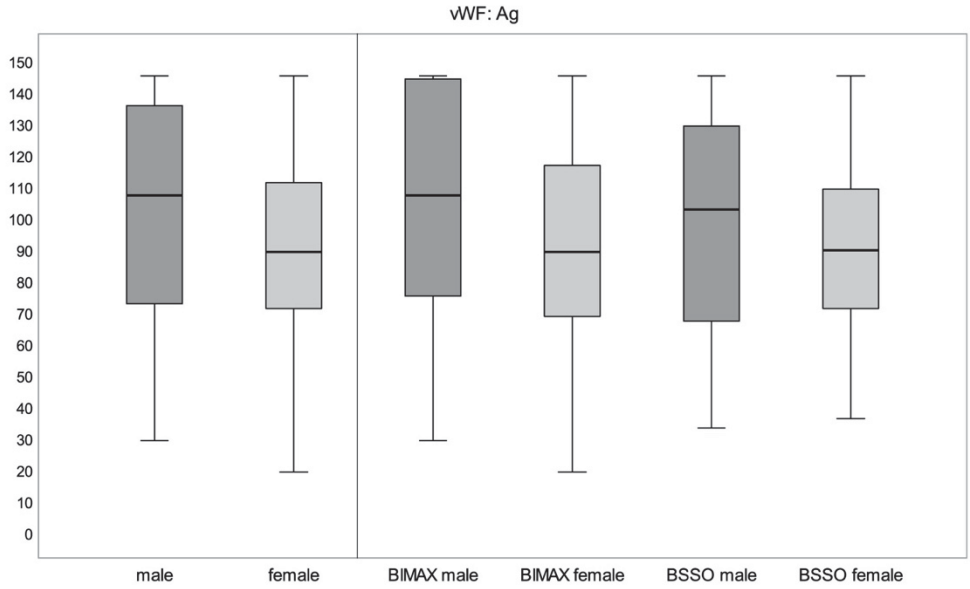

Figure 21: vWF: Ag (%) and patient gender, von Willebrand Factor – quantitative analysis

3.4.2.6.2 vWF: Akt.L (vWF-activity)

Statistically significant differences with reference to the vWF-activity in terms of male and female gender were found to occur (p=0.032). In males, higher activity of vWF in comparison with women was shown (male: 105.26% ±74.59; female: 80.97% ±34.27).

Interestingly though, these statistical differences between male and female gender were voided within the more detailed analysis according to the surgical technique (BIMAX: p=0.064; BSSO: p=0.272), (Figure 22).

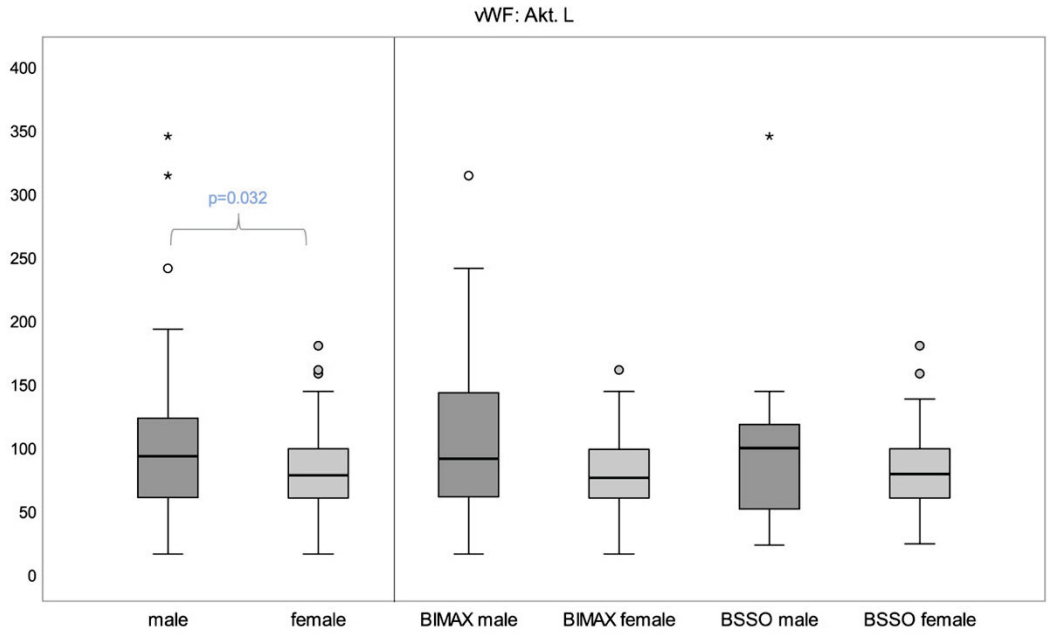

Figure 22: vWF: Akt. L (%); von Willebrand Factor - qualitative analysis

3.4.2.7 Coagulation factor VIII (FVIII)

With respect to coagulation factor VIII, no differences among males and females were detected (p=0.210), (Figure 23). In men, levels of FVIII amounted to 80.41% (± 49.52); in females, negligibly reduced percentages of FVIII activity (70.14% ± 30.34) in comparison with men were observed.

Neither in the bimaxillary-cohort, nor the BSSO-cohort any relevant gender-specific differences were observed (BIMAX: p=0.149; BSSO: p=0.777).

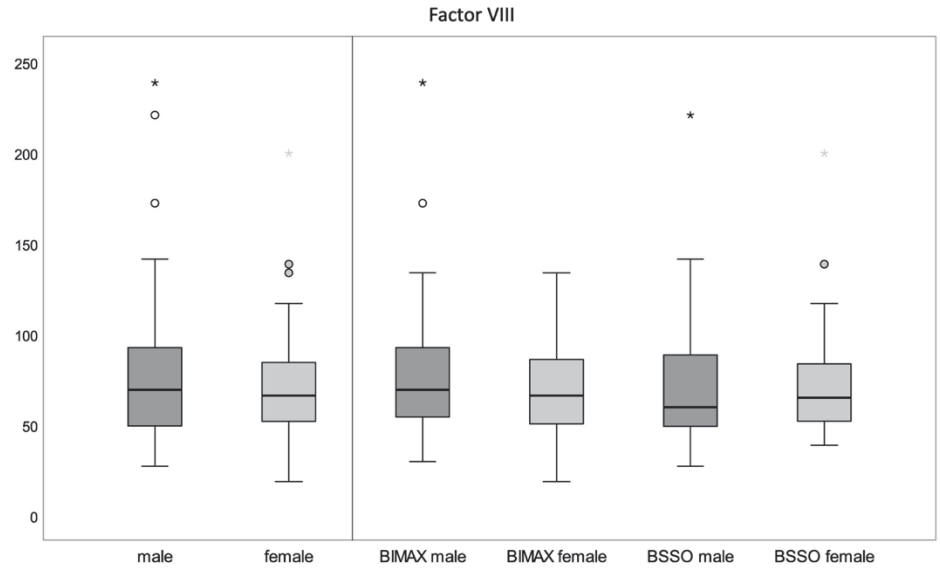

Figure 23: Coagulation factor VIII (%)

3.4.2.8 Coagulation Factor IX (FIX)

Coagulation factor IX, being associated with haemophilia B, did not differ significantly with regards to patient gender when combining both surgical techniques for statistical analysis (male 97.09 ± 20.75; female 91.52 ± 19.16; p=0.186), (Figure 24).
Within the bimaxillary-cohort, though, statistical tendency towards significantly higher levels of FIX were observed, with a p-value of 0.053 detected (male 102.76 ± 22.39; female 90.83 ±19.46). No such differences regarding the BSSO-cohort were determined (0.745).

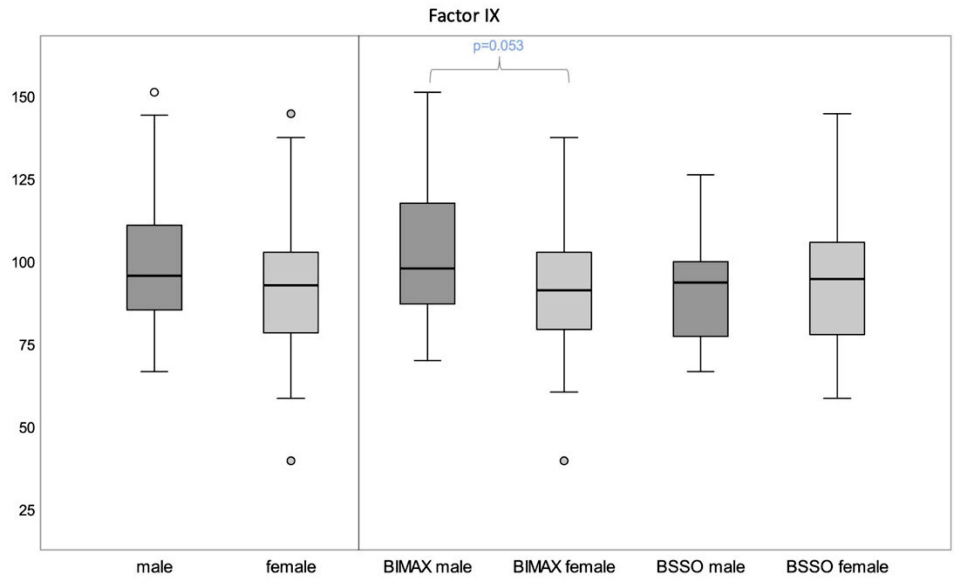

Figure 24: Coagulation factor IX (%)

3.4.2.9 Coagulation factor XI (FXI)

Overall, no relevant gender-specific differences between the male and female gender were detected with respect to factor XI (p=0.418), (Figure 25).

This conforms to the results found within the analysis of the bimaxillary-cohort (p=0.907) and the BSSO-cohort (p=0.263).

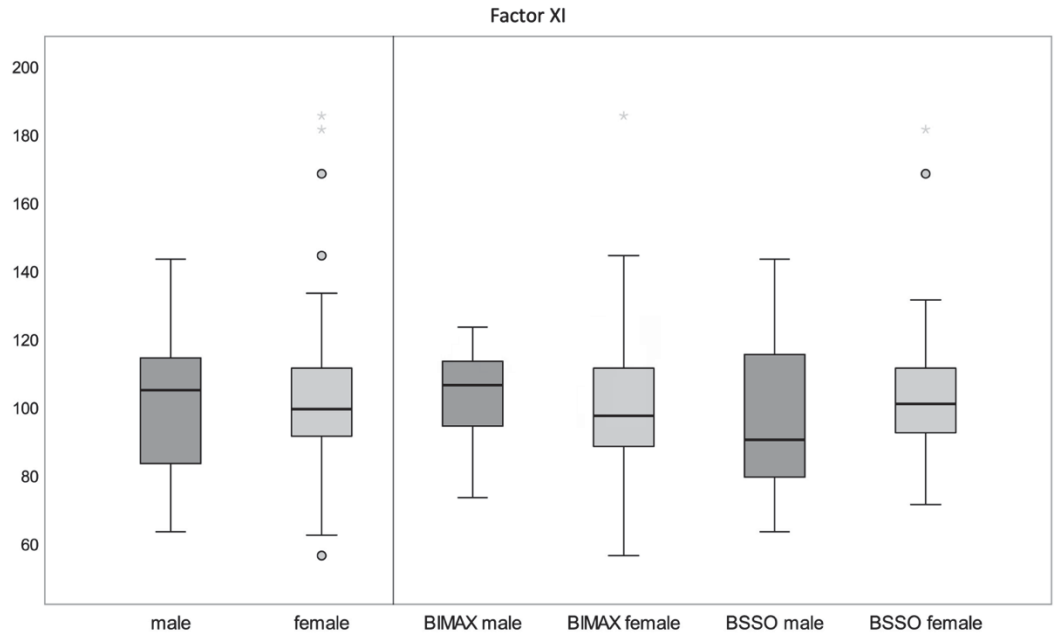

Figure 25: Coagulation factor XI (%)

3.4.2.10 Coagulation factor XIII

Detailed assessment revealed activated factor XIII to be within normal range for both the male and female gender (Figure 26). FXIII amounted to 117.9 (± 20.9) in female patients and 116.1 (± 25.1) in male patients. No statistically significant gender-related differences were found in this context (p=0.691).

A subgroup analysis taking into account the treatment modality applied, confirmed no statistically significant differences among males and females in this regard (BIMAX: p=0.385; BSSO: p=0.670). In women, the lowest level of factor XIIIa recorded amounted to 68; in men a minimum in terms of factor XIIIa of 63 was observed.

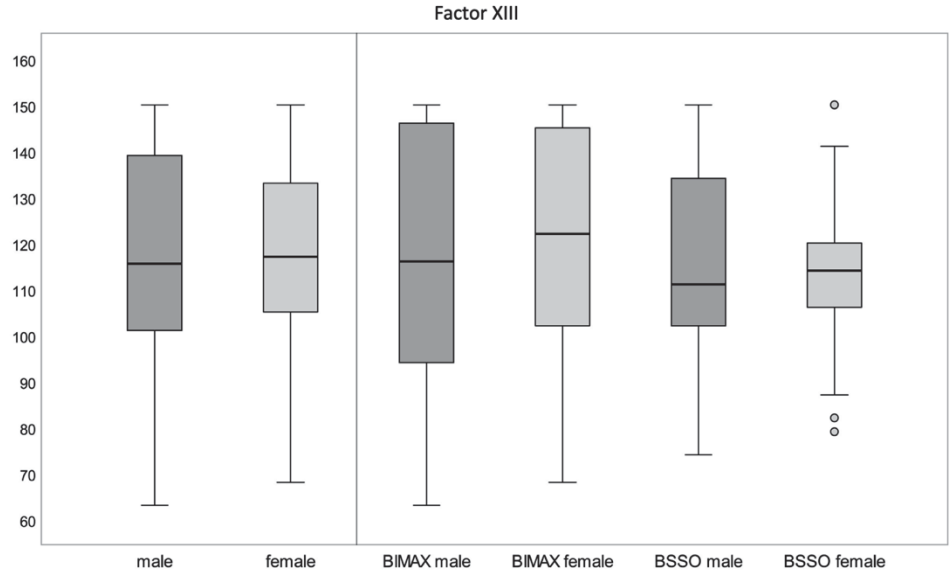

Figure 26: Coagulation factor XIII (%)

3.4.3 Endogenous Thrombin Potential (ETP)

The thrombin generation potential was measured preoperatively, on the day before surgery. Four parameters referring to the total thrombin formation and the peak height measurement were assessed according to gender and the surgical modality applied.

3.4.3.1 ETP- A area under the curve (%) (ETP-A-auc)

ETP-A-auc, indicating the total amount of thrombin formation, was shown to be within the normal range. As far as gender-specific differences go, no differences between male and female subjects were observed when pooling both surgical cohorts (male 84,48% ± 22.5; female 91.65% ± 19.46; p=0.108), (Figure 27).

Within the bimaxillary-cohort similar findings were shown, with no statistically significant differences detected (p=0.618).

In the BSSO-cohort, however, a statistical tendency towards significantly elevated levels of ETP-A-auc in female patients was observed (male 81,93% ± 25.15; female 93.74% ± 14.65; p=0.053).

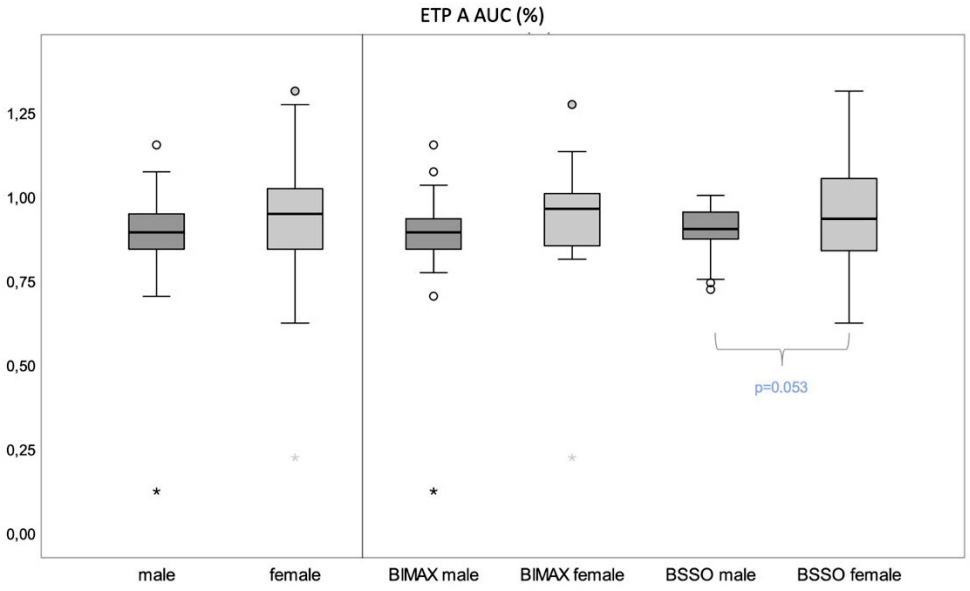

Figure 27: ETP-A-auc (%), net amount of thrombin formation

3.4.3.2 ETP- A-Cmax. (%) – thrombin-peak height

With regards to the peak height of the endogenous thrombin potential measured in the current study, overall, no gender-specific differences were detected (male 97.91% ± 32.53; female 96.49% ± 22.27; p=0.802), (Figure 28).

Similar statistical findings were drawn from the subgroup analysis according to the treatment modality applied (BIMAX p=0.167; BSSO p=0.237).

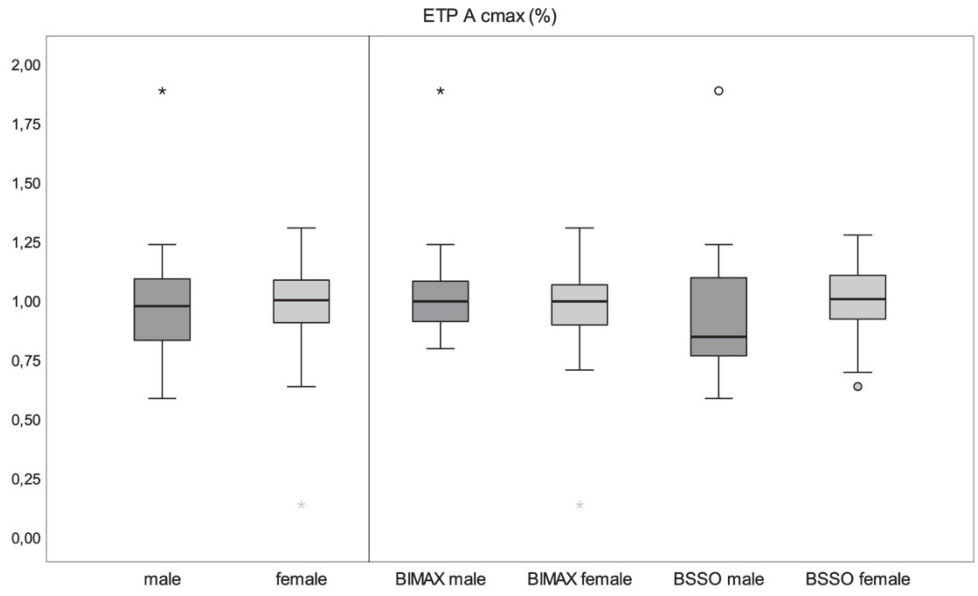

Figure 28: ETP-A-Cmax (%), peak height of thrombin.

3.4.3.3 ETP- B Area under the curve (%) (ETP-B-auc)

Regarding the measurement of ETP-B-auc, statistically significant differences between male and female subjects were detected (Figure 29). Within the pooled analysis of both treatment modalities combined, a significant p-value of 0.039 was determined, with females showing higher levels of ETP-B-auc (89.16 ± 16.01) than males (81.74 ± 17.87).
These gender-related findings were reinforced when solely considering the BSSO-cohort (p=0.018).

In the bimaxillary group no such differences were found to occur (p=0.589).

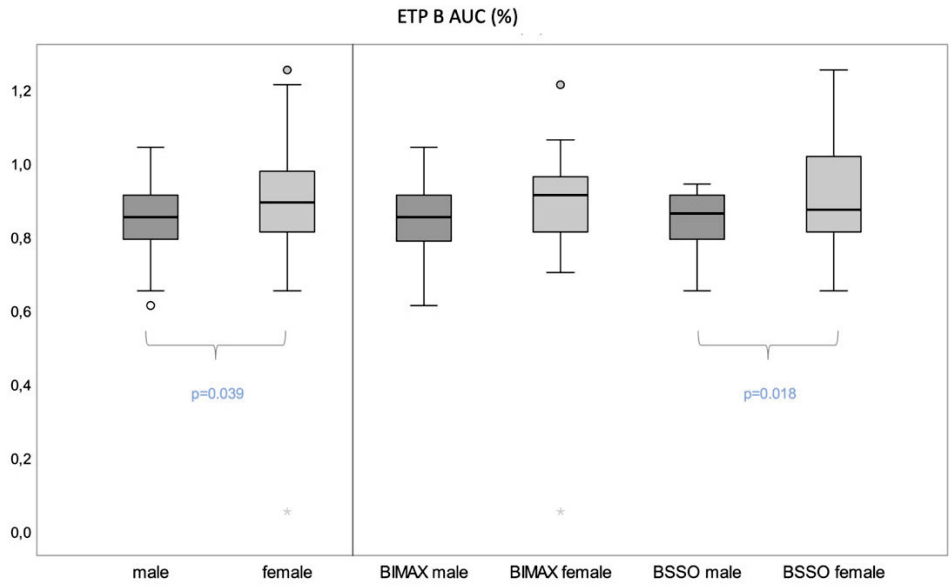

Figure 29: ETP-B-auc (%), net amount of thrombin generation.

3.4.3.4 ETP- B-Cmax. (%)

Turning now to the peak height measurement in terms of ETP-B-Cmax no relevant statistical differences between male and female patients were found in any of the comparisons made (Figure 30). Within the pooled analysis a p-value of 0.454 was determined.

In the bimaxillary-cohort and the BSSO-cohort, p-values amounted to 0.753 and 0.270, respectively.

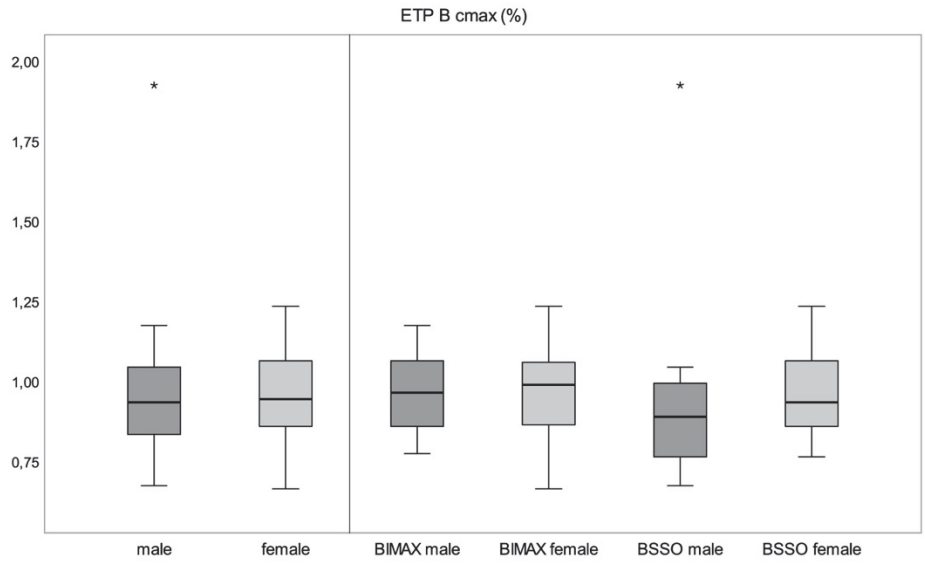

Figure 30: ETP-B-Cmax (%), peak height of thrombin.

3.4.4 Summarised Results

3.4.4.1 Overall

Both treatment modalities combined the following parameters were found to differ statistically significantly between the male and female gender:

- Haemoglobin ($p<0.001$); (m>f)
- Haematocrit ($p<0.001$); (m>f)
- aPTT ($p=0.002$); (m>f)
- vWF:Akt.L ($p=0.032$); (m>f)
- ETP-B auc ($p=0.039$); (f>m)

3.4.4.2 Bimaxillary-cohort

Within the bimaxillary-cohort the following parameters were found to differ statistically significantly between men and women:

- Haemoglobin ($p<0.001$); (m>f)
- Haematocrit ($p<0.001$); (m>f)

- Antithrombin-III (p=0.015); (m>f)

- Coagulation factor IX (p=0.053); (m>f), *statistical tendency*

3.4.4.3 BSSO

Regarding the BSSO-cohort, statistically significant differences referring to the following parameters were found:

- Haemoglobin (p<0.001); (m>f)
- Haematocrit (p<0.001); (m>f)
- aPTT (p=0.002); (m>f)
- ETP-B-auc (p=0.018); (f>m)

- ETP-A-auc (p=0.053); (f>m), *statistical tendency*

3.5 Correlation analysis

3.5.1 Gender-related differences in blood parameters and their influence on blood loss

In a further step, blood and coagulation parameters differing statistically significantly among males and females or showing a clear statistical tendency in this regard were correlated to blood loss relative to the treatment modality applied. All of the other blood and coagulation parameters assessed earlier were excluded from this analysis. Furthermore, the parameters haemoglobin and haematocrit were also left out, as from a scientific point of view, correlation analysis would not have added any value in this context.

For each of the parameters, which differed significantly in terms of patient gender, a correlation analysis between respective parameters and IOB was performed. In addition to that, these parameters were also correlated to the perioperative blood loss, measured 48-hours postoperatively (CBL-48h).

3.5.1.1 Bimaxillary-cohort

3.5.1.1.1 Intraoperative blood loss (IOB)

Within the bimaxillary-cohort Antithrombin-III (AT-III) was shown to differ statistically significantly between men and women, with men showing elevated levels in comparison with women (p= 0.015).
When further investigating effects of Antithrombin-III on the intraoperative blood loss in bimaxillary surgery no significant correlations between IOB and the level of AT-III were found (r=0.148; p=0.306). This was also true when analysing the data according to gender.

Furthermore, relevant gender-specific differences in terms of factor IX were shown (p=0.053) when comparing respective levels in male and female patients. Within the correlation analysis, however, no correlations with the intraoperative blood loss in any of the comparisons made were found to occur.

3.5.1.1.2 Calculated blood loss (CBL-48h)

While the level of Antithrombin-III was not shown to affect IOB, effects on the perioperative blood loss (CBL-48h) were observed. In this regard, blood loss appeared to be significantly affected by the level of AT-III (r=0.474; p=0.001). When additionally taking into account patient gender, statistically significant correlations between AT-III and CBL-48h were confirmed (r=0.439; p=0.013) in terms of the female gender (Figure 31). In contrast, no such correlations in males resulted.

In terms of factor IX, no correlations between CBL-48h and this coagulation parameters were found.

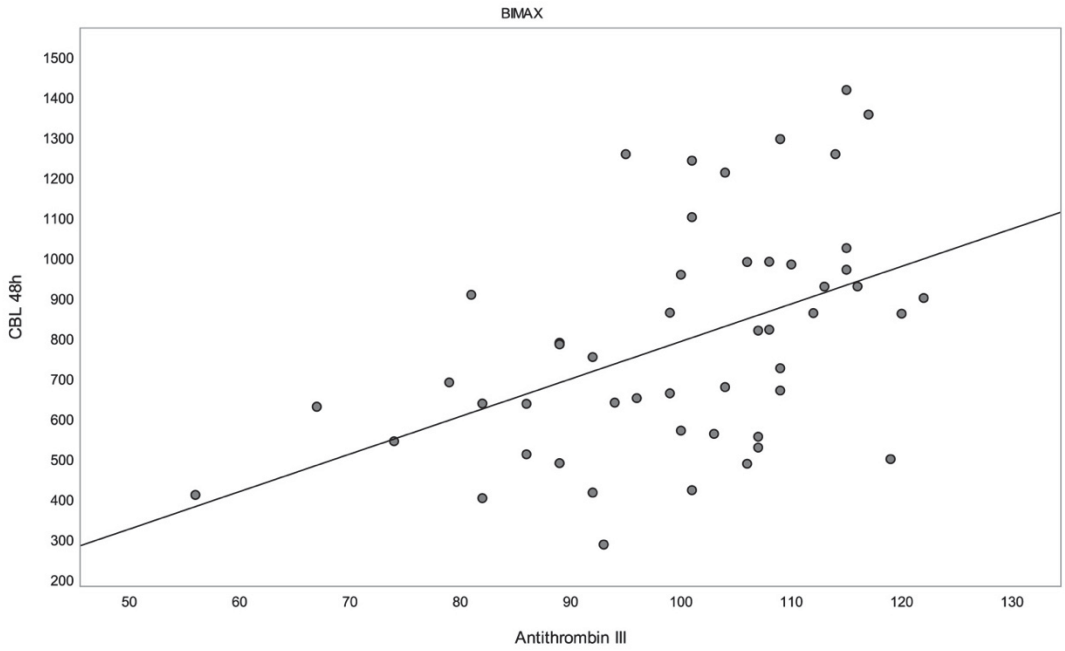

Figure 31: Correlation analysis between AT-III and CBL-48h

3.5.1.2 BSSO-cohort

In the BSSO-cohort, relevant gender-specific differences relative to the parameters ‘aPTT’, ‘ETP-A-auc’ and ‘ETP-B-auc’ were shown.

Within the correlation analysis, no statistically significant correlations between the IOB and any of the aforementioned parameters were observed.

Similarly, no correlations between CBL-48h and aPTT, ETP-A-auc and ETP-B-auc resulted.

3.5.2 Patient characteristics and blood loss

3.5.2.1 Operation time and blood loss

Statistically significant correlations were detected when correlating the length of the procedure with the bleeding volumes IOB and CBL-48h. This was done separately for the bimaxillary- and the BSSO-cohort to ensure valid statistical results.

3.5.2.1.1 Bimaxillary surgery

3.5.2.1.1.1 Intraoperative blood loss

In the bimaxillary-cohort, the length of the surgical procedure was shown to statistically significantly correlate with the amount of the IOB (r=0.477; p<0.001). When further taking into account a patient's gender, similar results were obtained. The operating time was shown to significantly affect the amount of IOB in both male and female patients (male: r=0.582, p=0.004; female: r=0.342, p=0.055), (Figure 32).

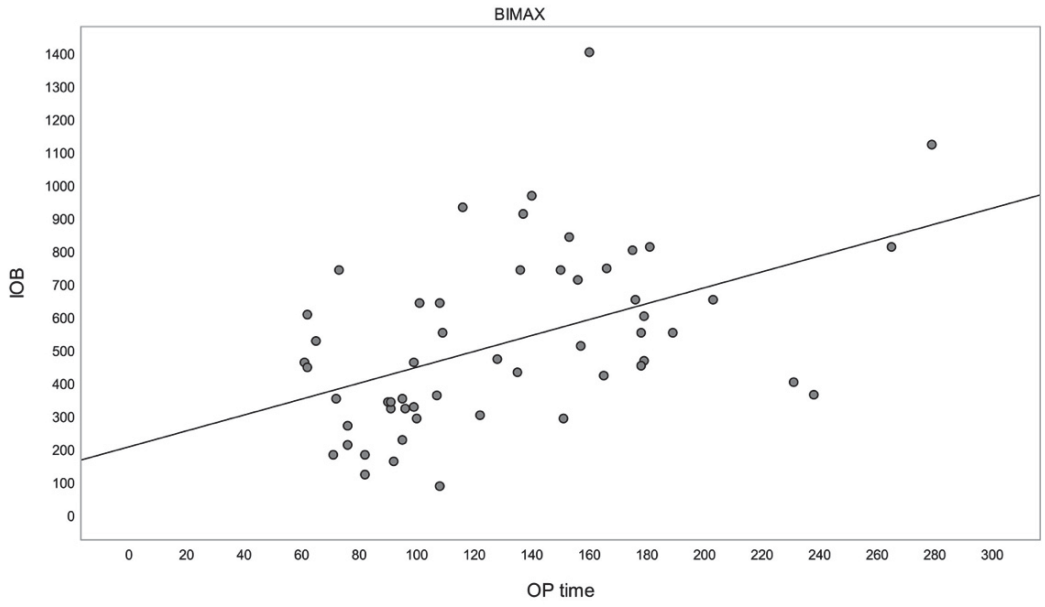

Figure 32: Figure to show the statistically significant correlation between the length of the procedure and the amount of intraoperative blood loss determined.

3.5.2.1.1.2 Perioperative blood loss (CBL-48h)

In terms of CBL-48h statistically significant correlations with the operating time were observed, when analysing the data without sub-specifying the bimaxillary-cohort according to gender (r=0.307; p=0.024), (Figure 33). These correlations were voided, when adding the factor 'gender' to the analysis.

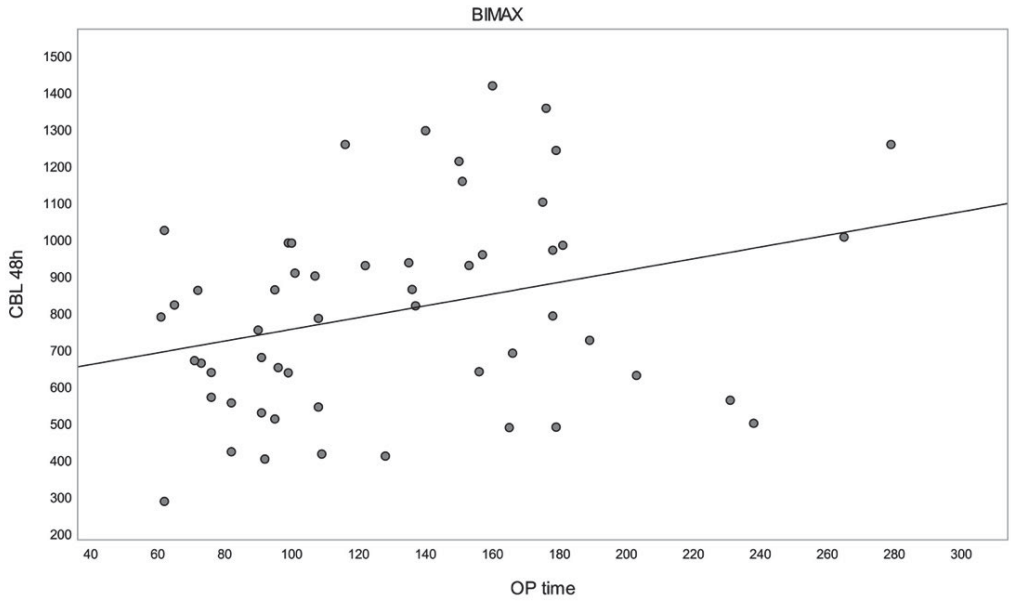

Figure 33: Figure to show the statistically significant correlation between the length of the procedure and the amount of perioperative blood loss (CBL-48h).

3.5.2.1.2 *BSSO*

3.5.2.1.2.1 *Intraoperative blood loss*

With regards to BSSO, the operation time (OT) was shown to statistically significantly correlate with the parameter 'IOB' (r=0.485; p<0.001), (Figure 34).

In terms of the male gender, no statistically significant correlations between OT and IOB within the BSSO-cohort were detected (r=0.438; p=0.090); in contrast, the intraoperative blood loss in females was shown to increase with the length of the surgical procedure. In this regard, a statistically significant correlation-coefficient of r=0.562 was determined (p<0.001).

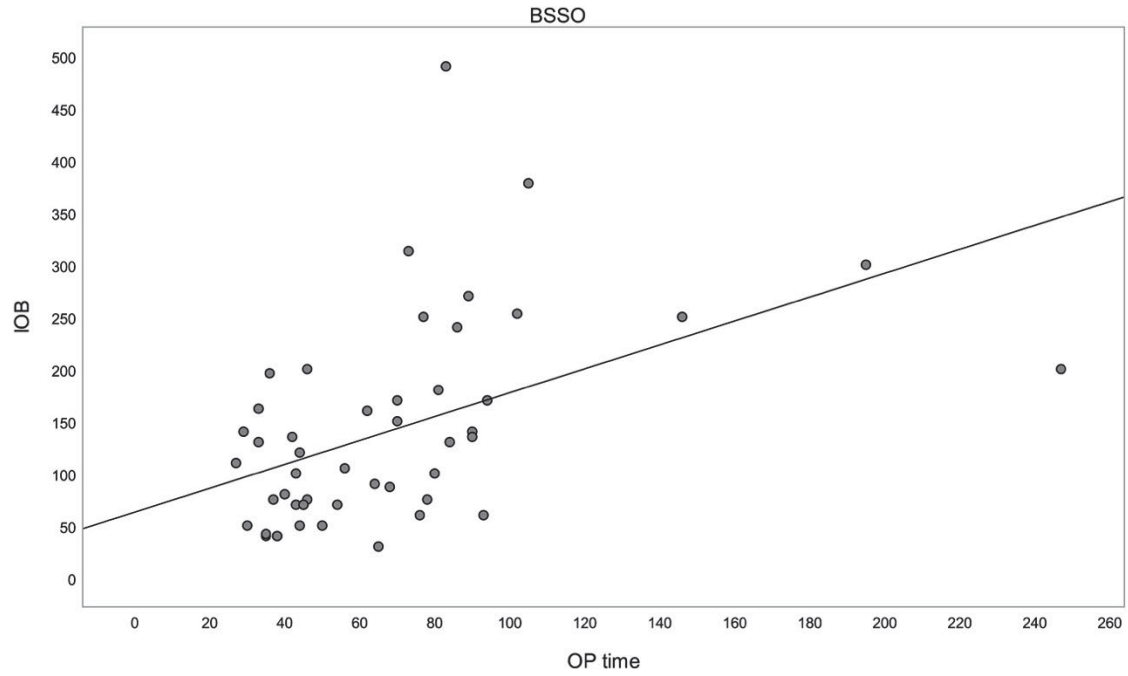

Figure 34: Figure to show the statistically significant correlation between the length of the procedure and the amount of intraoperative blood loss determined in the BSSO-cohort

3.5.2.1.2.2 *Perioperative blood loss (CBL-48h)*

Looking now at CBL-48h in the BSSO-cohort, a statistically significant correlation with the operation time in females was shown (r=0.439; p=0.011). No other correlations were found to occur.

3.5.2.2 Age and blood loss

When focusing on a patient's age according to patient gender and the treatment modality applied, no relevant correlations were found to occur, in terms of the parameters IOB and CBL-48h.

3.5.2.3 BMI and blood loss

Orthognathic blood loss did not appear to be affected by the patient's BMI according to our findings. This is because no statistically relevant correlations in any of the analyses made in this regard were established. Hence, neither male or female blood loss in terms of IOB and CBL-48h were shown to be influenced by a patient's BMI.

3.6 Regression analysis

Two regression analyses were performed, the first of which was performed relative to IOB. Coagulation parameters, differing statistically significantly between male and women were included in this analysis. What is more, those factors were considered, which were commonly described to affect blood loss in orthognathic surgery: gender (38, 69), operation time (37), age (78) and BMI (70).

The second regression analysis was performed on the basis of CBL-48h as the dependent variable.

3.6.1 Bimaxillary-cohort

In terms of the coagulation parameters analysed Antithrombin-III and coagulation factor IX, were identified as differing significantly between male and female gender. Hence, these two parameters were included in the regression analysis in the bimaxillary-cohort.

3.6.1.1 Intraoperative blood loss (IOB)

Regarding IOB (r-squared= 0.34), the patient's age ($p=0.044$) and the length of the surgical procedure ($p<0.001$) were found to be relevant. Gender, BMI, Antithrombin-III and Coagulation factor IX did not affect the intraoperative blood loss in this group.

3.6.1.2 Perioperative blood loss (CBL-48h)

With reference to CBL-48h (r-squared= 0.31), higher levels of Antithrombin-III were identified as having contributed to a surge in bleeding volumes. As opposed to IOB, the operating time and age did not affect blood loss in this analysis.

3.6.2 BSSO-cohort

Those parameters that showed statistically significant differences relative to gender were included in this analysis (aPTT, ETP-A-auc, ETP-B-auc). Similar to the bimaxillary group, the (38, 69), operation time (37), age (78) and BMI (70) were added to the analysis.

3.6.2.1 Intraoperative blood loss (IOB)

In terms of the intraoperative blood loss (r-squared= 0.31) the operation time was found to statistically significantly correlate with the amount of blood loss in this regard (IOB: p=0.002). No other correlations were identified with respect to the parameters chosen.

3.6.2.2 Perioperative blood loss (CBL-48h)

Similar to IOB, the operation time was found to be of relevance when focusing on CBL-48h (r-squared= 0.21; p=0.017)

4. DISCUSSION

Orthognathic surgical procedures are performed in highly vascularised midfacial areas and have, therefore, frequently been associated with excessive intra- and perioperative bleeding (47). Large bleeding volumes linked to orthognathic surgery are known to confer additional risks and negative side-effects and as such, great efforts have been made within this surgical field to try to reduce and predict the amount of blood loss (32, 34, 68, 69, 71).

With regards to the literature on this topic, numerous indicators and factors affecting the amount of blood loss related to orthognathic surgery, have been highlighted (68-71, 78). Though, very controversial findings have resulted. This can, among other things, be attributed to variability in terms of the study design chosen and the differing methodology applied, as far as the time point and the method to measure blood were concerned (58, 67, 68, 70). What is more, pooling of different surgical techniques and the use of varying perioperative measures and protocols, such as the administration of antifibrinolytics or similar, may have additionally added to the complexity in terms of comparing and interpreting study results (112). Against this backdrop, we aimed to analyse orthognathic blood loss in a homogenous study cohort in a clear and systematic manner, with a specific emphasis on patient gender. Additionally, our scientific focus was directed at identifying gender-related differences in terms of blood parameters and the haemostatic profile. In a further step, statistically significantly differing parameters were correlated with the amount of blood loss. This was done to study the effect of gender-related differences on orthognathic blood loss.

4.1 Blood loss

Within this study, blood loss was monitored by means of two well-established methods in the context of orthognathic surgery (38, 58, 68, 70). Three different time-points were chosen to gather detailed information on the amount of intra- and perioperative blood loss in connection with bimaxillary surgery and BSSO.

Blood loss was measured using the ‘subtraction method’ and applied immediately after mucosal closure had been performed. At this point, the amount of irrigation fluid used was subtracted from the total amount of fluid in the suction canister. In addition to that, the weight difference, with regards to surgical gauze, and the throat pack used were added to the amount of blood loss determined. This was done in line with the methodology used by Kretschmer, Secher and Ueki (38, 58, 62).

24- and 48-hour postoperative blood loss was calculated by means of the haemoglobin-balance method, recently highlighted as the most accurate method to determine

perioperative blood loss in knee arthroplasty (80). Calculation of blood loss with the aid of this formula requires prior estimation of the patient's total blood volume, which was established using Nadler's formula (84).

With regards to the amount of blood loss detected in our study, statistically significant differences between the bimaxillary-cohort and the BSSO-cohort were observed in all of the measurements performed. A steady increase in terms of the bleeding volumes was observed in both of the procedures analysed, referring to the different timings of the measurements. Unsurprisingly, patients undergoing bimaxillary surgery were found to bleed statistically significantly more in comparison with BSSO. One of the major contributing factors in this regard appeared to be the length of the surgical procedure. Completion of bimaxillary surgery took almost twice as long as that of BSSO, with a mean surgical time of 131.2 min (± 52.3) in the bimaxillary group and 68.1 min (± 40.5) in the BSSO-cohort ($p<0.001$).
Bimaxillary surgery necessitates a combination of two surgical procedures, which inevitably contribute to the complexity of the surgery and imply a wider surgical exposure of the midface. In addition to that, orthognathic surgical procedures involving the maxilla have regularly been associated with increased blood loss due to the abundant vascular anatomy and the limited accessibility to the source of bleeding (45). This also added to the vastly diverging bleeding volumes detected in our study, when comparing bimaxillary surgery with BSSO. These findings conform to those of other studies reporting on blood loss in orthognathic surgery and underline the importance of a homogeneous study population for statistical analysis of blood loss in terms of the surgical technique applied (37, 68, 70). What is more, they strongly support the hypothesis that pooling different surgical techniques will have a significant impact on the absolute bleeding volumes determined.

With reference to the bimaxillary-cohort a mean intraoperative blood loss of 520.5 ml (± 266.7) was found to occur in our study, when measuring blood loss according to the 'subtraction method'. These findings correspond to the ranges observed in other studies, using a similar methodology. In a study conducted by Secher et al., specifically assessing blood loss in bimaxillary surgery, detected an average blood loss of 403ml. Most of the Le-Fort-I osteotomies performed in their study included sectioning of the maxilla into four parts (38). Thastum et al., stated an average blood loss of 479ml (±325) with regards to bimaxillary surgery, however, it did not take into consideration the weight difference of surgical gauze and the throat pack (70). In addition to that, Le-Fort-I osteotomies were performed in three pieces in their study. Sectioning of the maxilla, as performed in the aforementioned studies, very likely affected the length of the procedure and therefore may have increased the amount of blood loss. Although the mean surgical time was found to range from 220 and 254 minutes

in respective studies, a significantly shorter amount of time was needed in our study to perform bimaxillary surgery (131 min) (38, 70). No antifibrinolytics but hypotensive anaesthesia were administered in the studies highlighted, which conforms to the methodology applied by our group. Significantly higher average intraoperative bleeding volumes were determined by Apipan et al., reporting a blood loss of 1177.5 ml in their study cohort without the use of antifibrinolytics (107). While this seems excessive, these results give some indication on the variability in terms of blood loss in bimaxillary surgery.

As previously mentioned, significantly lower bleeding volumes with respect to BSSO, with a mean intraoperative blood loss of 140.8 ml (± 96.7 ml), were found to occur. When comparing our findings to relevant literature on this topic, the bleeding volumes determined in our study appeared to be at the lower end of the spectrum (37, 57, 70). In this regard, Thastum et al. observed a blood loss of 196 ml (± 99) (70). Andersen et al. reached a very similar result with a blood loss of 197ml (±102) (57). In contrast, Schneider et al. observed a bleeding volume of over 400 ml (37). In terms of the operating time spent to complete the procedure, remarkable differences were observed: average operating times ranged from 101 to 178 minutes in respective studies, whereas completion of BSSO only amounted to 68min in our study. Hence, we claim that the decreased operating time observed in our study had a favourable effect on the amount of blood loss determined.

Issues with regard to the ‘subtraction method’ used to measure the intraoperative blood loss in the present study, particularly refer to the risk of underestimation of the actual amount of blood loss (62, 76). This is because bleeding into tissue spaces and the maxillary sinuses is not accounted for. Furthermore, this method does not incorporate blood loss after wound closure has been performed. It has previously been shown in other surgical specialties that the amount of undetected surgical blood loss may be significant in causing a variety of negative side-effects. In this context and despite low intraoperative bleeding volumes recorded, patients have frequently been found to develop postoperative anaemia. In more severe cases, even blood transfusions were found to be necessary (115, 116). Hence, we additionally applied a second, commonly used approach to monitor blood loss in orthognathic surgery that better incorporated undetected bleeding volumes, subject to the timing of the measurement. The ‘haemoglobin-balance method’ used for this purpose, determines blood loss based on pre- and postoperative levels of haemoglobin as well as the patient’s estimated total blood volume (80).

Blood loss was calculated 24- and 48-hours after surgery, by means of the aforementioned method. Similar to other studies in this field, neither blood transfusion nor admission to the ICU were necessary (58-60).

24-hours postoperatively, an average blood loss of 667.5 ml (± 296.5 ml) was determined with regards to the bimaxillary cohort; 48-hours after surgery the blood loss calculated amounted to 802.9 ml (± 275.3ml). With reference to the BSSO-cohort, a calculated blood loss of 298 ml (± 235.9 ml) and 495.2 ml (± 267.6 ml) for respective time-points was observed. On the basis of this significant increase in terms of the bleeding volumes in the first 24- to 48-hours after surgery, we suggest that the timing of the measurement is of utmost clinical importance.

Similar to our study, higher bleeding volumes were determined in related clinical using the same methodology, in comparison with studies in which the 'subtraction method' was used (57, 67, 70). Choi et al. standardly measured blood loss 48-hours postoperatively using a very similar formula based on the levels of haemoglobin (67). The time-point was chosen as normalised blood volumes were assumed at this stage. Bimaxillary surgical procedures were assessed in their study, however, variability, in terms of the actual techniques applied, was observed. What is more, their group relied on a different approach to estimate the patient's total blood volume, not taking into account the patient's height. An average bleeding volume of 1173.6 ml (± 700.1 ml) was reported (67). Stehrer et al., aiming to analyse and reliably predict blood loss in orthognathic surgery used the exact same approach in terms of the formulae to calculate blood loss and estimate a patient's total blood volume (68). In contrast to our study and that of Choi et al.'s though, Stehrer did not provide any details on the timing of the measurement (68). Blood loss in their study amounted to 494.1ml (± 223.4ml) with regards to bimaxillary surgery; when looking at the BSSO-cohort an average blood loss of 291.6ml (±167.7ml) was stated (68). While these differences in terms of the study design highlighted above appear to be subtle, divergences regarding the time-point used might have considerably affected absolute bleeding volumes determined. This is an important point to consider when comparing and interpreting respective study results.

With respect to other surgical specialties, several authors have explicitly focused on the analysis of the parameter 'hidden blood loss' (HBL), indicating the amount of undetected blood loss (115, 116, 144). Absolute differences between the intraoperative and perioperative blood loss calculated postoperatively were recorded for this purpose. Inconsistencies, in terms of the methodology applied to determine HBL can be found, when looking at the aforementioned investigations in more detail. However, all of these studies came to a similar conclusion: hidden blood loss accounts for a significant amount of determined perioperative blood loss (115-117, 144, 145). In this regard, Sehat et al. found

that a percentage share of 49% of the total perioperative blood loss calculated in knee arthroplasty was attributed to the parameter ‘hidden blood loss’ in their study (116). Ogura et al. analysed the hidden blood loss occurring in spine surgery by applying various formulae to calculate blood loss. Relevant differences were observed regarding the amount of the determined hidden blood loss, depending on the formula used to calculate the perioperative blood loss. In terms of the timing, the last blood sample before discharge was used for calculation of the perioperative blood loss (115). In contrast, Yang et al. relied on the lowest level of haematocrit recorded during the first five days after surgery to calculate the perioperative blood loss (144).

In our study, the hidden blood loss was calculated by subtracting the amount of the intraoperative blood (IOB) from the determined perioperative blood loss on the second postoperative day (CBL-48h). No statistically significant differences between the two treatment modalities applied were found to occur, with a mean HBL of 282.4 ml (± 244.8 ml) in the bimaxillary group and 354.3 ml (± 258.6 ml) in the BSSO-cohort. However, when further considering the percentage share of HBL in relation to CBL-48h, significant differences were observed: whereas HBL amounted to 35.2% regarding bimaxillary surgery, a percentage share of 71,5% of CBL-48h was attributed to HBL in the BSSO-cohort. These findings indicate, firstly, that a certain amount of blood loss remains undetected during orthognathic surgery and secondly, that after completion of surgery a certain amount of blood loss is to be expected. From our point of view, consideration of HBL appears to be particularly relevant in the context of orthognathic surgery, as more and more orthognathic procedures are currently performed in a day-surgical setting.

We acknowledge that the use of surgical drains might be helpful in terms of monitoring blood loss in the immediate postoperative period. In this regard, Ogura et al. even demonstrated, that the amount of blood loss monitored with the aid of surgical drains exceeded the amount of HBL calculated by means of formulae (115). While this is an interesting fact to consider, blood loss after spine and orthognathic surgery might not be comparable in this context. This is because surgical drainage in orthognathic surgery does not include bleeding into the maxillary sinuses nor from the nose, in addition to the blood volume being swallowed after surgery. Furthermore, issues regarding this method, including blockage and displacement of the surgical drain, have regularly been reported, which can aggravate underestimation of blood loss in orthognathic surgery.

Various studies added the use of antifibrinolytics to their perioperative protocol, which led to a significant decrease in the intra- and perioperative blood loss determined (34, 35, 38, 67). In this regard, most studies investigated the impact of intravenous tranexamic acid, administered preoperatively, on the aforementioned bleeding volumes (34, 35, 38, 67). While

blood loss reduction by means of tranexamic acid has been verified, the effect on HBL has not been investigated in the context of orthognathic surgery as of yet.
We therefore suggest that by additionally considering the amount of HBL, different dosing regimens and time points regarding the administration of antifibrinolytics may prove advisable to further reduce blood loss in orthognathic surgery.

The steady increase in blood loss in the first 48-hours after surgery, is corroborated by a significant decrease in haemoglobin- and haematocrit-levels observed in our study.
In terms of bimaxillary surgery, haemoglobin-levels were shown to decrease by 2.51 g/dl (± 0.89) in the first 48-hours after surgery; with regards to BSSO a less significant haemoglobin-drop of 1.58 (± 0.86) was observed. Levels of haematocrit were reduced by 7.06% (± 2.67) in the bimaxillary-cohort and 4.27% (± 2.80) in the BSSO-cohort. These differences between bimaxillary surgery and BSSO are consistent with the statistically significantly bleeding volumes determined regarding the two treatment modalities applied.
Faverani et al. quoted a similar reduction with regards to the aforementioned blood parameters, when measured immediately after surgery (56). Salma et al, reviewed the haemoglobin-decrease associated with different orthognathic surgical interventions in their study, but did not provide any information on the timing of the blood-sample (56). While the drop of haemoglobin-levels as well as postoperative levels correspond to our results, estimated bleeding volumes determined by means of the 'subtraction method' were found to be lower in comparison to our study, especially in the bimaxillary group. Choi et al. taking blood samples 4-, 24- and 48-hours after surgery, demonstrated a significant decrease in comparison with preoperative levels of haemoglobin and haematocrit; however, when focusing on the three postoperative time-points, no on-going decrease of blood parameters was found to occur (67). These findings are in contrast to our results.

4.2 Common factors influencing blood loss

Among relevant factors interacting with blood loss in orthognathic surgery, the length of the surgical procedure, together with the treatment modality applied were entrenched in our study. This has already been highlighted as one of the major sticking points in the previous section of the discussion, revealing highly statistically significantly differences in terms of blood loss between the bimaxillary cohort and the BSSO cohort. The bleeding volumes detected and the operating time needed to complete the respective surgical procedure were shown to differ enormously when comparing the two treatment modalities. In addition to the prolonged operating time it can be assumed that blood loss was additionally increased by a combination of mandibular and maxillary surgery in the case of bimaxillary surgery. This in

turn, created a larger, more extended operating field in a well-vascularised environment of limited accessibility which inevitably resulted in higher bleeding volumes. Similar conclusions were drawn from other research on this topic (37, 41, 58, 68, 77).

To eliminate the risk of bias, in terms of factors influencing blood loss, the effect of the operation length on blood loss was further investigated relative to the treatment modality applied. A longer operation time was unequivocally found to trigger an increase in intraoperative blood loss with reference to both treatment modalities. In contrast, the operation time appeared to have less of an effect on the amount of perioperative blood loss calculated. Within a detailed analysis in this regard, it becomes obvious that the length of the surgical procedure solely affected CBL-48h related to the bimaxillary cohort. While these findings underline the relevance of the length of the procedure in terms of blood loss, they further enable new perspectives on this subject.

Among other patient characteristics, age has previously been discussed as a relevant factor to consider, however, it has not been found to correlate with blood loss in the present study (78). Similarly, increased levels of BMI, previously identified as a favourable factor to reduce the relative blood loss, were not shown to play an important role in our study cohort (70).

Numerous studies investigated the effect of gender on blood loss related to orthognathic surgical procedures. It has been proposed on various occasions that men are likely to bleed more, when it comes to blood loss reported in other surgical specialties (146-148). With regards to orthognathic surgery conflicting results were stated. Whereas most studies did not find any differences between male and female subjects in terms of the blood loss detected, few trials reported statistically significantly increased bleeding volumes in males in comparison to their female counterparts (38, 61, 69, 70, 78). All of these clinical trials focused on the intraoperative blood loss measured immediately after surgery by means of the subtraction method. All studies, but two, analysed gender-specific differences on the basis of bimaxillary surgery (70, 78). Thastum et al. included bimaxillary procedures, Le-Fort I osteotomies and BSSO in their study (70). Moenning et al. pooled numerous surgical techniques for statistical analysis, therewith potentially camouflaging the effect of influencing factors (78).

With regards to our study, gender-specific differences detected were confined to the bimaxillary group only, whereas within the BSSO-cohort no statistically significant differences between men and women in any of the comparisons made were found to occur.

Relevant gender-specific differences in terms of bimaxillary surgery referred to various parameters: among other findings to be highlighted and in terms of the parameter IOB an increased intraoperative blood loss in males was found (p=0.056). It has to be acknowledged, though, that the operating time in men undergoing bimaxillary surgery exceeded that reported in women by approximately 25 minutes. No statistically significant differences were reported in this regard (p=0.076), although, this might still have contributed to a more pronounced intraoperative blood loss associated with the male gender. Similar results were shown by Olsen et al., having defined gender as the primary predictor variable in their study (69). In this context, the male gender was found to be linked to a significantly higher intraoperative blood loss in a homogenous study cohort undergoing bimaxillary surgery. These findings were explained by relevant gender-specific differences, in terms of the patient's coagulative profile, meticulously analysed and displayed. However, and what is more, longer operating times in men by approximately 30 minutes may have additionally biased results in their study. Findings reported by Thastum et al., were consistent with those of Olsen et al., showing gender-specific differences, in terms of the intraoperative bleeding volumes, with respect to bimaxillary surgery (69, 70). In their study, operating times of men equalled those of women (70). Similar to our findings, no statistically significant differences, in terms of relative bleeding volumes, resulted.
Regarding the study conducted by Rummasak et al., significant differences in terms of gender-specific blood loss in patients undergoing bimaxillary surgery were observed (61). While men were found to bleed more with respect to the total amount of blood loss detected, women were shown to be linked to a higher blood loss relative to the defined acceptable blood loss (61). Secher et al. conducted a placebo-controlled clinical trial investigating the effects of perioperatively administered tranexamic acid on gender-specific blood loss related to bimaxillary surgery (38). Interestingly though, statistically significant differences between the male and female gender were confined to the intervention group, which received tranexamic acid. Study cohorts were comparable in terms of the operating time, however, no further gender-specific subgroup analysis with respect to the length of the surgical procedure was conducted. Potential differences in this regard may have considerably affected study results.

In addition to relevant gender-specific differences detected in terms of IOB, the male gender was found to be associated with a significantly increased perioperative blood loss measured 48-hours after bimaxillary surgery (CBL-48h). A mean difference between male and female gender of 176.9ml was reported (p=0.019). As this is the first study to additionally consider perioperative orthognathic blood loss in relation gender, no comparisons to other studies in this field can be drawn. With reference to other surgical specialties, however, similar findings

regarding perioperative blood loss were observed. Hu et al., analysing blood loss related to knee arthroplasty reported increased bleeding volumes associated with the male gender (146). In their study blood loss was measured by means of the Gross-formula based on pre- and postoperative levels of haematocrit. Three time points to assess blood loss were chosen: blood loss was calculated on the first, second and third postoperative day. Within the multivariate regression analysis gender was found to statistically significantly correlate with the amount of blood loss. Kajja et al., investigating bleeding volumes with reference to femoral fractures, calculated blood loss 72-hours after surgery (148). Their results also suggested increased bleeding volumes in males in comparison to females. Hence, our results are consistent with other studies on this topic. Intriguingly, when solely focusing on the parameters 'Hb-drop' and 'Hct-drop' determined in our study cohort, the aforementioned gender-specific differences are voided. A comparable gender-specific decrease in haemoglobin and haematocrit levels in the first 48-hours after surgery were observed. Similarly, when considering the patients' blood loss relative to the estimated total blood volume, no differences between the male and female gender were found to occur. Analysis of hidden blood loss has recently been explored in the context of orthognathic surgery (112). Studies conducted in other surgical specialties have identified gender-specific differences in this regard. This, however, has not been the case with our study population. Neither in the bimaxillary cohort nor the BSSO-cohort were any gender-related differences in terms of HBL detected.

Olsen et al. held gender-specific differences in terms of the patients' haemostatic profile accountable for statistically significantly higher intraoperative bleeding volumes detected in males undergoing bimaxillary surgery (69). In this regard, thromboelastographic results and markers for fibrin turnover were found to differ significantly between the male and female gender. On a related note, thromboelastographic analysis had previously been entrenched as a reliable predictor for blood loss in orthognathic surgery (71).

Secher et al., hypothesized that female sex hormones and oral contraception which can affect a patients' haemostatic profile were linked to a significantly decreased blood loss in women in comparison with men in their study (38).

4.3 Gender-specific differences in blood parameters and the haemostatic profile

To investigate the underlying principles of gender-specific differences in terms of blood loss in our study, numerous blood and coagulation parameters were analysed. This was done to identify relevant gender-related differences in terms of a patient's haemostatic status, which might further correlate with the bleeding volumes detected.

Overall, few gender-related differences in terms of the blood parameters assessed were found in our study population. Unsurprisingly, preoperative levels of haemoglobin and haematocrit were shown to differ significantly with respect to gender, as reported by Olsen et al. (69). From a clinical point of view, these findings were irrelevant for the following reasons: (1) the differences did not affect blood loss; (2) all participants were shown to be within their normal limits in terms of the abovementioned parameters determined preoperatively; (3) the decrease in haemoglobin- and haematocrit levels did not differ significantly between the male and female gender and (4) none of our patients required blood transfusions postoperatively.

In terms of the platelet count, females were shown to present with higher levels in comparison with males, lacking statistical significance (p=0.065). It is a well-established fact that gender interferes with the platelet-biology in terms of platelet count and function, with females faring better in this context (149). However, within the statistical subgroup analysis of our study cohort which additionally took into account the treatment modality, these subtle gender-specific differences were voided. We therefore concluded that the platelet count is not accountable for gender-related differences in terms of bleeding volumes determined in our study.

With regards to the preoperatively measured routine coagulation parameters aPTT, PT and INR distinct differences between the male and female gender were found to be specific to aPTT. In this regard, females were shown to present with a shorter aPTT in comparison with men. These findings were consistent with those reported by Fourel et al. (150). Within the subgroup analysis which additionally took the treatment modality into consideration, these differences between the male and female gender were confined to the BSSO cohort only, whereas in the bimaxillary cohort no such differences resulted.

APTT, which monitors the intrinsic coagulation pathway, has been shown to reliably detect severe to moderate deficiencies in terms of various coagulation factors, such as coagulation factors II, V, VIII, IX and XI (121, 122). Mild forms of factor deficiency, however, which may still be clinically relevant, are likely to remain undetected when using this coagulation test. In addition to that, aPTT has not been found to be sensitive to hypercoagulative disorders, associated with an elevated risk of thrombosis. This means that while moderate to severe hypocoagulative disorders, such as factor deficiency, are reflected in a prolonged aPTT, hypercoagulative disorders are not necessarily linked to a shorter aPTT and may present with an aPTT within its normal limits (121). Tripodi et al. concluded that this coagulation assay reacts more accurately to deficiencies of procoagulant factors than to those of their anticoagulant counterparts (138). Such examples include deficiencies in the natural

anticoagulants Antithrombin-III, Protein S and Protein C, which ultimately trigger procoagulant imbalance and lead to an increase in thrombin formation. This procoagulant imbalance is associated with an elevated risk of thrombosis. In these cases, however, the aPTT is within its normal range, instead of significantly shortened (138).

In cases where a prolonged aPTT is detected, haemostasis needs to be investigated further in terms of more specific coagulation tests, as it solely indicates a qualitative or quantitative issue related to the aforementioned coagulation factors (122). It should be borne in mind that the aPTT was initially introduced as a test to verify the suspicion of excessive bleeding and monitor the effects of anticoagulative drugs. Furthermore, when used within the preoperative assessment of young and healthy individuals, as was the case in the present study, results need to be examined rather critically. This is because the prevalence of bleeding disorders is generally quite low in a normal population and, therefore, a prolonged aPTT does not necessarily imply a relevant bleeding disorder, but is more likely to indicate a false positive result. Nonetheless, abnormal findings with reference to this parameter impose a potential risk for the patient about to undergo surgery and, hence, further investigations are required. As a result of this, surgical procedures will often need to be delayed or rescheduled. Normal results, on the other hand, cannot exclude hypo- and hypercoagulative disorders with certainty, however, will falsely reassure the surgeon to go ahead with the procedure (122). To minimise the risk of relevant haemostatic disorders remaining undetected when it comes to elective procedures such as orthognathic surgery, we therefore suggest that a more specific haemostatic analysis should be considered within routine preoperative screening in this context.

With reference our study, gender-related differences in terms of aPTT may indicate subtle peculiarities in the haemostatic profile in terms of the male and female gender, which may potentially affect blood loss. In this context, a longer aPTT related to the male gender in comparison with the female gender appears to be reasonable as males have frequently been associated with a higher amount of surgical blood loss (38, 69, 146, 148). In our study, the male gender was found to be associated with higher intra- and perioperative blood loss in comparison with the female gender in the bimaxillary group, which is consistent with the abovementioned literature. In this patient cohort, however, no gender-related differences in terms of aPTT resulted. While this is surprising, a lack of statistically significant gender-related differences in this regard does not automatically imply that there were no variations in the haemostatic profile between the male and female gender, which may have significantly affected blood loss. The latter statement clearly refers to the limitations of this assay, which will be addressed as this discussion proceeds.

Turning now to the analysis of specific coagulation parameters assessed, only few gender-related differences were identified in our study.
In contrast to the results reported by Olsen et al., who evaluated the effect of specific blood parameters on blood loss in orthognathic surgery, no statistically significant differences between the male and female gender in terms of the parameter Fibrinogen were found to occur in our study (69). Significantly higher levels of Fibrinogen associated with the female gender were observed by Olsen et al., which were presumed to have had a favourable effect on blood loss. In terms of the haemostatic cascade, this hypothesis makes perfect sense, as clot formation is heavily reliant on Fibrinogen (124). Hence, higher levels of Fibrinogen may allow for more efficient haemostasis, which might further be held accountable for a reduction in blood loss. Olsen et al. were able to confirm this hypothesis in their study, as levels of Fibrinogen were shown to negatively correlate with the amount of intraoperative blood loss. It should be highlighted, though, that their study population was small and only comprised 41 patients, 15 of whom were women (69). Having said this, similar gender-related differences in terms of Fibrinogen were found in a study assessing 329 subjects, with females showing significantly higher levels in comparison with males (151). As previously mentioned, no differences between the male and female gender were observed in our study when comparing 38 male patients with 65 female patients.

With reference to the coagulation factors VIII, XI and XIII no gender-specific differences between the male and female gender were observed. In terms of the von Willebrand factor (vWF), gender-related differences exclusively concerned qualitative features of this parameter (vWF-Akt.L) with women showing a decreased activity in comparison with men. vWF is known to play an important role in the haemostatic cascade as it is closely associated with platelet aggregation and adhesion. Furthermore, it has been found to interact with the coagulation factor VIII, which prevents it from proteolysis (127). Quantitative or qualitative deficiencies related to the von Willebrand factor are common and are likely to be associated with an increased bleeding, the severity of which depends on the extent and the type of the deficiency (127). With vWF being deficient, VIII will not be sufficiently protected against proteolysis, which will further impair important haemostatic processes. This, in turn, will trigger bleeding. When assessing mean levels of the parameters vWF-Akt.L, vWF-Ag and VIII in our study, all were shown to be within the normal range. On closer inspection of levels of vWF and VIII determined in our study cohort, it is evident that a number of patients presented with a significantly reduced parameter activity, which may have triggered bleeding. This, however, was not the case in our study (unpublished data).

AT-III is a natural anticoagulant which counteracts haemostatic processes (126). When taking into account both surgical modalities, the parameter Antithrombin-III did not differ significantly in terms of gender. In contrast, levels of AT-III differed statistically significantly in the bimaxillary group, with levels in males outweighing those in females. Reflecting on the function of AT-III, in conjunction with the aforementioned gender-related differences detected in the bimaxillary group, a correlation between gender-specific differences in terms of the bleeding volumes determined and the level of AT-III seems likely. To prove this hypothesis a detailed correlation analysis was conducted, which will be discussed in the next section.

Routine coagulation tests such as aPTT and PT are associated with certain limitations, as previously discussed. These include poor sensitivity in terms of detecting hypocoagulative disorders and mild factor deficiency (121, 122, 138). Reasons for this are multifactorial but specifically rely on the design of these coagulation assays, in conjunction with the complexity of haemostasis. The process of coagulation necessitates the well-coordinated interaction of procoagulant and anticoagulant parameters, which eventually allow for a blood clot to form in the exact location of the injury. Quantitative or qualitative abnormalities with regards to any of the parameters involved may lead to an imbalance in the haemostatic cascade, triggering hyper- or hypocoagulative disorders (122, 138). To adequately identify these imbalances and assess their haemostatic function, it is important to gather precise information on all aspects of the coagulation cascade. APTT and PT, however, are static tests, in both of which the generation of fibrin is defined as their endpoint. This implies that relevant processes of the coagulation cascade, such as the formation of thrombin, cannot be fully evaluated by means of these classic assays. In more detail, aPTT and PT have been shown to only account for only 5% of the thrombin potential, whereas 95% of the thrombin potential remains unnoticed (137, 138). In addition to that, anticoagulant parameters have been found to require a longer time period to express their potential than the time frame provided by these tests. As a result, their effect on thrombin cannot be assessed in this regard (138).
Specific coagulation assays, which aim to analyse the interaction between procoagulant and anticoagulant parameters by means of thrombin, are referred to as thrombin generation assays (137, 139, 152). These assays are based on thrombin formation and decay and are triggered by procoagulant and anticoagulant parameters, respectively. Within these coagulation tests, the so-called thrombogram is generated, which comprises three parameters disclosing relevant information about a patient's haemostatic function: 'lag time', 'peak height of thrombin' and the 'endogenous thrombin potential', which is defined as the area under the thrombin curve. With regards to the literature, these parameters have proven essential in understanding interactions and underlying mechanisms related to various congenital or acquired haemostatic disorders (137, 138, 153, 154). What is more, gender-

related differences regarding the haemostatic profile have been identified on various occasions by means of assessing the thrombin potential. Consequently, the female gender has frequently been associated with a greater thrombin generation potential, a shorter lag time and a greater peak-thrombin formation in comparison with the male gender (137). From a clinical point of view, these findings indicate that women present with an ampler haemostatic profile than men, which may explain the differences in terms of the bleeding volumes detected regarding the male and female gender. Thrombin generation is known to be particularly reliant on the axis of coagulation factor VIII and protein C. Among others, this axis is easily affected by the intake of oral contraceptives, pregnancy and the menstrual cycle, which all interfere with protein C activity. Hence, a procoagulant imbalance results as protein C and its anticoagulant effect is downregulated, leaving factor VIII as the major driver of coagulation (138). While not discussed in detail in their paper, Secher et al. suspected that gender-related differences in terms of blood loss can be attributed to this mechanism (38). Although, as numerous other factors are capable of perturbating the balance of procoagulants and anticoagulants, the latter mechanism is only one of many possible explanations in this regard (138, 153).

In our study, the thrombin generation potential was analysed prior to surgery to gather information regarding a patient's haemostatic profile. Specific focus was allocated to the analysis of gender-specific differences in this regard which might have affected the amount of blood loss. The area under the thrombin curve (ETP-auc) and the peak of thrombin (ETP-Cmax) were assessed and further correlated with the orthognathic bleeding volumes detected. To the best of the authors' knowledge, this is the first study to analyse the thrombin potential in patients undergoing orthognathic surgical procedures. When comparing males with females without sub-specifying the data according to the treatment modality applied, statistically significant differences in terms of the parameter ETP-B-auc resulted. This specific parameter refers to the net amount of thrombin generated. According to these findings, females were shown to present with a higher thrombin potential in comparison with their male counterparts, as previously suggested. In contrast, no relevant gender-based differences regarding the peak height of thrombin were found. In terms of the parameter ETP-B-auc, these findings indicate that the conversion from Fibrinogen to Fibrin should have worked more efficiently in females than males. This in turn, should have led to a more effective clot formation and consecutive haemostasis, as this process is mediated by thrombin. With reference to blood loss, these findings could explain reduced bleeding volumes associated with the female gender. In the bimaxillary cohort, however, where gender-related differences regarding the intra- and perioperative blood loss were determined, no significant differences between the male and female gender in terms of the thrombin

potential were found. In marked contrast, differences in terms of the thrombin potential in the BSSO cohort were observed, while no gender related differences in terms of the bleeding volumes were detected.

4.4 Gender-related differences in blood parameters and their influence on orthognathic blood loss

4.4.1 Correlation analysis

To assess the effect of gender-based differences in terms of the haemostatic parameters evaluated on the bleeding volumes determined, a correlation analysis was performed. This was done separately for each treatment modality applied, to minimise the risk of bias owing to the pooling of the surgical techniques.
Within the bimaxillary cohort, relevant gender-related differences with regards to the bleeding volumes determined resulted with men associated with higher intraoperative and perioperative blood loss (IOB: p=0.056 and CBL-48h: p=0.019). Coagulation parameters showing relevant differences in terms of gender were correlated with blood loss in this cohort. These were AT-III and coagulation factor IX, both of which presented with higher plasma levels in males.

Surprisingly, the intraoperative blood loss (IOB) appeared unaffected by gender-based variations in terms of the haemostatic profile in our study, unlike the perioperative blood loss recorded (CBL-48h). In this regard, a significant correlation between the level of AT-III and CBL-48h was established, which indicated that higher levels of AT-III were linked to a greater perioperative blood loss.
Our findings related to IOB in patients undergoing bimaxillary surgery were in marked contrast to the results reported by Olsen et al. who investigated the effects of haemostatic parameters on intraoperative blood loss (69). While gender-related differences in terms of IOB and the haemostatic profile were reported in both studies, it was solely Olsen et al., who further entrenched significant correlations of the haemostatic parameters assessed with the IOB determined. In their study, haemostatic assessment was realised by means of thromboelastographic analysis and analysis of specific coagulation parameters such as Fibrinogen, D-Dimer and Prothrombin fragment 1+2. Thromboelastography, which is considered a global coagulation assay, assesses the viscoelastic properties of the blood together with fibrin polymerisation, on the basis of which detailed information regarding a patient's clotting dynamics can be gathered (152, 155). An ampler haemostatic profile in women in comparison with men was determined by Olsen et al., which was accountable for a significantly lower intraoperative blood loss in females (69). With reference to our study, a

different global coagulation assay was applied to quantify the haemostatic potential according to gender. The coagulation test used in this context is referred to as thrombin generation assay and relates to thrombin formation and decay (138). Thrombin generation, in turn, is known to strongly interrelate with a patient's haemostatic function. A greater thrombin potential indicates that more thrombin can be generated, which generally implies good coagulability. While a greater thrombin potential in women has frequently been described, no gender-based differences regarding the thrombin generation potential in the bimaxillary cohort were determined in our study (137).
Furthermore, when correlating the amount of IOB with the thrombin potential, no relevant correlations were established in this regard. What is more, no significant correlations between the thrombin potential and CBL-48h were found either.

In terms of the parameter AT-III, higher levels of this anticoagulant were linked to an increased perioperative blood loss in the bimaxillary cohort in our study (r=0.474; p=0.001). However, instead of contemplating increased levels of AT-III in this context, it is more sensical to discuss the other side of the coin. As previously highlighted, the anticoagulant pathway in females is highly sensitive to hormonal changes and the intake of oral contraceptives, which undoubtedly triggers procoagulant imbalance in the haemostatic profile. Hence, a reduced activity of AT-III in females (as in the present study) could explain a decrease in the perioperative blood loss. We further hypothesized that IOB was unaffected by the level of AT-III in our study, as the full procoagulant and anticoagulant potential might only be expressed after wound closure was performed. As a result of this, imbalances in this regard will only be evident at a later stage, which will most likely be reflected in the amount of perioperative blood loss.

In the BSSO cohort, plasma levels of aPTT, ETP-A-auc and ETP-B-auc were shown to differ significantly among males and females. While aPTT was longer in males, a greater endogenous thrombin potential associated with females was observed. These findings indicate that women presented with an increased procoagulant potential in comparison with men. Hence, a lower blood loss in women was to be expected. In contrast to the bimaxillary cohort, though, no gender-related differences in terms of blood loss were found to occur in this patient cohort (IOB and CBL-48h). Within the analysis, no significant correlations between the intra- and perioperative blood loss, and any of the haemostatic parameters differing in terms of gender were entrenched either. While these findings are contentious, it should be borne in mind that the operating time, as well as the extent of the surgical exposure, were significantly decreased in the BSSO-group in comparison with the bimaxillary cohort. As a result, a significantly lower blood loss in the BSSO cohort was observed. In

terms of the IOB, gender-specific differences in terms of the haemostatic profile detected might not have had the time to come into effect. With reference to CBL-48h, a significant increase in blood loss in the first 48-hours after surgery was detected. However, as the surgical exposure and the wound surface were rather small, it is proposed that no gender-specific differences resulted in this context.

4.4.2 Regression analysis

To further investigate the effect of relevant parameters on blood loss and better understand how these parameters compare and interrelate, a regression analysis was performed. Significantly different haemostatic parameters among males and females in addition to length of the procedure, patient gender, BMI and age were included in this analysis. Again, the statistical analysis was performed separately for each surgical method.

In terms of the IOB, the length of the surgical procedure appeared to be the parameter which affected blood loss the most. This implies that a longer operating time led to a more pronounced intraoperative blood loss, which was true for both surgical modalities. In addition to that, age turned out to be of relevance in the bimaxillary cohort. However, it is proposed that these findings are likely to be attributed to a longer operating time in older patients, as age is unlikely to affect blood loss. Sticking points with regards to these findings refer to the fact that gender-related differences in haemostatic parameters did not affect intraoperative bleeding in our study. As previously mentioned, these results are in marked contrast to the findings reported by Olsen et al. in whose study sex-related differences in the haemostatic profile were shown to considerably affect intraoperative bleeding (69).

With regards to CBL-48h, however, the natural anticoagulant AT-III was found to be most closely associated with the amount of perioperative blood loss in the bimaxillary group, whereas the length of the surgical procedure did not appear to be of relevance in this context.

4.5 Answers to hypotheses

- **Null-hypothesis**
  The null-hypothesis of our study, which suggested that there was no gender-related difference in terms of the bleeding volumes determined related to orthognathic surgery, was rejected. This is because statistically significant differences in terms of the

perioperative blood loss among males and females in the context of bimaxillary surgery were observed.

- **Alternative hypotheses**

The following alternative hypotheses were **verified:**

H1

Statistically significant gender-specific differences in terms of blood loss in patients undergoing orthognathic surgical procedures resulted in our study.

H2

Statistically significant differences regarding blood loss between the treatment modalities applied (BIMAX vs. BSSO) were found to occur, with bimaxillary surgery associated with significantly larger bleeding volumes in comparison with BSSO.

H3

Statistically significant gender-based differences in terms of the thrombin potential were detected, with women presenting with an ampler, more efficient haemostatic profile.

H5

Statistically significant gender-related differences with reference to the levels of the haemostatic parameters assessed were determined, which confirmed a more procoagulant haemostatic profile associated with females.

H6

Antithrombin-III was shown to statistically significantly correlate with the amount of perioperative blood loss related to bimaxillary surgery. Furthermore, gender-related differences in terms of plasma levels in this specific anticoagulant parameter were found, which were shown to affect blood loss in the current study. As a result of this, hypothesis ‘H6’ was verified.

H9

The length of the procedure was identified as a crucial factor regarding the amount of blood loss determined.

The following alternative hypotheses were **rejected:**

H4

The thrombin potential did not correlate with blood loss in the current study and was not identified as a reliable factor for predicting the amount of blood loss to be expected in the field of orthognathic surgery.

H7

In terms of HBL-48h, no gender-related differences were determined.

H8

With reference to RBL, no statistically significant differences among males and females resulted.

H10

This hypothesis was rejected because age was found to affect intraoperative blood loss, while a patient's BMI was found to be irrelevant.

4.6 Clinical implications of the study results

The findings of our study entail numerous clinical implications with reference to the perioperative management of patients undergoing orthognathic surgery, which will be discussed in the following areas.

- **Blood loss**

Based on the results of our study, a significant amount of orthognathic blood loss remains undetected, when solely focusing on measuring the intraoperative blood loss related to respective surgical techniques. This was true for both treatment modalities examined in the current study and was independent of patient gender. In more detail, when comparing IOB with CBL-48h, a difference in terms of the amount of blood loss determined of approximately 300ml was found (HBL-48h). This was because (1) the actual amount of intraoperative blood loss is often underestimated owing to the methodology of measuring blood loss and (2) blood loss appears to pertain after wound closure has been performed. The latter is of utmost clinical importance in this context as increasingly more clinical centres tend to perform orthognathic surgery on a day-surgical basis. While this appears to be a safe approach for mandibular surgery, where low intraoperative and reasonably low perioperative blood loss are determined, it is suggested that discharging a patient from hospital immediately after bimaxillary surgery could prove problematic on multiple fronts. This is because intraoperative blood loss associated with bimaxillary surgery was shown to considerably exceed that of mandibular surgery. It is acknowledged that this might be irrelevant for the immediate postoperative period, however, when adding an additional 300ml of blood loss in the first 48-hours after surgery, it might drastically affect a patient's well-being and quality of life. What is more, some patients might even require a blood transfusion owing to this increase in the bleeding volume observed. If still admitted to hospital and postoperative blood sampling was routinely performed in the first few days after surgery, it would easily be feasible to react to changes in a patient's blood count. In contrast, if discharged from hospital too soon, an increase in blood loss together with a decrease in relevant blood parameters, such as haemoglobin and haematocrit, could easily remain unnoticed. Hence, it is advocated that patients undergoing bimaxillary surgery should be closely monitored in the first couple of days postoperatively to minimise the risk of a patient's well-being deteriorating after surgery.

- **The use Antifibrinolytics (TXA)**

Antifibrinolytics, such as tranexamic acid, are widely used within the field of orthognathic surgery and have been shown to effectively reduce the amount of blood loss related to this

surgical field on numerous occasions (34, 35, 38). Despite this, the use of antifibrinolytics should be considered carefully, as these drugs involve additional risks and side-effects (100, 101). Especially in females, where an ampler, procoagulant haemostatic profile has frequently been described (as a result of interactions with female sex hormones and oral contraceptives with the anticoagulant pathway), one should be reluctant to administer antifibrinolytics if not absolutely indicated from a clinical point of view.

With reference to orthognathic surgery, the effects of antifibrinolytics on intraoperative blood loss have been studied on numerous occasions expect in one study that focused on perioperative blood loss instead (34, 35, 38, 67). Most studies indicated that antifibrinolytics were associated with a significant decrease of blood loss (34, 36). Interestingly, antifibrinolytics appeared to reduce blood loss in females more effectively than in males (38). With regards to our study cohort, no antifibrinolytics were administered. Despite this our findings allow for clinical suggestions in this context as blood loss was analysed at three different time points. In terms of IOB determined in our study, blood loss related to BSSO was very low owing to a short operating time and minimal surgical exposure. In contrast, significantly increased intraoperative blood loss with reference to the bimaxillary cohort resulted, when comparing the two treatment modalities. It was hypothesised that the length and the complexity of the procedure together with the amount of the surgical exposure of the midface were accountable for more pronounced intraoperative bleeding in this cohort. All of these factors inevitably interfered with haemostasis in the current study and made clot formation more challenging. Both surgical techniques applied in our study, were linked to a significant increase regarding the bleeding volumes determined in the first 48-hours postoperatively. In the context of bimaxillary surgery, a more pronounced perioperative blood loss was found in males in comparison with females.

Based on our findings, the following suggestions and considerations regarding the use of antifibrinolytics in the field of orthognathic surgery are advised:

- With reference to bimaxillary surgery, the use of antifibrinolytics to reduce intra- and perioperative blood loss is recommended. However, while most studies administered IV antifibrinolytics prior to surgery or at induction only, it is believed that a second dose administered in the immediate postoperative period would prove beneficial to better address perioperative bleeding (34, 36). Especially in males associated with increased perioperative blood loss in comparison with females, adaption of the antifibrinolytic dosing regimen may be sensible.

- In terms of the BSSO cohort, a low intraoperative blood loss was observed, which questions the benefit of using antifibrinolytics in this group. However, when the perioperative blood loss recorded is taken into account, antifibrinolytics in the immediate postoperative period might be indicated.

  To prove the hypotheses related to the use of antifibrinolytics in the field of orthognathic surgery further prospective research needs to be conducted.

- **Preoperative screening**

Orthognathic surgical procedures are considered elective, for the most part, which necessitates high safety standards and a low rate of complications. In this regard, the importance of adequate preoperative patient assessment should be highlighted. Preoperative analyses of blood parameters and haemostatic function have been entrenched as crucial factors to minimise the risk of excessive blood loss and associated side effects. As previously discussed, the use of routine coagulation parameters such as aPTT, PT and INR has proven contentious in the context of preoperative screening (122). This is because bleeding disorders are generally very rare in a healthy population and these tests are associated with significant limitations in terms of detecting relevant bleeding disorders. We therefore suggest that for the purpose of preoperative haemostatic assessment, additional parameters should be considered. Thrombin generation assays, as used in the current study, represent a reliable method to detect imbalances in terms of haemostasis (138). However, these tests might be unavailable within the clinical routine for the sole purpose of preoperative screening. Alternatively, thromboelastographic analysis may be used, which has previously shown to reliably predict blood loss in orthognathic surgery (71).
In addition to these global coagulation assays a more specific coagulation analysis might be advantageous to identify abnormalities and peculiarities with regards to a patient's haemostatic profile. With reference to our study imbalances in terms of the anticoagulant parameter AT-III were found to occur, which were shown to significantly affect blood loss. Hence, in addition to aPTT, PT and INR, the parameter AT-III should be assessed. With respect to other research on this topic, the parameter 'Fibrinogen' was found to be relevant (69). In females, it would prove beneficial to additionally take into account Protein C and S. As a result of a more detailed haemostatic analysis, patients associated with a higher risk of bleeding complications might be identified prior to surgery. Similarly, patients linked to procoagulant imbalances and an elevated risk of thromboembolic events may be identified beforehand. Consequently, the use of antifibrinolytics may possibly be optimised in the sense that these drugs would only be administered if a higher amount of blood loss was to be

expected and normal haemostatic function was to be assumed. Ultimately, a lower rate of complications together with a better quality of life in the postoperative period should be ensured by considering gender-specific aspects together with a patient's individual treatment needs.

4.7 Conclusion

This study aimed to investigate blood loss related to orthognathic surgical procedures with the scientific focus specifically directed on patient gender. This is because males have frequently been associated with greater orthognathic blood loss in comparison with females (38, 69). The underlying mechanisms have barely been investigated, however, gender-based differences in the haemostatic profile together with female sex hormones have been suggested as relevant contributing factors in this context (38, 69). To investigate this further, a detailed preoperative coagulation analysis was conducted to highlight gender-related differences regarding the haemostatic profile. Relevant parameters were then correlated with the amount of intra- and perioperative blood loss.

Relevant findings and conclusions:

- Orthognathic blood loss was shown to increase significantly in the first 48-hours postoperatively with reference to both surgical modalities assessed.

- Bimaxillary surgery was associated with a significantly greater blood loss in comparison with BSSO in all of the measurements taken.

- In terms of patient gender, no relevant differences regarding the intraoperative blood loss (IOB) resulted. In contrast, perioperative blood loss determined 48-hours after surgery (CBL-48h) was shown to differ statistically significantly among males and females. These findings, however, were specific to the bimaxillary cohort with males linked to a significantly greater perioperative blood loss than females.

- Several relevant gender-specific differences in terms of the haemostatic profile were detected. However, few significant correlations between haemostatic parameters which differed significantly among males and females and the amount of blood loss were established. Furthermore, an analysis of the thrombin generation potential, which allows for a detailed assessment of a patient's haemostatic profile, highlighted differences in terms of haemostasis among males and females. Thrombin generation, however, was not shown to correlate with the amount of blood loss in our study. In contrast, a significant correlation between the anticoagulant parameter Antithrombin-III (AT-III) and

CBL-48h in the bimaxillary cohort was determined. It was hypothesised that decreased levels of AT-III associated with females may have triggered procoagulant imbalance, which may further be held accountable for gender-related differences in terms of CBL-48h in this cohort.

- The operating time was found to significantly correlate with blood loss. In terms of IOB, the length of the procedure was found to affect intraoperative bleeding in both surgical cohorts considerably. In contrast, the operation length did appear to be irrelevant regarding the perioperative blood loss associated with bimaxillary surgery. In this context, gender-related peculiarities related to haemostatic function were shown to significantly affect blood loss.

- In this, patient reported outcome measures (PROMS) were not investigated, which might have provided a better understanding of how the amount of intra- and perioperative blood loss affects a patient's quality of life. In this regard, the effect of gender-specific differences in terms of orthognathic blood loss on a patient's well-being could have been disclosed. This, however, represents future work of our research group.

- Further prospective studies and detailed research into haemostasis and blood loss in the field of orthognathic surgery are still needed to better understand underlying mechanisms related to our findings. This will help to establish predictive factors regarding excessive blood loss in orthognathic surgery and identify at-risk patients. In addition, this will allow accurate preoperative screening and optimise the use of perioperative measures, such as tranexamic acid, to reduce and prevent blood loss where indicated.

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