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DISSERTATION

Complication rate analysis of proximal humerus fracture surgery in elderly patients –
Guiding the benefit-risk assessment for an immunomodulatory therapy

zur Erlangung des akademischen Grades
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List of Abbreviations

AE	Adverse event
AMG	Arzneimittelgesetz (Medicinal Products Act)
AO	Arbeitsgemeinschaft für Osteosynthesefragen
AVN	Avascular necrosis
BCRT	Berlin Institute of Health Centre for Regenerative Therapies
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (Federal Institute for Drugs and Medical Devices)
BMD	Bone mineral density
BMI	Body mass index
CMSC	Center for Musculoskeletal Surgery
cPGI	Carbaprostacyclin
CT	Computerized Tomography
CV	Curriculum vitae
EMA	European Medicine Agency
EudraCT	European Union Drug Regulating Authorities Clinical Trials database
FDA	Food and drug administration
GCP	Good Clinical Practice
GCP-V	GCP-Verordnung (Good Clinical Practice regulation)
GLUT1	Glucose transporter 1
GMO	Genetically Modified Organisms
GMP	Good manufacturing practice
HED	Human Equivalent Dose
HMOX	heme oxygenase
IB	Investigational Brochure
ICD 10	International Classification of Diseases, Tenth Revision
IFN-ɣ	Interferon gamma
IMP	Investigational Medicinal Product
IMPD	Investigator Medicinal Product Dossier
IV	Intravenous
K wire	Kirschner wire

LAGeSo	Landesamt für Gesundheit und Soziales (The state office for health and social affairs)
MRI	Magnetic Resonance Imaging
MSCs	Mesenchymal stem cells
NYHA	New York Heart Association Classification
ORIF	Open reduction and internal fixation
PGI2	Prostaglandin I2 (Prostacyclin)
PHF	Proximal humerus fracture
PHILOS	Proximal humeral interlocking system
PPH	Primary Pulmonary Hypertension
RANK	Receptor activator of nuclear factor kappa-B (κB)
RANKL	Receptor activator of nuclear factor kappa-B (κB) ligand
RSA	Reverse shoulder arthroplasty
SAE	Serious adverse event
SAP	Systems, Applications & Products in Data Processing
TAD	Tip apex distance
T_{EMRA}	Terminally Differentiated Effector Memory CD8+T
TNFα	Tumor necrosis factor alpha
T_{reg}	Regulatory T cell subtype
VEGF	Vascular endothelial growth factor
μCT	Micro-computed tomography

Zusammenfassung

Hintergrund:

Die proximale Humerusfraktur (PHF) ist die dritthäufigste traumatische Knochenfraktur in der älteren Bevölkerung. Etwa 70% der dislozierten PHF, die einer chirurgischen Behandlung bedürfen, treten bei älteren Patienten auf. Es wird erwartet, dass sich die Inzidenz der PHF in den nächsten drei Jahrzehnten verdreifachen wird. Die Behandlung von PHF bleibt problematisch, hauptsächlich aufgrund des Fehlens eines Konsens über die optimale Behandlungsstrategie. Leider ist das Ergebnis nach PHF ungünstig mit einer hohen Komplikationsrate, die zwischen verschiedenen Studien über bestimmte Behandlungsmethoden und zwischen verschiedenen Zentren variiert.

Ziel dieser Studie ist es, die Ergebnisse der chirurgischen Behandlungsstrategien, einschließlich der Komplikations- und Revisionsraten, der beiden am häufigsten durchgeführten chirurgischen Eingriffe (winkelstabile Plattenosteosynthese (PHILOS, Synthes) und Arthroplastik) bei älteren Patienten mit PHF zu analysieren. Darüber hinaus wurde der klinischen Translationsprozess eines neuen immunmodulatorischen Therapieansatzes, der die Heilungsergebnisse für die identifizierte Patientengruppe verbessern kann, in dieser Arbeit initiert.

Methodik:

Es wurde eine retrospektive Analyse aller im Centrum für Muskuloskeletale Chirurgie der Charité – Universitätsmedizin Berlin zwischen März 2017 und Juni 2018 wegen PHF chirurgisch behandelten Patienten durchgeführt, welche ein Follow-Up von mindestens sechs Monaten aufwiesen. Es wurden nur Patienten eingeschlossen, welche mit (PHILOS) oder einer Endoprothese versorgt wurden. Zusätzlich wurden die methodischen Aspekte der klinischen Umsetzung des präklinischen Wissen eines immunmodulatorischen Mittel, Iloprost, in eine solide klinische Studie beschrieben, und die erforderlichen Zulassungen von den zuständigen Behörden eingeholt.

Ergebnisse:

Es konnten 88 PHF bei 87 Probanden mit einem Durchschnittsalter von 72,9 Jahren in die Analyse eingeschlossen werden. Die Studie zeigte, dass die Gesamtkomplikationsrate bei 4-teiliger PHF, die mit PHILOS behandelt wurde, die höchsten Werte (68.8%) aufwies und damit auch höher als die Komplikationsrate bei endoprothetisch versorgten Patienten (19%) war. Die Tiefenanalyse zeigte aber auch, dass die Komplikationen nach Plattenosteosynthese einen geringeren

Schweregrad als die Komplikationen nach Endoprothese aufwiesen. Eine Revision erfolgte nur bei 8 von 19 Komplikationen (42%) in der PHILOS- im Vergleich zu 5 von 5 (100%) in der Endoprothetik-Gruppe. Basierend auf diesen Werten wurde ein hoher medizinischer Bedarf für neuartigen additive Therapien für die osteosynthetische Behandlung von höhergradigen PHF identifiziert.

Der Translationsprozess der immunmodulatorischen Therapie erforderte eine detaillierte Bestimmung der richtigen Dosis und des Dosierungsschemas sowie die Identifizierung von Ein- und Ausschlusskriterien, die Auswahl repräsentativer Endpunkte und die Erstellung einer Nutzen-Risiko-Bewertung. Die behördliche Zulassung wurde von LaGeSo und BfArM erfolgreich erhalten.

Schlussfolgerung:

Ältere Patienten mit 4-teiliger PHF, die mit PHILOS behandelt wurden, zeigten die höchste Komplikationsrate, und könnten durch eine additive lokale immunmodulatorische Therapie profitieren.

Abstract

Introduction

Proximal humerus fracture (PHF) is the third most common traumatic bone fracture in the elderly population. The incidence of PHF is expected to have tripled in the next three decades. About 70% of displaced PHF that need surgical treatment occur in elderly patients. The treatment of PHF remains problematic, mainly due to the lack of a consensus on the optimal treatment strategy. Moreover, the outcome after PHF surgery is currently unfavorable, with a high complication rate that varies between different studies for a given method of treatment and between different centers.

This study aims to measure the outcome of surgical management strategies, including complication and revision rates, of the two most commonly performed surgical procedures (angle stable plate osteosynthesis (PHILOS, Synthes), and arthroplasty) in elderly patients with PHF. Additionally, the clinical translation process of a novel immunomodulatory approach that may improve the healing outcomes for the identified patient group has been established.

Methodology

A retrospective medical record analysis was performed at the Center for Musculoskeletal Surgery of the Charité - Universitaetsmedizin Berlin, where patients aged 60 years or older with PHF who underwent operative treatment from March 2017 to June 2018, with either PHILOS or arthroplasty and with a follow-up period of at least six months, were included. In addition, the methodological aspects of clinically translating pre-clinical knowledge of an immunomodulatory agent, Iloprost, into a sound clinical trial to obtain the necessary approvals from regulatory authorities, were described.

Results

A total of 88 PHFs in 87 subjects with a mean age of 72.9 years were recorded. The study revealed that the overall complication rate in 4-part PHF treated with PHILOS recorded the highest values, 68.8%, compared to 19% in arthroplasty cases. Further analysis showed that the nature of complications after PHILOS was less severe than the ones after arthroplasty, and revisions were performed in 8 out of 19 cases (42%) in the PHILOS group compared to 5 of 5 (100%) in arthroplasty. These observations indicated a high medical need for enhancing bone healing in osteosynthesis patients.

To conduct a clinical trial with Iloprost, a detailed estimation of proper dose and dose regimen, identifying inclusion-exclusion criteria, selecting representative endpoints, and establishing a

benefit-risk assessment were performed. Regulatory approval was successfully obtained from relevant authorities.

Conclusion

Elderly patients with 4-part PHF treated with PHILOS yielded the highest complication rate and could benefit from the local administration of Iloprost.

Chapter 1 : Introduction

1.1 Proximal Humerus Fractures (PHF)

Traumatic fractures are among the most common injuries worldwide (1). In the USA, up to 25% of the population may suffer from musculoskeletal injury per year (2). In Germany, about 1.6 million fractures have been reported annually (3). The fracture number is expected to increase because of the extended life expectancy and consecutive age-related disorders affecting the musculoskeletal system, such as osteoarthritis and osteoporosis (4).

One of the most common traumatic fractures in the elderly population (>65 years old) is the fracture of the proximal humerus (PHF), which is ranked third after hip fracture and distal radial fracture, respectively (5, 6). The most common mechanism of trauma in this age group is mainly a simple fall from patients' height onto an outstretched hand (6, 7) (Figure 1). In Finland, a retrospective study on the patient (>18 years old) who suffered from PHF between the years 2006 and 2010 showed an overall incidence of 114 and 47 fractures per 100,000 person-years in females and males, respectively (8). This incidence increased with age and has been linked to osteoporosis, which is more common in females representing 75% of cases (7). This has been confirmed in a study conducted between 1992 to 1996 in Edinburgh, Scotland, where the incidence of PHF was 260 per 100000 persons/year in females aged 80 - 89 years and 109 per 100000 persons/year in males of the same age group (8).

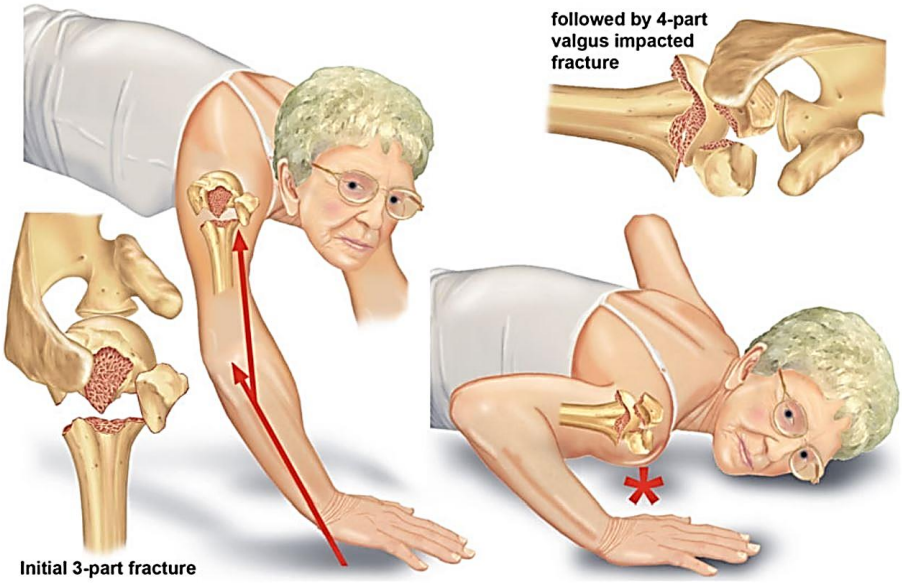

Figure 1: Mechanism for low energy proximal humerus fracture in elderly individuals (9)
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PHF is considered a growing challenge for health systems due to the continuous increase of cases every year. For instance, the incidence of PHF is expected to triple within the next three decades, due to the cumulative aging of the world population (10, 11). By 2050, it is expected that half of the German population will be over 50 years old (12). In Germany, the 2019 population profile shows that 18.1 million people were above 65 years old, representing 22%. According to predictions of the Federal Statistical Office, the number of people above 65 years old is expected to reach 38% by the year 2040 (13). The one-year mortality rate for PHF patients is 9.8%, while the five-year mortality rate is 28.2% (14).

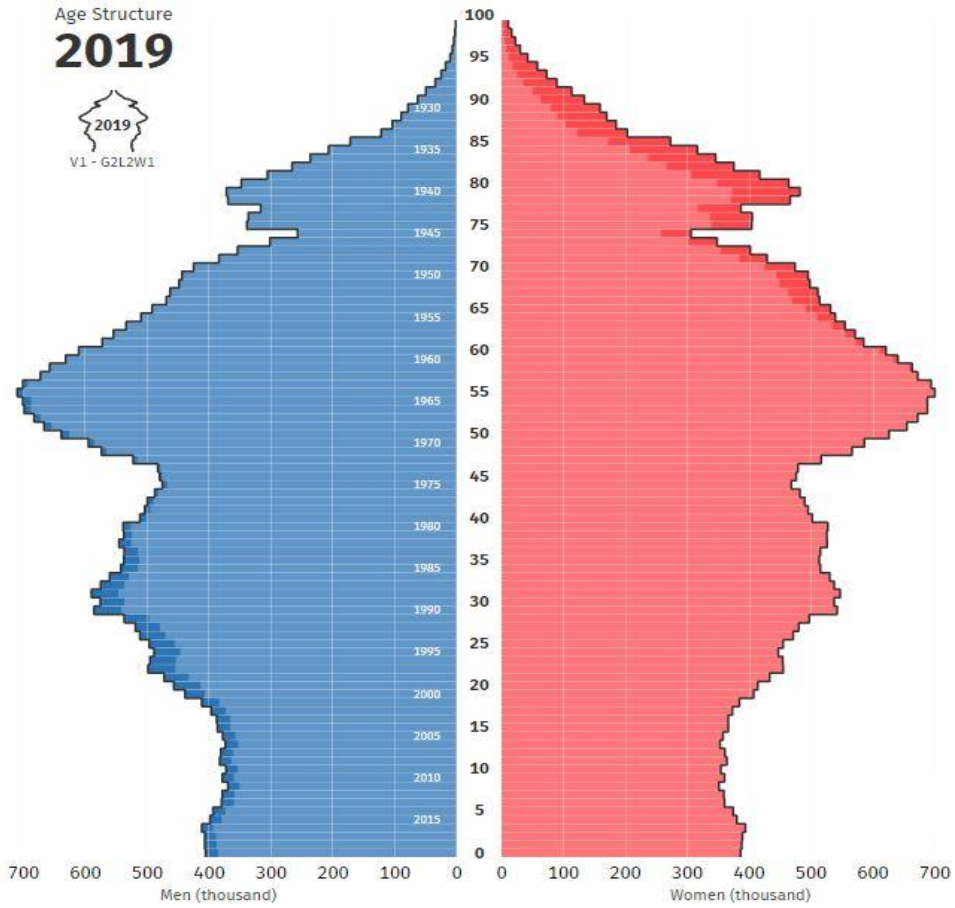

Figure 2: 14th coordinated population projection for Germany. (Statistisches Bundesamt; www.destatis.de) (13)

1.2 Classification of PHF

PHF is mainly classified according to the Neer classification (15), which is the most frequently used classification in addition to the AO classification. The Neer classification of PHF refers to the four main anatomical parts of the proximal humerus: humeral head, humeral shaft, greater tuberosity and lesser tuberosity. A fracture is considered displaced if there is a fragmental displacement of more than 1 cm or angulation of more than 45 degrees (15) (Figure 3). The fracture classification, according to the number of the displaced fragments, highlights the severity and complexity of the fracture pattern with the advancement of the classification grade (16). Although

the outcome of PHF could be affected by many variables such as patient age, bone quality, comorbidity, and fracture reduction, the link between the number of displaced fragments according to Neer classification has been shown to be negatively correlated with the functional outcome (17, 18). Moreover, fracture severity, according to the Neer classification, has been previously used as a predictive value for the occurrence of complications (19).

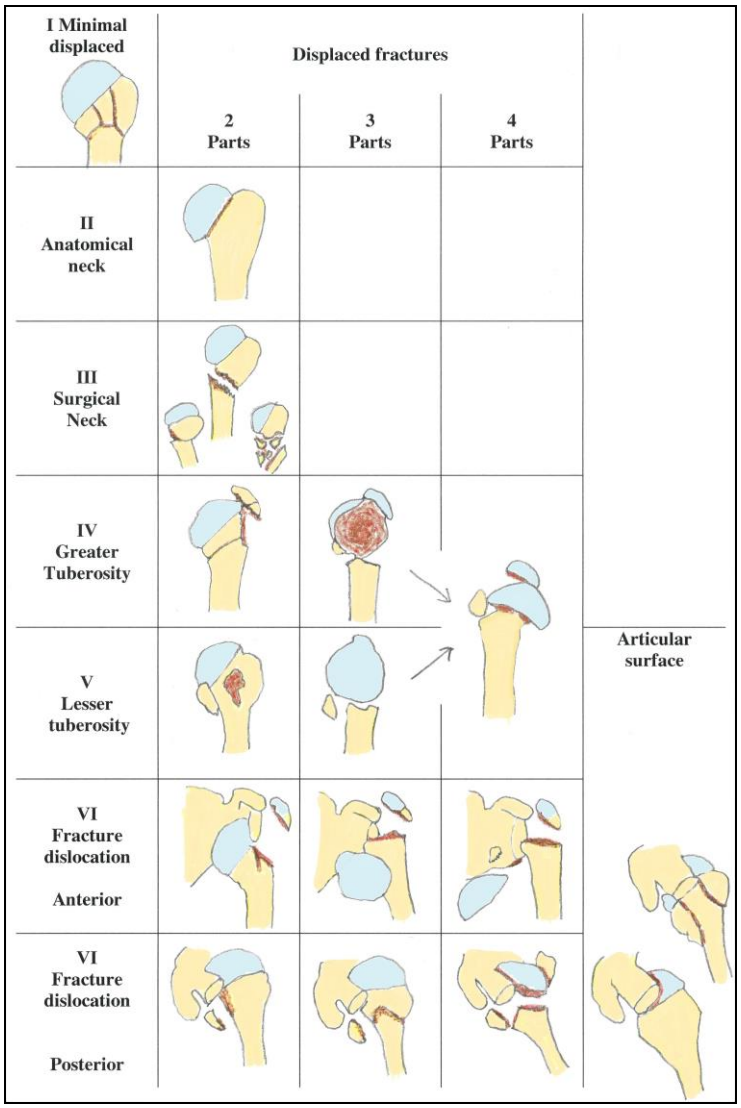

Figure 3: Neer Classification of proximal humerus fractures (15)

1.3 Current clinical management strategies

Although the management of proximal humerus fractures has been studied intensively over the years, it remains one of the unsolved orthopedic problems mainly due to the absence of clear evidence-based guidelines for treatment (20). This is reflected in the lack of a consensus on the optimal treatment strategy among the scientific community (17). Currently, surgeons rely on a combination of factors when deciding on the most suitable management strategy, such as

classification of the fracture, degree of fracture comminution, patient bone quality (osteoporosis), patient age, physical capacity, and functional demand of the patient (21).

1.3.1 Surgery as the first-line of treatment

The current treatment strategy for PHF does not involve pharmacologic treatments since no drugs exist that are able to stimulate bone healing in fractured patients sufficiently, especially compromised elderly patients. Surgery remains the first-line treatment in displaced 3-part and 4-part PHF (22–24). Many surgical options for the treatment of PHF have been described in the literature, which can be categorized into fixation and arthroplasty. Fixation comprises the stabilization of the fragments of fractured bones by implants, such as closed reduction and percutaneous K wire fixation, open reduction and fixation with tension bands, bone sutures, cerclage wires, minimally invasive screw fixation, T-plates, intramedullary nails, and locking plate fixation. Arthroplasty comprises partial (hemiarthroplasty) or total (reverse or anatomical shoulder arthroplasty) replacement of a joint (25–28).

PHF is common in elderly females, above all, because osteoporosis is the pathological basis of the fractures (29). As a result of osteoporosis, the cancellous bone trabeculae decrease in both number and thickness, which in turn leads to poor bone quality and a decrease in bone mass (30). The osteoporotic proximal humeral bone could be described as an eggshell with the lowest bone density being in the central part of the humeral head, which is nearly devoid of bone. Therefore, the management of PHF should include the evaluation of the bone mineral density and treatment of the possibly existing osteoporosis (31). This would also reduce the incidence of potential hip fractures, which increase by 500% in the first year following PHF (32). Bad bone quality leads to poor screw purchase and endangers the fixation stability in the gold standard of fixation, angle stable plate osteosynthesis (33).

Proximal humeral locking plates

Proximal humeral locking plates, such as the (PHILOS) plate (Synthes, Switzerland), are commonly used for the fixation of PHF (34). PHILOS allows for the positioning of multiple head screws in predefined directions, which in turn enable a good purchase of screws in the bone. Moreover, the screw heads are locked in the plate producing a one unit device, giving the maximum possible hold of the fracture fragments after fixation (35). Osteosynthesis with the PHILOS is the most common fixation method for 2-part and 3-part and, in some cases, for 4-part fractures when it is still possible to reconstruct the humeral head (36). Although frequent complications after PHILOS plate osteosynthesis reached 49%, in some studies as discussed in the

following section (17), PHILOS has the advantage of preserving the natural anatomy of the bone and satisfactory functional outcome (18, 37).

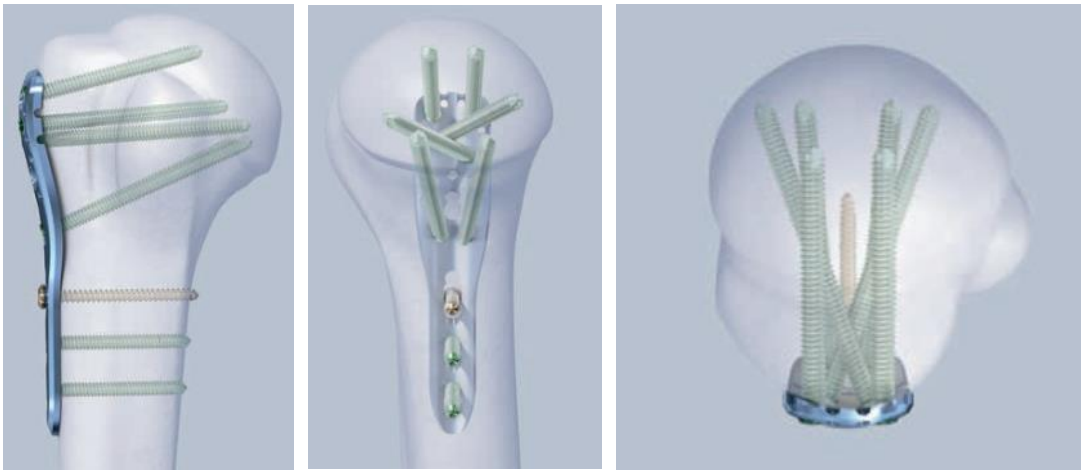

Figure 4: PHILOS plate (DePuy Synthes® Switzerland) (38)

Arthroplasty

Hemiarthroplasty is the replacement of the damaged humeral head with a metal joint prosthesis. The procedure has shown to be a good treatment option for complex 3-part and 4-part fractures (31). A randomized controlled study comparing the outcome of both hemiarthroplasty and conservative management in 4-part PHF showed that the range of motion was similar in both groups. However, the hemiarthroplasty group showed less postoperative joint pain compared with nonoperative conservative management (39). Nevertheless, hemiarthroplasty is considered inferior or at least similar in terms of the range of motion when compared to conservative non-operative treatment of 4-part PHF in the elderly for the long-term (39).

1 2

Figure 5: Hemiarthroplasty 1. GLOBAL® FX™ (DePuy Synthes® Switzerland) (40) 2. Zimmer Anatomical Shoulder™ Fracture System (41)

Reverse shoulder arthroplasty (RSA) uses prosthesis components to replace the glenoid fossa as well as the humeral head, reversing the bearing partners of the shoulder joint (Figure 6). The design of the RSA enhances mechanical stability and moves the center of rotation medially and inferiorly,

thereby improving the function of the deltoid muscle through increasing its lever arm, which in turn compensates for the potential loss of rotator cuff function following the fracture (42). Although RSA has been shown to be an effective procedure in cases of complicated shoulder fractures (43), it is technically demanding, and patients are left with limited options in the case of implant failure (31).

1 2

Figure 6: reversed shoulder arthroplasty
1. **(**DELTA XTEND™) Reverse Shoulder System (DePuy Synthes® Switzerland) (44) 2. Zimmer® Reverse System (45)

1.3.2 PHF evaluation and patient considerations

PHF typically occurs in elderly female patients after simple falls (46). These falls have a high risk of fracture incidence, which is reflected by the fact that the elderly with an active lifestyle suffer more frequently from PHF (6, 47, 48). The treatment of choice for PHF management requires a proper assessment with careful evaluation considering not only the fracture pattern and classification but also, and of high importance, the patients´ expectations. The patient evaluation process should begin with the patient history with particular attention on the independency level of the patient, the presence of previous injuries, especially rotator cuff tears and previous neurological injuries, and the patient's functional demands (49) and tolerability of the planned rehabilitation program (31). Then, the patient should be examined thoroughly, among others, for their general condition, presence of chronic diseases that could affect wound healing, immune system status, and usage of particular medication such as steroids (50). Furthermore, careful local examination of the affected arm, such as finding out if it is the dominant side, timing and mechanism of fracture, presence of other injuries, vascular status, and neurological examination (49, 51).

A detailed osteoporosis assessment is considered a fundamental step in the patient evaluation process as osteoporosis is not only connected to the actual PHF but also could affect the incidence of possible following osteoporotic fractures (52, 53). Proper X-ray for the shoulder joint should be obtained in anteroposterior, lateral, and axillary views, in case of complex fracture patterns, also Computerized Tomography (CT) imaging, for better-visualization and planning.

Moreover, Magnetic Resonance Imaging (MRI) could help in assessing the rotator cuff status, since tears accompany the PHF in up to 40% of patients (54). Rotator cuff tears at time of injury were found to be significantly linked to patients' functional loss at one year follow-up (55).

1.3.3 Individualization of the treatment

The treatment choice process for PHF in the elderly is not a simple choice and varies significantly from one patient to another, particularly for 4-part PHF (31). This variation could be explained by the absence of strict treatment guidelines for PHF treatment. Previously, the choice of treatment was mainly determined depending on fracture radiology and on fracture classification. However, this concept has been revised as depending only on these two factors was found to be unreliable and unreproducible (56–58).

The controversies in treating PHF in the elderly started early in deciding whether surgical or non-surgical treatment is a better choice for the patient. Some PHF patients have an obviously clear indication for surgery such as in open fracture, pathological fracture, vascular injury, or neurological injury which could require surgical fixation to secure the repair (36), while other patients have a relatively clear indication for nonsurgical treatment, such as in non-displaced fractures or patients with cerebral stroke or other permanent neurological impairment at the same fracture side. These patients would not gain benefit from surgical management. Moreover, conservative treatment may be preferred for unstable patients and who could only be treated surgically if the patient’s general condition improved (50). However, also in patients treated with arthroplasty, the surgeon should consider that early surgery (within the first four weeks) is an essential variable for the functional outcome (59, 60).

The surgical treatment options for 4-part PHF encompass mainly angle stable plate osteosynthesis and arthroplasty. Murray *et al.* (36) describe criteria that could help in the proper selection of the surgical treatment for the 4-part PHF. The criteria categorize the indication into either PHILOS plate or primary arthroplasty with cases where both treatment modalities are indicated (figure 7).

It has been reported that PHF patients above 50 years old with a head split injury should be treated with arthroplasty; this was explained by the significant damage of the articular surface that could affect the fragments vascularity, which in turn increases the incidence of avascular necrosis (61).

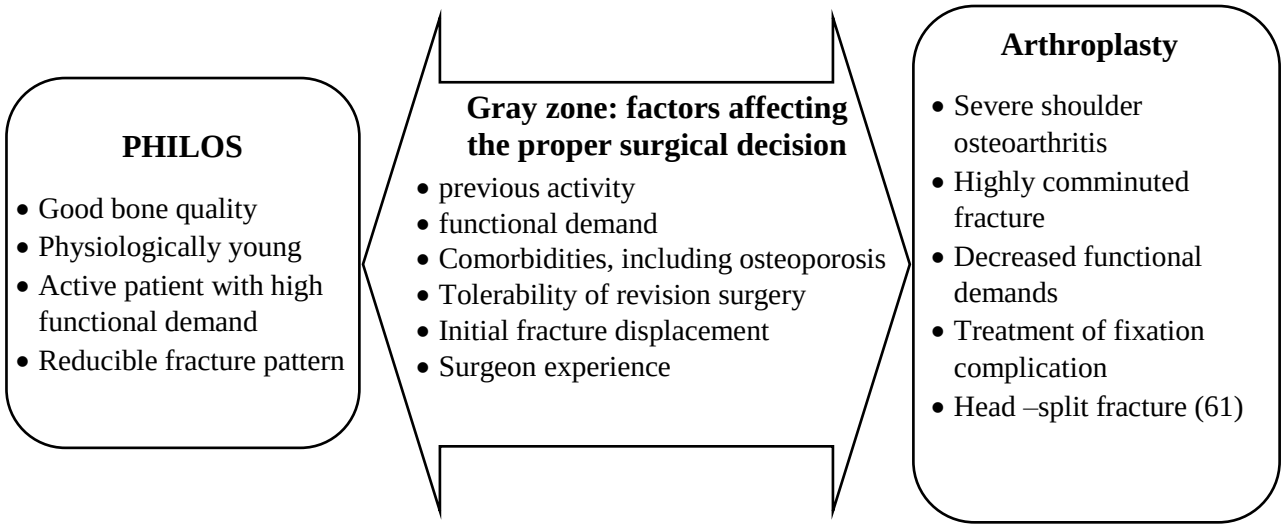

Figure 7: Criteria of treatment choice for the 4-part PHF

1.4 Treatment outcomes and complication rates

The outcome of fracture treatment depends on several factors that can be categorized into i) patient factors: age, presence of chronic diseases, bone quality, and degree of fractures, or ii) treatment-related factors: choice of either conservative or operative treatment, the operative technique used and skills of the surgical team.

The complication rate of managing PHF varies markedly between different studies for a given specific method of treatment. For example, the overall complication rate for locking plate fixation was reported to be between 9.7% and 57% (Table 1). Similarly, the complication rate of RSA was reported to fall between 4.8% and 68% and reached in some studies up to 75% (62, 63). The reason for this difference in complication rates between studies could be attributed to the heterogeneity of the studies concerning several factors, such as the age of the study population, fracture classification, the method of outcome evaluation, and the difference in studies follow-up duration (64). Compared to RSA, hemiarthroplasty previously showed a higher revision rate and inferior results concerning pain relief, patient satisfaction, and range of motion (65–68). A decrease range of motion in hemiarthroplasty mainly occurs in the long run (mean 3.7 years) (69), which is due to possible mal-union of the humeral tuberosities (29, 70, 71). This, in turn, leads to a defective range of motion, especially in raising the hand above the head (31).

Table 1: Reported complication rates for locking plate fixation in proximal humerus fracture

Study	Complication rate	Number of patients	Median follow-up	Ref.
Aksu *et al.*	9.7%	103	19 months	(72)
Koukakis *et al.*	15%	20	16.2 months	(73)
Agudelo *et al.*	19%	153	55 months	(74)
Haasters *et al.*	21.4%	646	12 months	(75)
Egol *et al.*	23.5%	51	16 months	(76)
Hepp *et al.*	31.3%	83	12 months	(77)
Owsley *et al.*	36%	53	44 months	(78)
Klug *et al.*	37.8%	66	12 months	(64)
Jost *et al.*	57%	121	22 months	(79)

The complication rate of managing PHF does not only vary between methods of treatment, as stated above, but also between different clinical centers. For instance, tertiary hospitals are known to receive and manage more complex referral cases that could explain higher complication rates. For these reasons above, it is challenging to infer accurate rates of complication of managing PHF from the literature. A more effective approach would be to rely on the data derived from each center.

1.5 Complication patterns and associated risk factors

1.5.1 Complication patterns

There are different surgical procedures for PHF, particularly for complex injuries in the elderly, with different complications for each procedure. Nevertheless, the common possible complications after surgical treatment of PHF can be summarized as follows:

a) **Loss of reduction:** Loss of reduction can be considered a severe complication and one of the most frequent causes of revision surgeries (18). It can be diagnosed with either fracture angulation of ≥ 10 degrees in any direction or loss of the humeral head height ≥ 5mm (80). It is a common complication, particularly in elderly patients, which is linked to osteoporosis. The prevention of loss of reduction is difficult, and loss of reduction frequently ends with low functional outcomes (81).

b) **Infection:** Infection after proximal humerus fracture treated with open reduction and internal fixation (ORIF) is a feared complication. However, the soft tissue coverage and good blood supply of the surgical site prevent against infection, and therefore infections are relatively infrequent after osteosynthesis with 2.9% of all procedures (75, 82, 83). The

reversed shoulder arthroplasty showed an infection rate of about 0.76% at 90 days follow-up, which increased to 2.4% after the first year, and to 6.74% at 2-year postoperative follow-up (62). Infections can be divided into acute or delayed infections (84). Delayed infections can either occur as low-grade infections up to three years after ORIF or arthroplasty, and are caused by intraoperative contaminations of the implants, or as haematogeneous infections after many uneventful years, especially after arthroplasty. The latter are mostly caused by transient bacteremia. The infection management is always a complicated and costly process which always includes surgical intervention with the procedures depending on the type of infection. The required surgical procedures include debridement, together with either implant retention in acute infections, or removal of all implants in chronic infections (85, 86).

c) **Screw cut-out and long screw:** penetration of the screw tip out of the medial cortex of the humeral head is a potential complication following surgery (87). This complication is common in elderly patients and linked to low bone quality and osteoporosis and occurs mainly in unstable fractures (78). Screws cut-out is associated with delayed healing and/or bone necrosis as a sequence of loss of reduction with protrusion of the upper screws into the joint. The screw penetration into the joint space could also occur at the time of surgery as a technical error (88).

d) **Pseudo-arthrosis:** A relatively uncommon complication of PHF, is pseudo-arthrosis after ORIF. Pseudo-arthrosis is a form of nonunion, where fibrous tissue is formed between tissue fragments. Pseudo-arthrosis occurs more frequently with Neer type II surgical humeral neck fractures, which could be explained by excessive mobility in the fracture site (89). The pseudo-arthrosis treatment can be difficult due to local factors such as connectivity of the fracture to the synovium and the stress forces produced by muscles and ligaments around the fracture. Moreover, osteoporosis and cavitation of the humeral head pose challenges, which increase with aging. The surgical treatment of the pseudo-arthrosis is challenging and can lead to an unfavorable functional outcome even when treated with arthroplasty (89, 90).

e) **Nonunion:** Nonunion is defined as the failure of bone trabeculation to cross the fracture gap. The clinical presentation usually includes persistent pain and loss of function of the shoulder. Nonunion frequently requires revision surgery (91). Therefore fracture nonunion has been defined by Calori *et al.* (92); this is the fracture that will not unite without further intervention.

f) **Avascular necrosis:** Avascular necrosis (AVN) of the humeral head can be defined in traumatic cases as bone death following deprivation of blood supply. The dead bone under stress forces is prone to flattening and collapse, leading to an abnormal shape of the humeral head and joint incongruity and is often associated with pseudo-arthrosis (31).

g) **Pseudo-paralysis:** Pseudo-paralysis can be considered as one of the unsolved challenging complications of shoulder surgery (93). The definition of pseudo-paralysis varies in the literature, but it is mainly defined as the loss of active shoulder elevation more than 90 degrees with free passive motion. Pseudo-paralysis is linked to rotator cuff tear and leads to low functional outcomes affecting the quality of life (93).

h) **Instability and Dislocation:** Instability refers to the inability to keep the humeral head in the glenoid fossa (94). Instability is one of the most common and challenging complications that can follow arthroplasty and one of the leading causes of revision surgery (95, 96). The management of joint instability needs careful evaluation of the cause and managing the predisposing factors such as humeral shortening, excessive medialization, together with the proper choice of the implant and soft tissue management (96). Shoulder dislocation after arthroplasty can occur either early in the first three months or delayed (after 3 months). Early dislocation can be managed conservatively with closed reduction under anesthesia, providing there is no relevant biomechanical problem causing the dislocation (97). Delayed dislocation usually requires revision surgery after careful evaluation of the cause of instability (96).

1.5.2 Associated risk factors

The overall healing capacity is known to be decreased in the elderly; this decrease could affect bone healing and lead to delayed healing or even nonhealing with its subsequent complications, as stated above (98). Aging is linked to many physiological changes that could affect bone healing. Many studies have evaluated the differences in the bone healing process between young and elderly and revealed several causes of delayed bone healing in the elderly (99–101). PHF is common in old age females and strongly linked to low bone mineral density as one of the fragility fractures. Its correlation with fragility is even more pronounced than the linkage with fractures of the hip, distal radius, or spine (29). Therefore, the management of PHF should include not only the evaluation of bone mineral density and treatment of the possible existing osteoporosis but also a thorough analysis of fragility and possibilities of prevention strategies (31, 102).

The relationship between the immune system in the elderly and the bone healing process has been previously established (100, 101, 103). This link has been established concerning physiological

bone turnover as well as pathologically as in fragility fractures (104). The initial inflammatory phase of bone healing has a significant role in initiating bone healing cascade (105). Typically in the bone healing cascade, the initial pro-inflammatory phase is followed by the anti-inflammatory phase; this switch is critical for proper bone healing (99). The initial inflammatory phase of bone healing is a necessary step to initiate the healing cascade via sending a chemotactic signal, which helps to invite more cells, especially endothelial cells, to the fracture hematoma (106). This phase has been shown to reach its peak within the first 24 hours following bone fracture (107), then declines, and the inflammatory cytokines start to decrease with a predominance of the anti-inflammatory cytokines. The upregulation of the anti-inflammatory factor expression is associated with an increase of expression of angiogenic factors such as heme oxygenase (HMOX), Vascular endothelial growth factor (VEGF), and Glucose transporter 1 (GLUT1), which are beneficial for bone healing (107, 108). It has been previously reported that a prolonged pro-inflammatory phase could impair angiogenesis and disturb the osteogenic processes leading to a delay in the healing progression of long bone fractures or could even lead to non-unions (101, 107, 109, 110).

Moreover, the initial inflammatory phase of bone healing is typically characterized by a large population of macrophages of the M1 phenotype, which has the ability to release cytokines that trigger and promote the inflammatory response (111, 112). Later in the anti-inflammatory phase of bone healing, the macrophages are mainly of the M2 phenotype, which releases growth factors and anti-inflammatory cytokines (111, 113). The switch between M1 into M2 at the proper time is of great value in regulating the inflammatory phase and affects the bone healing process (99). However, with aging, the ability to control the pro-inflammatory phase is decreased, leading to a prolonged and high amplitude pro-inflammatory phase, which in turn negatively affects bone healing (99, 114–116).

Additionally, immunologically restricted patients such as those with autoimmune diseases or malignancies often suffer from delayed or insufficient fracture healing, which has been found to be due to the vigorous inflammatory activity on cellular and humoral levels at fracture sites (117). The analysis of the fracture hematomas and/or the surrounding bone marrow of these patients showed a significant difference in the initial inflammatory phase compared to the healthy control group. The immunologically restricted patients show a higher population of immune cells with high levels of pro-inflammatory cytokines, which could be one of the reasons that explain healing problems in such patients (117).

Differences between young and old cell populations strengthen this assumption; as with aging and the continuous exposure to pathogens, the memory T cell population such as Terminally Differentiated Effector Memory CD8+T (T_{EMRA}) increases, leading to a high CD8/ CD4 ratio.

CD8+T (T_{EMRA}) cells have been proven to play a crucial role in controlling bone cells through specific cytokines that control the osteoclasts via specific receptor activator of nuclear factor κB (RANK) on the cell surface (118). These cells release RANK-ligand (RANKL) that is capable of stimulating osteoclasts and hence increasing bone resorption, which, as a result, delays the healing process (119). The link between CD8+T (T_{EMRA}) cells and the delayed union has also been further proven through the finding of a high population of CD8+T (T_{EMRA}) cells in the delayed bone healing fracture site (109, 120). Similarly, fractures in an animal model with a low population of CD8+ show enhancement of the bone healing process (101).

Moreover, CD8+T (T_{EMRA}) cells were found to be enriched in fracture hematomas; these cells were the major producers of Interferon gamma /Tumor necrosis factor-alpha (IFN ɣ/ TNFα), which inhibit osteogenic differentiation and the survival of human mesenchymal stromal cells (101). On the other hand, the T regulatory (T_{reg}) subtype revealed a positive impact on both wound and bone healing (121–125). Additionally, bone healing capacity was found to be improved in the (T_{reg}) high population animal model (121–125). Therefore, balancing the CD4+ T_{regs} / CD8+ effector memory cell ratio could enhance the local fracture milieu and control the inflammatory phase in a way that could benefit the bone healing process in elderly patients (107, 126).

1.6 Novel concepts in bone regeneration

PHF in elderly patients has shown increased rates of healing delays with the consequence of fracture complications. As given in detail above, the complication rate due to deficient bone quality in elderly patients has reached up to 57% for surgically treated patients (79). Therefore, these patients exhibit a high medical need for a biological solution. Immunomodulatory therapy has emerged as a potential therapeutic strategy that can benefit fracture patients with unfavorable immune responses. Such therapies are expected to reduce the risk of delayed bone healing in fracture patients with a potential dysregulation of the immune reaction and altered immune cell compositions in the fracture site through downregulating CD8+ cytotoxic cells, which has a potentially unfavorable effect on bone healing. Moreover, immunomodulatory therapy reduces the TNF-α and IFN- γ secretion of T cells and further supports macrophage polarization towards an anti-inflammatory type. In other words, immunomodulatory therapy aims to downregulate the inflammatory phase, which is known to be of high amplitude and long duration in this specific age group due to an over-reactive immune response (101, 120, 127).

In recent years, the potential role of Iloprost as an immunomodulatory agent was found to be promoting an anti-inflammatory and immunosuppressive effect (128, 129). Iloprost is a synthetic analogue of prostacyclin PGI2, a product of the cyclooxygenase pathway metabolizing arachidonic

acid constitutively in human cells, which dilates systemic and pulmonary arterial vascular beds. The U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA) approved Iloprost in 2004 and 2003, respectively, for the treatment of Primary Pulmonary Hypertension (PPH). Moreover, Iloprost has been used in cases of Buerger disease (thromboangitis obliterans), scleroderma, and ischemia (130), in addition to severe pain caused by sickle cell crisis, Reynaud phenomenon and systemic lupus erythematosus. Iloprost acts by causing vasodilatation in the microcirculation, reducing the capillary permeability, preventing the adhesion of thrombocytes, improving the viscosity of distal vessels, and reducing the generation of oxygen free radicals and leukotrienes (131, 132). Furthermore, the drug has been previously used as an off-label treatment for bone marrow edema in initial cases of osteonecrosis and showed promising therapeutic results (132–135).

The immune-modulatory effect of Iloprost has been investigated in the context of bone regeneration (100). *In vitro* studies confirmed the immune-modulatory properties of Iloprost and the postulated positive osteogenic effect (100). In a proof of concept *in vivo* study, the local Iloprost application in a mouse osteotomy during the early bone healing phase showed a positive impact on bone healing, where Iloprost within a fibrin-based release system was inserted during surgery into an osteotomy gap of a mouse to delay the release of Iloprost to the surgical site (100). This delay allowed the initial pro-inflammatory phase to continue and initiate the healing cascade of the local fracture milieu (107, 126). Another preclinical experiment in a sheep model was performed, where Iloprost was applied in a hydrogel scaffold during surgery in the bone drilled hole (136). No adverse effects nor local toxicities were observed with the local application of Iloprost in this large animal model, which could be evidence for the local safety of Iloprost application.

1.6.1 The potential benefits of Iloprost in bone healing

According to the performed and published preclinical findings to date, the benefits of Iloprost as an immune-modulatory agent in inducing bone regeneration can be summarized as follows:

1.6.1.1 Immunomodulatory Effects of Iloprost on cytokines:

Iloprost reduced the concentration of secreted IFNγ and TNFα of T cells creating a favorable milieu for MSC differentiation. This effect has been tested *in vitro* on murine MSCs in two different Iloprost doses (300 nM and 3 μM) (100). Both cytokines (IFNγ and TNFα) have a significant role as signaling molecules in bone repair, particularly in the early fracture healing phase with overly high amounts of them negatively affecting bone repair by diminishing the formation of the mineralized matrix by MSCs (101).

1.6.1.2 Immunomodulatory Effects of Iloprost on CD8+ T cells:

The preclinical data showed that the presence of 3 μM Iloprost affects the isolated CD8+ T cells leading to a decreased secretion of IFNγ and TNFα (100). CD8+ T cells are one of the primary producers of pro-inflammatory cytokines in the early bone repair phase (101).

1.6.1.3 Immunomodulatory Effects of Iloprost on macrophages:

Iloprost led to the downregulation of pro-inflammatory and the upregulation of anti-inflammatory cytokines by MΦ, M1, or M2 polarized macrophages. Macrophages are responsible for the clearing of the cell debris through early infiltrating the fracture area and are necessary for the recruitment of further cells adverse for the progression of the healing cascade due to their secreted cytokine profile (4).

1.6.1.4 Iloprost showed no negative impact on the osteogenic and chondrogenic differentiation capacity of MSCs:

MSCs are the precursor cells for both cartilage-producing chondrocytes and bone-forming osteoblasts. Iloprost showed no negative effect on the osteogenic capacity of MSCs when added to monolayers of MSCs that have been cultured for 14 days in osteoinductive media (100). Additionally, Iloprost did not hinder the chondrogenic differentiation of MSCs (100).

1.6.1.5 Iloprost promotes fracture healing *in vivo*:

Iloprost embedded in a fibrin clot (used as a delayed-release system) was inserted during surgery in an osteotomy gap of a mouse osteotomy model system. This delayed release allows the initial pro-inflammatory phase to proceed and to initiate the healing cascade. Micro-computed tomography (μCT) analysis 21 days post-surgery showed an improved healing outcome of the mice receiving Iloprost in comparison to the control group (an increase of both bone volume and total callus volume as well as the ratio of bone volume/total callus volume) (100). Additionally, histomorphometric (IHC) analysis of the tissue distribution around the gap after 21 days in the Iloprost treated group showed a significantly higher amount of mineralized bone and cartilage tissue (100). Finally, IHC analysis of about three days post-osteotomy showed the starting shift of the pro-inflammatory into the anti-inflammatory phase in the mouse osteotomy model system (100).

1.6.1.6 Iloprost effect on bone microcirculation:

Iloprost has also been previously used as an off-label treatment for bone marrow edema in early cases of osteonecrosis and showed promising therapeutic results (132–135, 137). Iloprost has been successfully used as an IV infusion to treat AVN safely with minor and totally reversible side

effects (138). Iloprost vasodilator effect is found to enhance microcirculation and increase local blood flow (138) by causing vasodilatation in the microcirculation, reducing the capillary permeability, preventing the adhesion of thrombocytes, improving the viscosity of distal vessels, and reducing the generation of oxygen free radicals and leukotrienes (131, 132).

1.7 Clinical trial approval

The process of clinical trial approval falls under the Directive 2001/20/EC (139) of the European Parliament and Council, which regulates the performance of clinical trials in humans while protecting their rights and dignity according to the Declaration of Helsinki (140, 141) and the good clinical practice guidelines (142). In order to obtain approval for a clinical trial in humans, the applicant should demonstrate a profound benefit-risk assessment and guarantee participant rights, well-being, and data protection throughout the entire clinical trial. Two simultaneous application processes need to be initiated and approved before starting a clinical trial, one at the ethics committee and the other at the relevant competent authority, which in this case was the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte BfArM) because the immunomodulatory agent Iloprost is a small molecule drug.

1.7.1 Ethics committee

The state office for health and social affairs (Landesamt für Gesundheit und Soziales (LAGeSo)) sets up the ethics committee of the state of Berlin to evaluate clinical trial applications, according to the German regulation for approval, and implements the clinical trial with medicinal products in humans (GCP-V) (143) and section 42 of the Medicinal Products Act (Arzneimittelgesetz – AMG) (144). Within ten days of receiving the application, the ethics committee is responsible for informing the sponsor that either a correct application has been received, or for asking the sponsor to resubmit any missing documents within fourteen days. The review timeline is one month between the formally completed submission and the first oral consultation for a Phase 1 mono center clinical trial. During the evaluation process of the application, the ethics committee can only request additional information from the sponsor once. The deadline for the ethics committee response is postponed until the additional information is received. Then, the ethics committee sends their reasoned assessment to the sponsor and the competent higher federal authority.

The following is the list of documents that are required to be submitted to the ethics committee in both paper and electronic forms:

1. Cover letter, which should include study data such as the name of the study, EudraCT number, sponsor name, study center, the list of all documents, their version and date, in addition to the confirmation that the electronic and paper versions are identical.

2. Application checklist form (list of all required documents) according to the 12th amendment to the AMG and the GCP-V (143, 145).
3. EudraCT: (European Union Drug Regulating Authorities Clinical Trials database). This is a registry of the interventional clinical trials operated by the European Medicines Agency and used by the member state competent authority to approve and monitor the clinical trial.
4. Module 2 (general overview of the trial)
5. EudraCT confirmation letter sent to the applicant after initiation of the new EudraCT
6. Sponsor responsibility (principal investigator authorization letter)
7. Study protocol German summary that should include the general outlines of the study protocol
8. Study protocol: this is one of the most crucial documents in the clinical trial application. It should include: a) general data as the name of the study, sponsor, monitor, principle investigator, and the clinical lab or other technical departments. b) background information of the investigational product. c) study objectives d) study design e) participants selection criteria f) assessment of efficacy and safety as well as study statistics g) data access, handling, record keeping, quality control measurements, ethics considerations, financial overview, and publication policy (GCP) (142)
9. Risk-benefit assessment
10. Investigational Brochure (IB) and the professional drug information: the IB should include all relevant clinical and nonclinical data on the investigational drug and the rationale for conducting the clinical trial. In the case of using previously marketed drugs, the summary of product characteristics should be attached. In the case of using an already marketed drug in a new indication, the IB should be prepared to be specific for the new use. (GCP) (142)
11. Patient information sheet and the informed consent form. Both documents should be written in easily understandable language for the patients with detailed information about the trial, mentioning the possible risks and benefits of the investigational drug in addition to including all data rights and responsibilities of the participants, and providing data on study insurance.
12. The study insurance documents. According to the (AMG), the insurance for the clinical trial should be at least 500000 Euro per study participant, which can be paid in cases of permanent disability or death in connection with clinical trials (144)
13. Principle investigator and sub-investigator (deputy) qualification documents and confirmations (CV, training like the GCP, financial interest, privacy and data protection agreement)

14. Study financial cost estimate: an overall study financial overview

1.7.2 Federal Institute for Drugs and Medical Devices (BfArM)

According to the GCP-V (143) and the section 40- 42 of the (AMG)(144), the relevant competent authority in this trial is the Federal Institute for Drugs and Medical Devices (BfArM) because the investigational drug (Iloprost as an immunomodulatory agent) is a small molecule drug. After receiving the application, the BfArM will respond to the sponsor within ten days either that their application is complete, or inform the sponsor of any missing document. The sponsor then has fourteen days to resend the missing document to the BfArM. Starting from the date of formally complete clinical trial submission, the BfArM has 30 days to evaluate the application and either approve or object to the conduct of the clinical trial. In the event of objecting to the trial, the sponsor has 90 days to reply to the BfArM objections by submitting additional documents/information. Finally, the BfArM has fifteen days to give the final decision of the whole application process. The ethics committee will also receive a copy of this final BfArM decision.

The following list of documents should be prepared in accordance with the European Commission 2010/C82/01 (146) and submitted to the BfArM in both paper and electronic forms:

1. Cover Letter
2. EudraCT and confirmation letter for the EudraCT number
3. Study protocol
4. Investigator's Brochure (IB)
5. Investigator Medicinal Product Dossier (IMPD). This is a critical document in the clinical trial application; the IMP is defined in the Directive 2001/20/EC (139) Article 2 (d) as "a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with marketing authorization but used or assembled (formulated or packaged) in a way different from the authorized form, or when used for an unauthorized indication, or also when used to gain further information about the authorized form (139)". This means that even the reference products such as placebo should be considered as an IMP. The given data should include all data regarding the general information and structure of the drug, detailed manufacturing data including materials and steps control, characterization, impurities, control of the drug substance, reference standards, container closure system, and data on the drug stability (147). In the case of using a previously authorized drug, in the clinical trial, it is sufficient to mention the marketing authorization number and marketing authorization holder, and it is possible

to refer to the data of preclinical and clinical use of the drug as well as data regarding the toxicology and safety profile of the drug (147).

6. Risk and benefit assessment

   This section includes a detailed analysis of the data, either clinical or nonclinical, to elaborate on the benefits and risks of using the investigational product. Risk and benefit assessment should mention any previously terminated clinical trial for safety issues discussing the cause of termination. The safety margin should also be mentioned by discussing the clinical relevance of any previously available clinical or non-clinical data (146). Moreover, it is essential to prove that the expected benefits outweigh the possible potential risks during the whole trial period during the trial (139).
7. Non Investigational Medicinal products dossier (Non-IMPD) if applicable. The non-IMPD is a medicinal product, for example, that is used in the trial as a concomitant (148).
8. Good manufacturing practice (GMP) (Manufacturing authorization as a proof for GMP compliance, this is only applicable if the previously authorized drug will not be used in its original form)
9. Labeling (if applicable)
10. Administrative Documents (sex distribution, further treatment, data protection declaration, costs declarations)
11. Scientific Advice (if applicable)
12. Genetically Modified Organisms (GMO) (if applicable)

1.8 Aims of the study

Regardless of the availability of different treatment options for PHF, the choice of the optimal management approach remains debatable, especially for patients above 60 years of age with 3-part and 4-part fractures, (149). Notably, members of this age group more frequently have a more experienced adaptive immune system than a younger collective, and the accompanied unfavorable immune response can lead to delayed bone healing, as discussed above (100, 107, 126). Moreover, the analysis of all surgical procedures outcomes and complication rates significantly differ between different treatment centers. Analyzing the outcome results and complication rate of current surgical management strategies for elderly patients with PHF in a leading academic center, where a high competence in fracture management with osteosynthesis and arthroplasty is present, could serve as a reference for the evaluation of the respective surgical techniques. The correlation of patients with a certain fracture pattern with the outcome can assist in considering their enrolment in an intervention study for the planned immunomodulatory therapy.

As such, the aim of this thesis was divided into two stages. The first aim focused on measuring the outcome of surgical management strategies, including complication and revision rates, of the two most commonly performed surgical procedures (angel stable plate osteosynthesis and arthroplasty) in elderly patients with PHF via a retrospective database analysis performed at the Center for Musculoskeletal Surgery at the Charité - Universitätsmedizin Berlin. This enabled us to identify the group of patients who could gain benefit from novel therapeutic approaches improving bone healing. The second part of this study focused on translating a scientifically sound novel immunomodulatory approach from the pre-clinical stage to phase I, IIa clinical trial that could improve the healing outcomes for the identified group of patients.

Specific aims:

1. To perform a literature review and identify gaps in knowledge regarding the current management strategies of PHF, the magnitude of complications following PHF management in elderly patients, and the potential benefits of immunomodulatory therapies.
2. To assess which surgical procedure for PHF is associated with lower complication and revision rates based on fracture classification.
3. To propose a novel therapeutic approach (immunomodulatory therapy) by translating preclinical data into a clinical trial that may help in improving the outcome of elderly patients with PHF.

Chapter 2 : Methods

The methodology of the study is divided into two sections. The first section focuses on a literature review and retrospective medical record analysis. This research was based on a review of data from patients suffering from PHF who had been surgically treated between March 2017 and June 2018 at the Center for Musculoskeletal Surgery (CMSC) of the Charité - Universitätsmedizin Berlin. The second part focuses on the development of a scientifically sound clinical testing strategy for an investigational immunomodulatory molecule.

2.1 Retrospective study

2.1.1 Literature review and formulating the research question

Research on PHF is known to lack comparative trials and having been performed on heterogeneous study populations, leading to the unavailability of reliable clinical recommendations (150). On the other hand, there is a rapid expansion in the literature for PHF focusing on new technologies and procedures. Due to this diversity in treatment strategies, and a substantial lack of clinical reports describing complication rates, particularly in elderly patients, performing a literature review to map and fill in the apparent knowledge gaps was seen as a necessary first step. PubMed, EMBASE, and MEDLINE databases were searched for the literature reporting on elderly patients treated surgically for (PHF). The search specifically focused on prospective clinical studies and retrospective observational studies investigating the outcome and the complication rate of surgical treatment of PHF. The search words included (proximal humer* fracture OR humer* head fracture AND age* OR elder* OR old* AND surgical OR surgery OR operat* AND treatment OR management OR outcome). The search scope was narrowed to the English language literature and from 2000 to 2020 (08.09.2020). Search filters applied were full text available, clinical trial, randomized control trial, review, and exclude duplication. The inclusion criteria were randomized control studies and cohort studies that recruited patients 60 years old or above, received operative treatment for PHF with any comparator, and follow up of at least one year. The exclusion criteria were case report studies (Figure 8).

The literature review has led to the formulation of the following study questions:

“Which group of patients in the elderly population with PHF have the least favorable clinical outcomes after surgical intervention?” and:

“Which clinical trial design investigating a local immunomodulatory therapy would have the potential of showing an effect on the outcome of PHF treatment?”

The research question was designed following the 'PICOT' model as follows:

- the patient population being studied: elderly patients suffering from PHF
- the intervention: treated with arthroplasty or ORIF (PHILOS)
- the condition: PHF based on Neer classification
- the outcome of interest: complication rates
- the timing of the analysis: six months after surgery

The complications discussed in this study were of Grade 2 or higher according to the surgical complication classification described by Dindo *et al.* (151). According to Dindo *et al.*, Grade 1 is any abnormal postoperative deviation, which includes events of minor risk that does not require therapy except simple medications such as analgesic, antipyretic or antiemetic. Grade 2 includes complications that may need either medical treatment (except the simple medications of Grade 1) or prolonged hospital stay by two times more than the average hospital stay of a similar procedure. Grade 3 encompasses any complication that could require invasive intervention. In contrast, Grade 4 is any complication that could lead to organ resection or permanent disability, and Grade 5 is any complication that could lead to death (151).

2.1.2 Study center

This study was based on single-center retrospective research. The study was carried out in the Center for Musculoskeletal Surgery (CMSC) of the Charité - Universitätsmedizin Berlin, considered as one of the largest orthopedic and trauma centers in Germany. The center is located at both Charité campuses, Mitte and Virchow Klinikum, with more than 8200 hospital admission cases and about 8500 surgical procedures every year.

2.1.3 Study design

This study is a retrospective medical record review study. The center's medical database was searched for all primary treatments of PHFs between March 2017 and June 2018 in patients aged 60 years or older utilizing the corresponding ICD-10 codes. All individual patient identifiers were removed, and patients' data were given a serial identification number (anonymized) when included in the study.

2.1.4 Patient selection

Patients aged 60 years or older with PHF who underwent operative treatment from March 2017 until June 2018 were the target group for this study. One hundred and five patients with PHF were identified. Patient selection was based on the ICD-10 coding (152) (S42.2 fracture of upper end of the humerus):

S42.20: Fracture proximal humerus (part unspecified)

S42.21: Head fractures including proximal epiphysis, proximal humerus fracture with two to four fragments

S42.22: Surgical neck

S42.23: Anatomical neck

S42.24: Greater tuberosity

S42.29: Other and multiple parts, includes: lesser tuberosity

2.1.4.1 Inclusion criteria

(1) A diagnosis of PHF

(2) Received surgical treatment with arthroplasty (hemiarthroplasty or reverse shoulder arthroplasty (RSA)) or ORIF with PHILOS (Synthes® GmbH, Switzerland)

(3) Completed a follow-up of six months

(4) Complete medical records were available

2.1.4.2 Exclusion criteria

(1) Age younger than 60 years old

(2) Treatment for PHF other than arthroplasty or PHILOS

(3) Follow-up of less than six months or insufficient data

All surgically treated PHF cases were screened to preserve the observational nature of the study. X-rays were used for the radiological confirmation of the diagnosis (PHF) and the identification of fracture-healing complications. Complications were also retrieved from the medical record database. All available X-rays and CT scans were analyzed carefully, together with the radiological reports as well as the medical file registry.

2.1.5 Data search and collection

Data collection, curation, and evaluation were performed between November 2018 and February 2019. Data search was performed on the medical records included in the (SAP, Walldorf, Germany) system of the Charité. The management plan for all eligible patients was carefully reviewed within the electronic medical file. The relevant data were collected with particular attention to the data of physical examination reports, operative notes, discharge letters, follow up visit reports, radiological examinations, and radiological reports.

The observed cases were mainly evaluated for:

- method of treatment
- fracture classification
- complications
- revision surgeries

2.1.6 Patients data verification

Data collection was performed in an Excel database sheet explicitly designed for this study. During data collection, patients were listed in the sheet according to the date of the surgical procedure they had received. The diagnosis included in the medical record for each patient and the performed surgical procedure were checked against the X-ray documentation.

The X-rays of eligible patients were examined to determine the fracture classification following the Neer fracture classification. Cases that were classified according to the AO classification were changed to the equivalent Neer classification. Finally, information on the course of fracture healing and associated complications were extracted and confirmed from the outpatient follow-up visits, the follow-up radiographs, and operation reports for the revision surgeries.

2.1.7 Statistical analysis

The collected data were imported from the Excel sheet to STATA statistical program for statistical analysis. The applied version of the Stata program was Stata version 15.1 (Copyright 1985-2017 StataCorp LLC- College Station, Texas 77845 USA). The logistic regression model allowed the identification of the possibility of complication occurrence with specific fracture classification. Moreover, it enabled the quantitative detection of the strength of association between the factor (fracture type and surgical technique) and the predictor (rate of complications). To determine the statistical significance of these correlations, an odds ratio with a confidence interval of 95% was calculated. P values of 0.05 were considered statistically significant.

2.2 The development of a scientifically sound clinical testing strategy

After identifying patients who were associated with a high rate of complications and high revision rate, the existing pre-clinical knowledge established about the local Iloprost application as an immunomodulatory agent, was translated into a clinical strategy to test this novel therapeutic approach in a human study. The clinical trial aims at investigating the safety of the local use of the drug and its ability to improve healing outcomes in PHF patients by modulating the prolonged and excessive pro-inflammatory reaction after fracture and surgery. The preclinical studies performed

by researchers at the Berlin Institute of Health Centre for Regenerative Therapies (BCRT) confirmed the immune-modulatory properties of Iloprost and the postulated positive osteogenic effect (100). Used locally to enhance bone healing, Iloprost is registered under patent number EP17188813.4 (European patent number) and CH833/2016 (Charité number). The work performed within the framework of this thesis focused on the utilization of the performed nonclinical studies to determine the clinical testing strategy in PHF patients, which eventually enabled us to submit a formally complete clinical trial application to the authorities and obtain the necessary approvals. To reach this aim, several aspects related to translational research and clinical trial design had to be investigated and devised. As part of this thesis study, the following tasks were identified:

1) performing a literature review on the immunomodulatory characteristics of Iloprost
2) determining the dose regimen (duration of treatment, formulation, and method of delivery)
3) determining the dosage of the investigational drug
4) selecting clinically representative endpoints and relevant controls
5) identifying inclusion and exclusion criteria to assess the suitability of the study population
6) identifying potential harms (adverse event (AE) and serious AE (SAE))
7) establishing clinical monitoring measures during the infusion of Iloprost
8) establishing an overall benefit-riskassessment of the investigational drug

Chapter 3 : Results

3.1 Literature review

The search yielded 1776 records in PubMed and 1396 records in Medline and EMBASE, which were reduced to 311 after applying the search filters. Subsequently, abstract screening of the 311 records was conducted using the inclusion /exclusion criteria, further reducing the number to 64 search results.

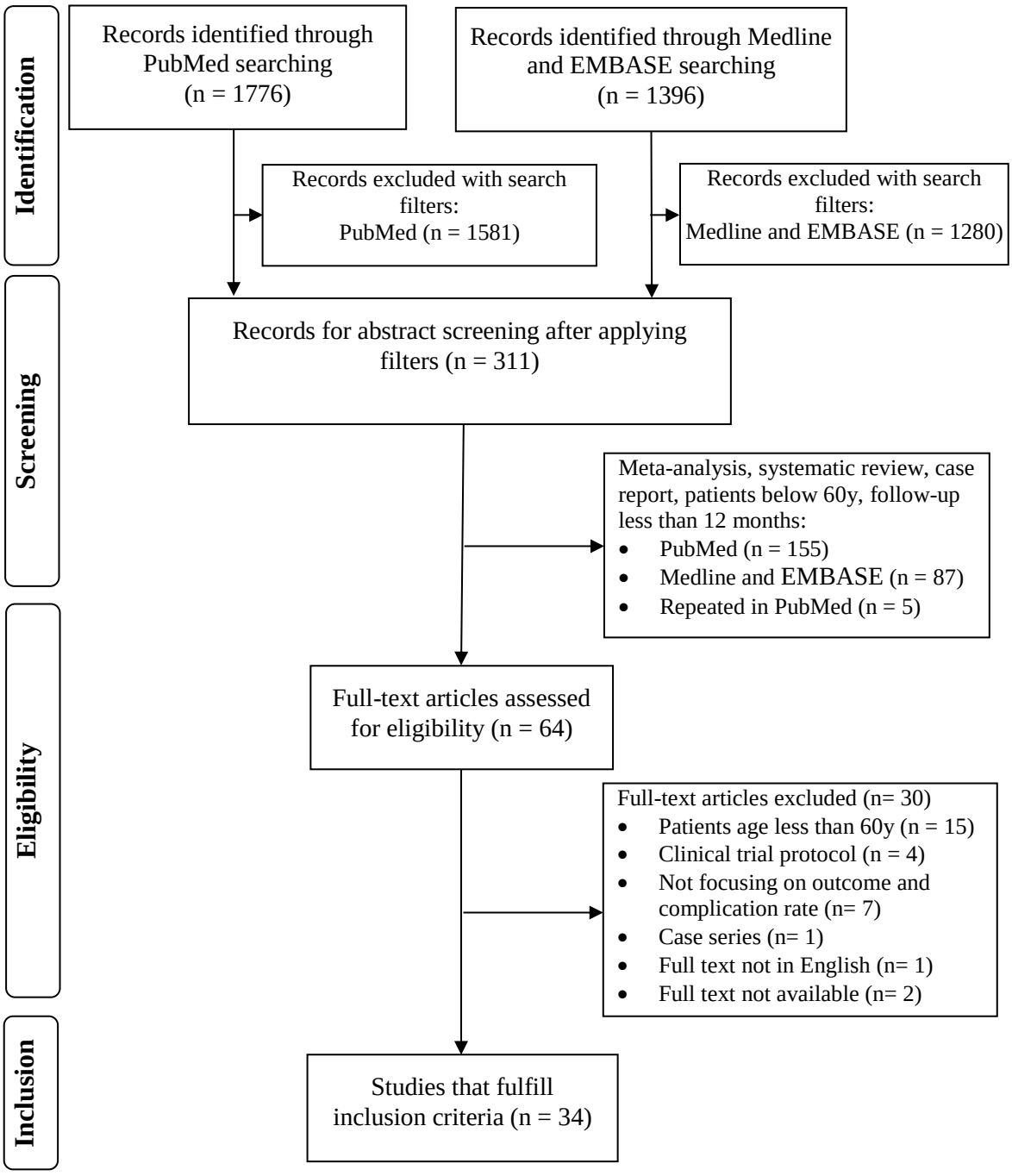

Figure 8: Flow diagram of the literature review

Full-text screening removed 30 records for not meeting the inclusion criteria, leaving 34 studies to be included in the analysis. In the 34 studies, a total of 2121 patients were included, out of which 471 were treated with PHILOS, 158 were treated with an intramedullary nail, 271 were treated with pinning either percutaneous or with tension band, 254 were treated with Hemiarthroplasty, and 375 were treated with reversed shoulder arthroplasty (RSA). The remaining patients received various treatments, such as different plates (other than PHILOS) or treated conservatively as a comparator (Figure 8, Table 2).

Table 2: Overview of the studies included in the literature review

Author	Year	Study category	Aim of the study Intervention / Comparator	Patient No.	Completed follow up	Treatment method										Follow up in months
						Conservative	Pinning	IMN	HSP plate	PHILOS (augmented screw)	Others	PHILOS	NCB-plate	Hemiarthroplasty	RSA	
Fraser A.N. *et al.* (153)	2020	RCT	To compare the outcome of RSA / angular stable plate	124	104							60			64	24
Chen H. *et al.* (154)	2020	Cohort study	To introduce a new technique - PHILOS augmented with titanium mesh	22	22						22					12–16
Lopiz Y. *et al.* (155)	2019	RCT	To compare the outcome RSA / conservative for 3- or 4-part PHFs	62	59	32									30	12
Launonen A.P. *et al.* (156)	2019	RCT	PHILOS / conservative for displaced type 2 PHF	88	72	44						44				24
Hengg C. *et al.* (157)	2019	RCT	PHILOS / PHILOS with screw augmentation	67	55					28		27				12
Plath J E. *et al.* (158)	2019	RCT	To compare the outcomes of IMN / PHILOS	81	55			28				27				12

Blonna D. *et al.* (159)	2019	Retrospective Cohort study	To report the outcomes for pins stabilized with an external fixator for PHF / -	188	188		188									24
Chivot M. *et al.* (160)	2019	Multicenter retrospective study	(RSA) / conservative in displaced 3-part or 4-part PHF	60	60	32									28	24
Zhao L. *et al.* (161)	2019	Retrospective cohort study	PHILOS / PHILOS combined with fibular allograft in 3- and 4-part PHF	42	42						21	21				12
Simovitch R.W. *et al.* (162)	2019	Retrospective, multicenter cohort study	To evaluate tuberosity union in RSA for 3- and 4-part PHF	55	55										55	33.7
Wei Ge *et al.* (163)	2017	Comparative study	IMN / PHILOS/ conservative	198	184	43		72				69				24
Chen A.C. *et al.* (164)	2017	Retrospective cohort study	To evaluate the efficacy of PHILOS using the anterolateral deltoid splitting approach	21	21 above 60y							21				24
Sebastia-Forcada E. *et al.* (165)	2017	Case-control study	Primary RSA / RSA as a revision (only primary cases are included)	30	30										30	24-60
Obert L. *et al.* (166)	2016	Retro. and prospective cohort study	To compare the outcomes RSA in 4-part fracture in nine centers	73	73										73	12-24

Youn S.M. *et al.* (167)	2016	Retrospective cohort study	To evaluate the outcomes of uncemented RSA	33	20										20	36-90
Sebastiá-Forcada E. *et al.* (168)	2014	RCT	To compare the outcomes of RSA / Hemiarthroplasty	62	61									30	31	24 - 49
Buecking B. *et al.* (169)	2014	RCT	Deltoid-split approach / deltopectoral approach for PHILOS	120	90								90			12
De Kruijf M. *et al.* (170)	2014	Retrospective analysis	To assess the safety and functional outcome of surgical treatment of PHF in the elderly.	64	64		15	4			5	24		16		12
Liu Q. *et al.* (171)	2013	Clinical trial	To compare the outcome of the TRIGEN IMN / PHF	64	54			54								12
Cuff D.J. *et al.* (172)	2013	Cohort study	To compare the outcome of Hemiarthroplasty / RSA	53	47									23	24	24
Shulman B.S.*et al.*(173)	2013	Retrospective review	Age effect on patients outcome of PHILOS treated PHF	45	45							45				12
Cai M. *et al.* (174)	2012	RCT	2-year outcomes of PHILOS / Hemiarthroplasty for PHF	32	27							13		19		24

Boons H. W. *et al.* (175)	2012	RCT	Outcome after 4-part PHF treated either conservative / Hemiarthroplasty.	50	50	25								25		12
Carbone S. *et al.* (176)	2012	Clinical trial	The outcome of 2 different types of percutaneous pinning in 3- or 4-part PHF in the American Society of Anesthesiologists score 3 or 4	58	54		58									24
Fjalestad T. *et al.* (177)	2012	RCT	PHILOS / conservative for displaced 3- and 4-part PHF	50	48	25						25				12
Liu Z. *et al.*(178)	2011	RCT	PHILOS / minimally invasive injectable graft	50	50					29		21				12-25
Olerud P. *et al.* (39)	2011	RCT	2-year outcome of Hemiarthroplasty /conservative for a displaced 4-part PHF	55	49	25								24		24
Voigt C. *et al.* (179)	2011	RCT	Polyaxial /non polyaxially locked screw-plate systems	56	48				20			28				12
Fjalestad T. *et al.* (180)	2010	RCT	To evaluate the costs and health outcome for surgical / conservative	50	50	25						25				12
Klein M. *et al.* (181)	2008	Clinical trial	To evaluate the outcome after RSA	20	20										20	33

Owsley K.C. *et al.* (78)	2008	Retrospective review	To assess the radiographic and clinical results of treating PHF with PHILOS	21	21							21				12
Krause F.G. *et al.* (182)	2007	Clinical trial	To compare the stability of 2 fixation techniques for the tuberosities in 3- or 4-part PHF treated with Hemiarthroplasty.	58	58									58		32
Agorastides L. *et al.* (183)	2007	RCT	To compare 2 mobilization regimens after Hemiarthroplasty for acute 3- and 4-part PHF.	59	49									59		12
Cheng-Chang Lu *et al.* (184)	2004	Clinical trial	To investigates intramedullary pinning with tension-band wiring for PHF treatment	10	10		10									20.6
Total				**2121**	**1935**	**251**	**271**	**158**	**20**	**57**	**48**	**471**	**90**	**254**	**375**	

RCT: randomized control trial; RSA: reversed shoulder arthroplasty; PHF: proximal humerus fracture; IMN: intramedullary nail

Table 3: Overview of reported complications in the PHILOS studies

Author	Patient number	Complication percentage (%)	Type of locally reported complication for PHILOS											
			Nonunion	Reduction loss	Malunion/ Deformity	Primary implant malposition	AVN	Impingement	Cut-out	Head impaction	Hematoma	Infection	Others	
Fraser A.N. *et al.* (153)	60	18.3	1						9			2	2	One periprosthetic fracture One rotator cuff tear
Launonen A.P. *et al.*(156)	44	7							2				1	One peri-implant fracture
Hengg C. *et al.*(157)	27	38.9		2			2	2	3	1	1		1	One soft tissue complication
Plath J. E. *et al.* (158)	27	34.3		3		6	2		12				4	One axillary n. lesion One adhesive capsulitis Two tuberosity resorptions
Zhao L. *et al.*(161)	21	33.3					1		3				3	Three cases of head collapse
Wei Ge *et al.*(163)	69	26.1	2	4			6	3	14			1		
Chen A. C. *et al.* (164)	21	43					2		7					
De Kruijf M. *et al.*(170)	24	8.3	2											
Shulman B.S. *et al.*(173)	45	22.2											10	Unspecified ten complications
Cai M. *et al.*(174)	13	23	1									1	2	Two fixation failures
Fjalestad T. *et al.*(177)	25	60							7				8	One hardware failure Seven partial axillary n. injuries
Liu Z. *et al.* (178)	21	28.6		3			2		2					
Voigt C. *et al.* (179)	28	29.2		3	5	1	5		13					
Fjalestad T. *et al.* (180)	25	40	2				21						1	One fixation failure
Owsley K.C. *et al.* (78)	21	57		9					9					
Total	**471**	**Avr. ± SD 31.3 ± 15.2**	**8**	**24**	**5**	**7**	**39**	**5**	**81**	**1**	**1**	**4**	**32**	

Table 4: Overview of reported complications in the intramedullary nail studies

Author	Patient number	complication percentage	Type of locally reported complication for intramedullary nail									
			delayed union	reduction loss	Malunion / deformity	primary implant malposition	AVN	impingement	cut-out	screw backing out	Others	
Plath J. E. *et al.* (158)	28	33.3		2		4	1		2		3	Three tuberosity resorptions
Wei Ge *et al.* (163)	72	18.1		2			2		6		3	Three rotator cuff injuries
De Kruijf M *et al.*(170)	4	-										
Liu Q. *et al.* (171)	54	14.8	1		2		1	1	1	2		
Total	**158**	**Aver. 24.1**	**1**	**4**	**2**	**4**	**4**	**1**	**9**	**2**	**6**	

Table 5: Overview of reported complications in the Hemiarthroplasty studies

Author	Patients number	Complication percentage	Type of locally reported complication for Hemiarthroplasty								
			Nonunion (GT)	Reduction loss (GT)	Primary implant malposition	Impingement	Hematoma	Infection	Subluxation	Others	
Sebastiá-Forcada E. *et al.*(168)	30	30						1		8	One intraoperative fracture, one stiffness, six proximal migration
De Kruijf M. *et al.*(170)	16	12.5						1		1	One axillary n. damage
Cuff D.J. *et al.*(172)	23	39					1			9	Nine tuberosity resorptions
Cai M. *et al.*(174)	19	15	1					1		4	Two fixation failures, one dislocation, One loosening
Boons HW *et al.* (175)	25	32	2	5	4					4	One head stem separation One proximal implant migration Two partial GT resorptions
Olerud P. *et al.* (39)	24	29.2		5		1				3	One complete resorption of GT Two partial resorptions of the GT
Krause F.G. *et al.* (182)	58	25.9		13						27	12 tuberosity resorptions, one periprosthetic fracture, eight stiffnesses, two partial axillary n. injuries, two aseptic loosenings, two cable wire breakages
Agorastides I. *et al.* (183)	59	20.4		4					6		
Total	**254**	**Aver. ± SD 25.5 ± 9**	**3**	**27**	**4**	**1**	**1**	**3**	**6**	**54**	

GT: greater tuberosity

Table 6: Overview of reported complications in the reverse shoulder arthroplasty (RSA) studies

Author	Patient number	Complication percentage	Type of locally reported complication for RSA					
			Hematoma	Infection	Dislocation	Nerve injury	Others	
Fraser A.N. *et al.* (153)	64	10.9		2		2	3	One glenoid fracture, two periprosthetic fracture
Lopiz L. *et al.* (155)	30	6.7				2		Two suprascapular nerve injuries
Chivot M. *et al.* (160)	28	7			2			
Simovitch R.W. *et al.* (162)	55	12.7				1	20	One ulnar n. neuropraxia, 6 radiolucencies, 7 GT nonunion, 7 GT malunions
Sebastia-Forcada E. *et al.* (165)	30	13.3			2		2	One humerus shaft fracture, one acromial fracture
Obert L. *et al.* (166)	73	37.8	1		2	3	8	Three brachial plexus paralyses, one humeral fracture, two stiffnesses, four ossifications, one malunion GT
Youn S. M *et al.* (167)	20	10					2	Two cases at risk of loosening
Sebastiá-Forcada E. *et al.* (168)	31	6.5	1	1				
Cuff D.J. *et al.* (172)	24	8	1			1	6	Four tuberosity resorptions, one apical pneumothorax, one periprosthetic fracture, and one ulnar paresthesia
Klein M. *et al.* (181)	20	15		2	1			
Total	**375**	**Aver. ± SD 12.8 ± 9.3**	**3**	**5**	**7**	**9**	**41**	

GT: greater tuberosity

3.2 Retrospective study

One hundred and five patients with PHF who underwent operative treatment from March 2017 until June 2018 were screened. Out of these identified and screened case files, ten cases were excluded from the study for not meeting the full inclusion criteria either because the PHF was associated with shaft fracture extension, or the data was not sufficient to obtain a complete case assessment. Subsequently, out of the remaining 95 cases, seven were further excluded for receiving a surgical procedure other than arthroplasty or PHILOS (such as intramedullary nail (4), minimally invasive fixations (2), and fixation with a plate other than PHILOS (1). These seven cases receiving other treatment for PHF than PHILOS and arthroplasty were excluded from the analysis since their small number warranted them unsuitable as comparators. Therefore, a total of 17 patients were excluded from the study, leaving a study cohort of 88 PHF in 87 patients (Figure 8). In order to examine the representativeness of the selected sample, the distribution of gender, age, and admission duration were assessed.

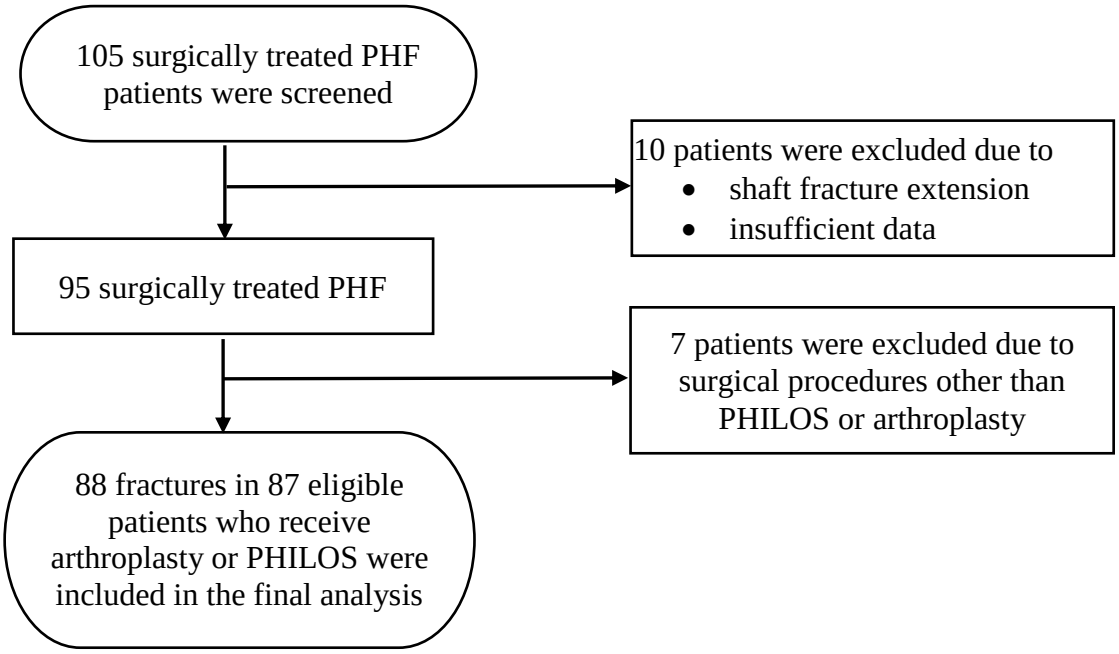

Figure 9: The retrospective medical record review study flowchart

Eighty-seven subjects met the inclusion criteria for this study and had complete clinical and records, including a follow-up period of at least six months, of whom 22 (18 females and 4 males) were treated with arthroplasty and 65 (44 females and 21 males) with PHILOS. The study population had an expectedly higher proportion of females (70.4%). The average patient age was 72.9 years, 74.1 for patients who received arthroplasty and 72.5 for those who received the PHILOS plate. Table 2 presents a summary of the demographic and clinical characteristics of the study patients. In 83 patients, the cause of the fracture was due to a fall of low energy impact. In

three cases, the fractures resulted from a polytraumatic event, and in two patients, the cause of fracture was not specified. According to Neer's classification system, one fracture was classified as 1-part (1.1%), six fractures as 2-part (6.8 %), 44 fractures as 3-part (50%), and 37 fractures (42%) as 4-part fractures.

Table 7: Demographic and clinical characteristics of the study patients

Characteristics		PHILOS group	Arthroplasty group	Total
Number of PHF		66 (75%)	22 (25%)	88 (100%)
Age (years)	Min (years)	60	66	60
	Max (years)	93	85	93
	Mean±SD	72.5±5.1	74.1±7.9	72.9±7.33
Number of fractures per gender (n/%)	Female	44 (67.7%)	18 (81.8%)	62 (71.3%)
	Male	21 (32.3%)	4 (18.2%)	25 (28.7%)
Hospital stay (days)	Min (days)	3	4	3
	Max (days)	43	38	43
	Mean±SD	10±7.95	11±7.32	10.34±7.78

3.2.1 Treatment approach and fracture classification

Twenty-two patients (about 25%) with PHF underwent hemiarthroplasty or RSA, while the majority of study patients (about 75%) were treated with PHILOS fixation. It should be mentioned that all arthroplasty prostheses used in this study, were reversed shoulder arthroplasty except for only three hemi shoulder arthroplasty prosthesis cases, which used in 4-part fracture management. The complexity of PHF increases in patients with more displaced fracture fragments (16). The 3-part fractures accounted for the largest group (n = 44; 50%), and 37 fractures (42%) were 4-part fractures. The most common treatment for 3-part fractures was the PHILOS (n = 43, 97.7%), while more cases with 4-part fractures were treated with arthroplasty (n=21, 56.7%), compared to ORIF (n = 16; 43.3%; Table 3).

Table 8: Treatment approach (arthroplasty vs. PHILOS) and fracture classification

Classification	Treatment		Total
	Arthroplasty, no. and percentage	PHILOS, no. and percentage	
1-part	0 0%	1 100%	1 100%
2-part	0 0%	6 100%	6 100%
3-part	1 2.27%	43 97.73%	44 100%
4-part	21	16	37

	56.76%	43.73%	100%
Total	22 25%	66 75%	88 100%

3.2.2 Overall complication rates

Assessing the complication rate in PHFs treated with one of the two surgical approaches, either arthroplasty or PHILOS, was one of the main aims of this study. For the analysis, complications were defined as any adverse events directly related to the performed surgical procedure (64). Dindo *et al.* (151) classify the surgical complication into four main categories according to the treatment needed to manage these complications, where Type 1 is a minor event deviation requiring no surgical intervention, while Type 2 and higher are considered major and need active management and/or further surgical intervention (151). The complications included in this study were all of Grade 2 or higher according to the surgical complication classification described by Dindo *et al.* (151).

All available radiographs and medical records were evaluated to determine the type of complication in each case. Overall, complications were seen in 24 cases (27.3%) of the total study patients, of which 19 patients underwent PHILOS fixation, and five underwent arthroplasty. The complication rates were higher in the PHILOS group, comprising 28.7% (19 of 66) compared to 22.7% (5 of 22) in the arthroplasty group (Table 4).

Table 9: Overall complication rates according to surgical technique

Treatment	Complication, fracture no. and percentage		Total
	No	Yes	
Arthroplasty	17 77.27%	5 22.73%	22 100%
PHILOS	47 71.21%	19 28.79%	66 100%
Total	64 72.73%	24 27.27%	88 100%

3.2.3 Complication frequencies in each fracture type

A careful analysis of the radiographs and the medical records of each patient was performed in order to identify the complications that had occurred within each fracture type (1-part, 2-part, 3-part, and 4-part). The majority of postoperative complications were reported in 4-part fractures, comprising 40.5% (15 of 37) compared to 18% (8 of 44) in 3-part fractures (Table 5).

Table 10: Complication frequencies in each fracture type

Classification	Complication no. and percentage		Total
	No	Yes	
1-part	1 100%	0 0%	1 100%
2-part	5 83.33%	1 16.67%	6 100%
3-part	36 81.82%	8 18.18%	44 100%
4-part	22 59.46%	15 40.54%	37 100%
Total	64 72.73%	24 27.27%	88 100%

A logistic regression analysis was then conducted to investigate the correlation between the complication rates and the fracture type or the surgical approach (both PHILOS fixation and arthroplasty). The hypothesis was that the surgical approach performed, as well as the type of fracture, influenced the rate of complications. The analysis showed that osteosynthesis (regardless of the fracture classification) had odds of complications 5.4 times the odds of complications in shoulder arthroplasty (OR 5.45, 95% CI: 1.32, 22.41). This difference was statistically significant (P-value, 0.019). Additionally, the analysis showed that 4-part fractures had odds of complications 7.4 times the odds of the complications in 3-part fractures (regardless of the treatment approach) (OR 7.42, 95% CI: 2.10, 26.20). This difference was statistically significant (with P-value 0.002).

Table 11: Logistic regression model for complications concerning either treatment or fracture classification.

Logistic regression				Number of obs		81
				LR chi 2 (2)		11.03
Log likelihood = -42.811666				Prob > chi 2		0.0040
				Pseudo R2		0.1141
Complication	Odds Ratio	Std. Err.	z	P > |z|	[95% Conf.	Interval]
PHILOS	5.447749	3.931353	2.35	0.019	1.324189	22.41219
4-part	7.415561	4.776572	3.11	0.002	2.098269	26.20758
_cons	.0416994	.0340794	-3.89	0.000	.0084038	.2069117
Note: _cons estimates baseline odds.						

3.2.4 Complication frequencies in each fracture type and surgical approach

Postoperative complications were then distributed according to the surgical treatment performed and the type of fracture for a better understanding of the relationship between these different

variables. Fifty-eight percent of patients (11 of 19) with 4-part fractures vs. 36.8% of patients (7 of 19) with 3-part fractures treated with PHILOS fixation suffered from postoperative complications. In the arthroplasty group, four out of all five patients recorded with complications suffered from 4-part fractures (Table 7).

Table 12: Complication frequencies in each fracture type and surgical approach

	1-part fracture		2-part fracture		3-part fracture		4-part fracture	
Complication / Treatment	yes	no	yes	no	yes	no	yes	no
PHILOS (n= 66)	-	1	1	5	7	36	11	5
Arthroplasty (n=22)	-	-	-	-	1	0	4	17
Total		1	1	5	8	36	15	22

A logistic regression analysis was then conducted to investigate the correlation between the fracture type and complication rates for PHILOS fixation. The hypothesis was that in PHILOS fixation, the type of fracture could influence the rate of complications. In this analysis, the correlation was examined between the rate of complication and 4-part fracture classification (in comparison to 3-part fracture) for patients who underwent PHILOS plate. This analysis focused only on the PHILOS group due to the higher number of patients and the rate of complications. The model showed that 4-part fractures, when treated with PHILOS fixation, had odds of complications 11 times the odds of complications in 3-part fractures (OR 11.31, 95% CI: 2.99, 42.85). The difference was statistically significant (P<0.0001).

Table 13: Logistic regression model for the effect of fracture classification on the complication rate for PHILOS fixation.

PHILOS						
Logistic regression				Number of obs		59
				LR chi 2 (1)		14.50
Log likelihood = -29.040934				Prob > chi 2		0.0001
				Pseudo R2		0.1998
Complication	Odds Ratio	Std. Err.	z	P > |z|	[95% Conf.	Interval]
4-part	11.31429	7.68659	3.57	0.000	2.987763	42.84579
_cons	.1944444	.0803211	-3.96	0.000	.0865329	.4369278
Note: _cons estimates baseline odds.						

The next logistic regression analysis (Table 9) examined the correlation between the rate of complication and the PHILOS fixation (in comparison to arthroplasty) for patients who suffered from 4-part fractures. The model showed that patients with 4-part fractures, when undergoing PHILOS fixation, had nine times the odds of suffering from a complication than when undergoing arthroplasty. (OR 9.35, 95% CI: 2.05, 42.66) The difference was statistically significant (P<0.004).

Table 14: Logistic regression model for the effect of surgical technique on complication rate in 4-part fracture

Classification 4-part						
Logistic regression Log likelihood = -20.162549				Number of obs		37
				LR chi 2 (1)		9.64
				Prob > chi 2		0.0019
				Pseudo R2		0.1929
Complication	Odds Ratio	Std. Err.	z	P > |z|	[95% Conf. Interval]	
PHILOS	9.35	7.240865	2.89	0.004	2.049371	42.6582
_cons	.2352941	.1307574	-2.60	0.009	.0791739	.6992619
Note: _cons estimates baseline odds.						

These results confirmed that the treatment modality, as well as the type of fracture, influenced the rate of complications. In patients with Type IV fractures, PHILOS fixation was associated with higher complication rates.

3.2.5 Complication patterns and revision surgeries

The primary cause of postoperative complications in the PHILOS group was the loss of reduction, which amounted to 33.3% (9 of 27) of all reported complications. A single patient could have more than one complication reported. Loss of reduction was the most prevalent complication in the PHILOS group (n=9), followed by infection (n=4). Complications in the arthroplasty group, with the majority being infections, were mainly observed in 4-part fractures. Overall, the PHILOS group showed a higher number of complications (n=22) compared to the arthroplasty group (n=5). The complicated arthroplasty cases included two RSA cases and three hemiarthroplasty cases. All three of these cases of hemiarthroplasty were used to treat 4-part PHF and ended with complications and revised later on with total shoulder arthroplasty. This means that, between the 4-part PHF treated with arthroplasty, there were four complicated cases; three of them were treated with hemiarthroplasty, and only one case was treated with RSA. The complications seen in hemiarthroplasty cases were instability, dislocation, and infection.

Table 15: Complication patterns for each surgical approach

Type of complications	PHILOS group			Arthroplasty group		Total
	2-part	3-part	4-part	3-part	4-part	
Loss of reduction	1	2	6	NA	NA	9
Infection	-	2	2	1	2	7
Screw cut-out	-	3	1	NA	NA	4
Pseudo-arthrosis	-	-	1	NA	NA	1
Pseudo-paralysis	-	-	1	-	-	1
Dislocation	NA	NA	NA	-	1	1
Primary long screw	1	-	-	NA	NA	1
Instability	NA	NA	NA	-	1	1
Avascular necrosis	-	1	-	NA	NA	1
Nonunion	-	-	1	NA	NA	1
Total	2	8	12	1	4	27

NA, Non-applicable

Among all study patients, 13 (14.7%) underwent at least one revision surgery in order to manage the postoperative complications. The PHILOS revision cases were loss of reduction in four cases, nonunion in two, infection in one, and primary long screw in one case. Arthroplasty revision cases included infection in three, instability/dislocation in two cases. The overall (3- and 4-part fracture) revision surgery rate was higher after arthroplasty (22.7%) than after the PHILOS plate fixation (12.1%) surgeries. In 4-part fractures the number of revision surgeries was higher in the PHILOS treated group (5 of 16 patients) compared with the arthroplasty group (4 of 21 patients).

Table 16: Number of revision surgeries per treatment type and per fracture classification

Treatment	Total number of fractures	Number of revision surgeries			Total
		2-part	3-part	4-part	
PHILOS	66	1	2	5	8 (12.1%)
Arthroplasty	22		1	4	5 (22.7%)
Total	88	1	3	9	13 (14.7%)

To further illustrate the different types of reported postoperative complications and some of the management approaches, three cases were selected from the study patients and described in more detail. The first case (Figure 9) was a patient who underwent PHILOS fixation and suffered from loss of reduction one month following the surgery. The loss of reduction was, in this case, the displacement of the greater tuberosity from the anatomical position. This patient underwent revision surgery.

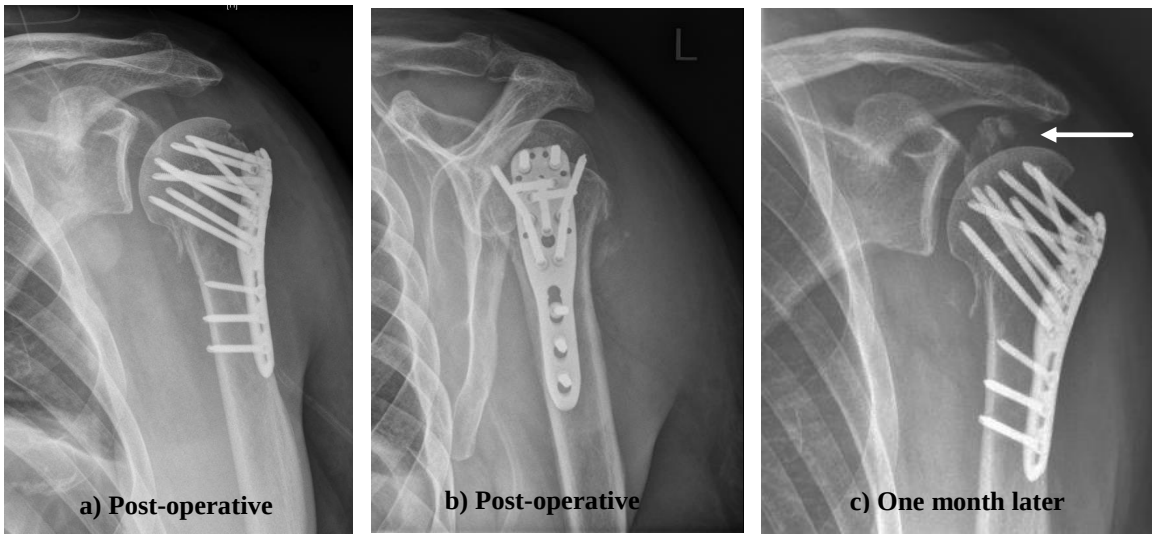

Figure 10: Loss of reduction in PHILOS plate fixation.
(a) and (b) postoperative, (c) one month later shows loss of reduction of the greater tuberosity (white arrow).

The second case was also a patient who underwent PHILOS plate fixation. Similar to the first case, the patient suffered from a loss of reduction two months after surgery (Figure 10 D). In addition, it could also be observed that the patient had multiple screws cut-out through the subchondral bone lamella of the humeral head (Figure 10 C & E). The X-ray series of this patient showed multiple postoperative complications (loss of reduction and cut-out).

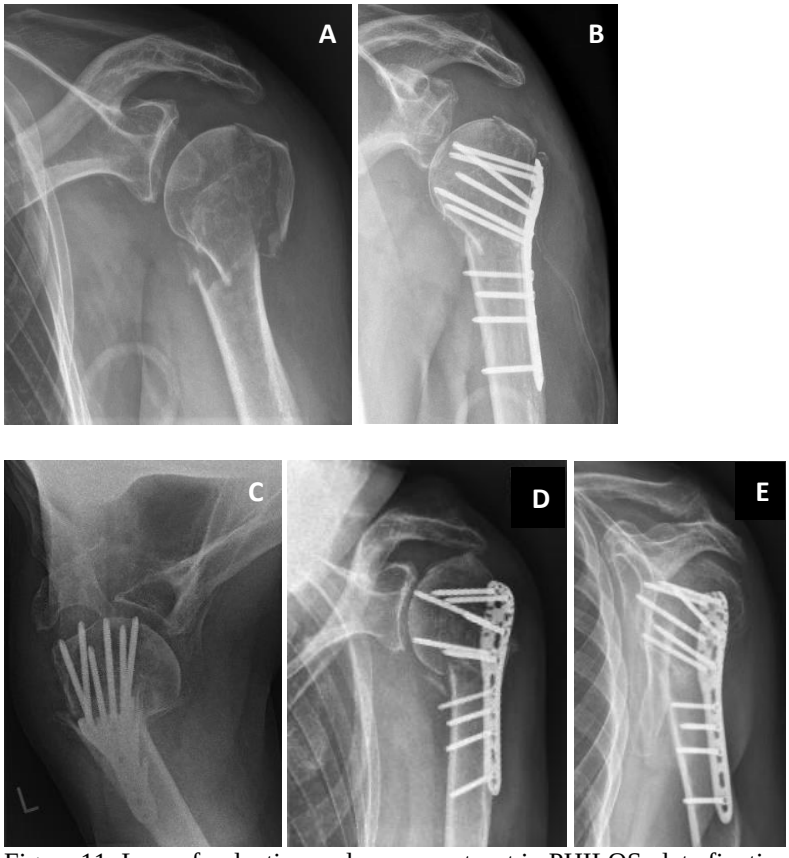

Figure 11: Loss of reduction and screws cut-out in PHILOS plate fixation.
(A) after fracture (B) after fixation. (C), (D) and (E) after two months of follow-up

The third patient underwent hemiarthroplasty and suffered from infection (Figure 11). This case was treated with a two-stage exchange procedure. In the first stage (Figure 11 B), removal of the infected prosthesis and bone cement was performed, followed by the debridement of the wound to remove any infected or dead tissue. Then a cement spacer impregnated with antibiotics was inserted, and the patient was given a course of antibiotics to control and treat the infection. In the second stage (Figure 11 C), a new prosthesis was inserted (RSA).

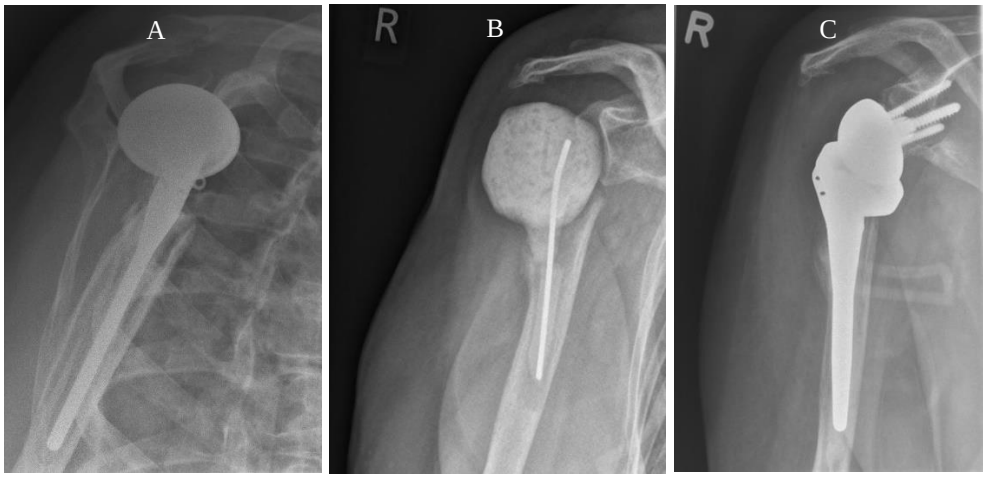

Figure 12: Infected shoulder hemiarthroplasty.
(X-ray A) Hemiarthroplasty, axial view, (X-ray B) spacer after infection, (X-ray C) revised by RSA after treatment of infection.

Clinical relevance of the retrospective study sample:

As indicated in table 1, the PHILOS's overall complication rate as reported in selected studies ranged between 9.7% and 57%, with the average complication rate being 27.9% and a standard deviation of 14.5%. We relied on these calculations to test the clinical relevance of our sample size. We then calculated the confidence interval of the included sample using the following equation (185).

$$CI = P \pm d$$

$$d = Z * \sqrt{\frac{P(1-P)}{n}}$$

Where d is the margin of error, Z is the Z score for a level of confidence, P population proportion, and n is the sample size (186). The confidence level is 95%, the proportion is 27.9%, and the sample size is 66. This has resulted in a margin of error of 10.82%, which can be interpreted as a 95% chance that the real complication rate is within ±10.82% of the measured complication rate. This margin of error (10.82%) was more conservative than the wide range of complication rates

observed in the literature (31.3 ± 15.2). In other words, the study sample of 66 can be considered appropriate to provide clinically relevant results.

In this calculation, we relied only on cases that received the PHILOS since reversed shoulder arthroplasty overcame the healing problem from the start by replacing the fractured head with the prosthesis. Thus, the outcome of the arthroplasty treated cases was not the primary focus of this retrospective study. Therefore, we provided a descriptive analysis of these cases. However, the finding that three out of the four arthroplasty cases with complications were Hemiarthroplasty is noteworthy to our mind. Unlike the reverse shoulder arthroplasty, tuberosity healing in Hemiarthroplasty is of great importance for a satisfactory clinical outcome. Tuberosity nonunion or malunion could be considered the most common cause of Hemiarthroplasty failure (29, 182, 187, 188). Delayed healing of the greater tuberosity leads to malposition and migration of the tuberosity, which may cause prosthesis subluxation (183). Moreover, tuberosity migration and malposition decrease the lever arm of shoulder abductors and change the tension of the rotator cuff complex. This significantly increases the needed torque for shoulder mobilization, which considerably affects the rotator cuff function and shoulder range of motion (189). Therefore, hemiarthroplasty patients could be considered as a potential future targeted population for healing enhancement therapy.

3.3 Devising a clinical testing strategy

The retrospective medical record analysis enabled us to determine the baseline complication rate and the rate of revision surgeries after PHF surgeries. Moreover, it helped us to identify the group of patients with the least favorable outcome after PHF surgery. Patients (> 60y) who underwent angle stable plate fixation, particularly with 4-part fractures, were associated with higher complication rates (figure 12). Within the ORIF group, as shown above, 3-part fractures were also associated with a significant complication rate. The most frequent complication in PHILOS groups (3-part and 4-part fractures) was related to compromise bone healing. Therefore, improving bone healing in these PHILOS treated group would be of great advantage. Hence, both cohorts, patients with 3- and 4-part fractures were identified as an optimal target group for an intervention trial.

The second part of this study focused on translating a novel, potentially beneficial treatment (immunomodulatory therapy) from the preclinical stage into the clinic by designing a clinical trial to investigate the potentially positive effect of immunomodulatory therapy on bone healing in PHF patients. In this context, a clinical trial was designed to check the safety as well as the efficacy of a local use of Iloprost, an analogue of prostacyclin PGI2, as an immune-modulatory therapy to enhance bone healing. The expected effect of local Iloprost application at the fracture site is the

enhancement of the local fracture milieu and the control of the early inflammatory phase. In order to investigate this approach in the identified patient population within the parameters of a clinical trial, several points had to be addressed adequately, such as the determination of dosage and dose regimen, choice of clinically representative endpoints, and establishing a benefit-risk assessment profile for the investigational product. Finally, the study entailed creating and submitting documents needed for clinical trial approval to the relevant authorities (please see sections 1.7.1 and 1.7.2).

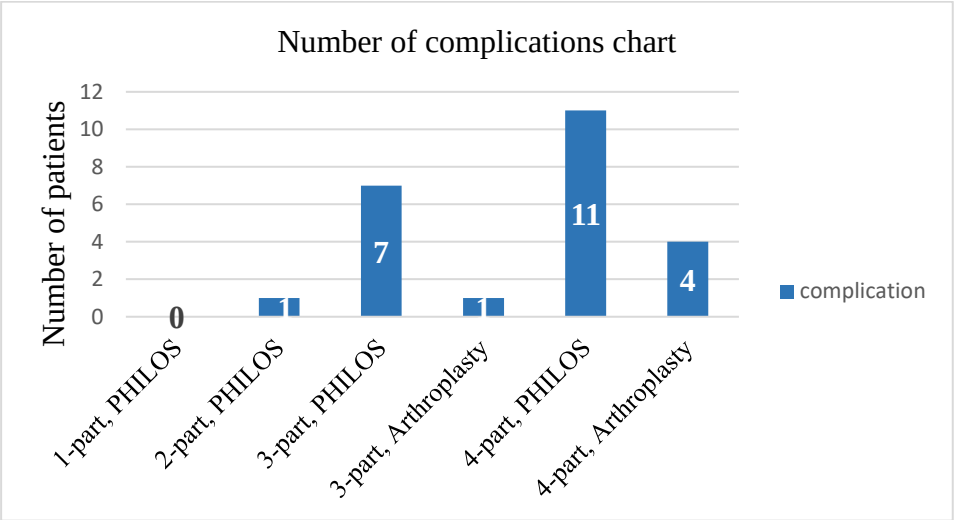

Figure 13: Number of complications in each fracture type and surgical approach

3.3.1 The proposed study design

The Iloprost study is a Phase I/IIa, prospective, mono-center, randomized, open-labeled, controlled study investigating the safety and clinical efficacy of Iloprost local application at the proximal humeral fracture site.

Study hypothesis: The local administration of Iloprost as an immunomodulatory agent at the fracture site is safe and could enhance bone healing in elderly patients with PHF.

Patients who fulfill the inclusion and exclusion criteria will be eligible to enter the study. Patients will be randomized on a 1:1:1 basis to one of the three arms (two treatment arms and one control arm).

The first intervention group will receive an open reduction and internal fixation (ORIF) with an angular stable plate (PHILOS) + Iloprost treatment. Patients will locally receive a dose of 0.125 ng/kg/min of Iloprost over 24 hours via a catheter and an electronic pump system. The catheter will be inserted during the surgical procedure. The infusion of Iloprost will start 24hrs post-operatively, and the dose will be delivered over 24h.

The second intervention group will also receive ORIF with an angular stable plate (PHILOS) + Iloprost treatment. Patients will locally receive a dose of 0.25 ng/kg/min of Iloprost over 24 hours

via a catheter and an electronic pump system. The infusion will start 24hrs post-operatively, and the dose will be delivered over 24h.

Control intervention: Patients will receive only ORIF with the PHILOS.

Follow-up per patient: 52 weeks, of which 26 weeks include active study participation. At the study end, a telephone call with the patients to ensure safety assessment.

Duration of intervention per patient: local Iloprost application for 24 hours starting 24 ±2 hours after surgery

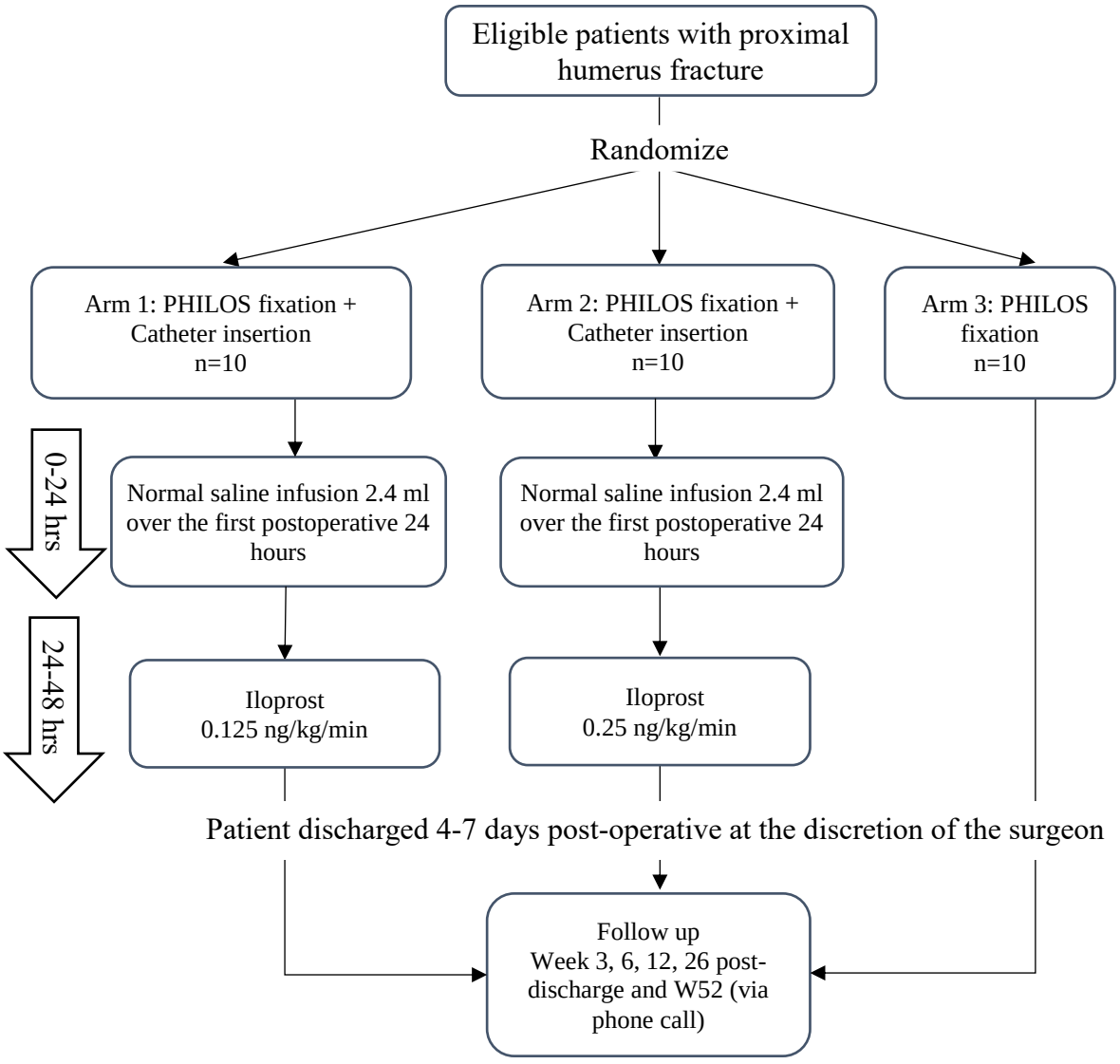

Figure 14: Flow diagram of the study design

3.3.2 Determining the dosage of the investigational drug

The choice and calculation of the dose used in this clinical trial relied on a multi-faceted strategy that took into consideration the benefit-risk profile of the drug. In particular, factors such as bone

vascularity, rate of infusion, and previous data from the animal model were taken into consideration as follows:

a) Given the high vascularity of bone, applying Iloprost locally to the site of fractures is expected to reach the vascular system. Applying Iloprost in this fashion is, to a great extent, similar to its market use via the intravenous route. To ensure patients' safety, the same recommended IV dose used in pulmonary hypertension was opted for the local infusion. Nevertheless, the drug is not expected to reach the same blood concentrations as in the case of IV infusion due to the slower infusion rate (see under b).

b) Iloprost has narrow therapeutic indices and a short half-life of 30 min. and is infused IV over six hours in its standard indication (190). Since Iloprost is known to show a reduction in tolerability and increase of side effects at higher infusion rates, the infusion period was extended from 6 to 24 hours to lower the possibility of any systematic toxicity and in order to increase the exposure time of the fracture to the drug. In this way the infusion rate (mL/hour) was reduced four-fold.

c) There is a direct correlation between body weight and bone mineral density (BMD) (191, 192). Bodyweight and body mass index (BMI) act as modifiable factors in the determination of bone mineral density (193). For instance, every unit increase in BMI was associated with a rise of 0.008 g/cm2 in L1-L4 BMD, 0.017 g/cm2 in femur neck BMD, and 0.018 g/cm2 in total hip BMD (194). Therefore, applying Iloprost according to body weight is expected to tailor the efficacy profile of the drug to each patient individually, an approach that would yield the best clinical outcome while mitigating any potential risks.

d) Finally, the dose was calculated to a greater extent based on the animal study that was previously performed by the research group in the (BCRT) (Box 1). The bone formation was analyzed in a drill hole model for four different groups in a sheep model: empty, gelatin only, gelatin with Iloprost, and bone graft group. The empty control group and the gelatin group were used as negative controls. Even though the efficacy of Iloprost using this dose was not found to be superior to controls because Iloprost was immediately given following bone injury and not as in the proposed trial after 24 hours, this dose did not show any toxicity and was well tolerated. Therefore, given all of these factors (a) to (d), it was evident that the weight adapted I.V. dose of Iloprost will be the most efficient and reliable dose to be used in the planned study.

Box 1: Dose calculation for the planned study

The human dose was calculated based on the Iloprost dose investigated in a sheep model after calculating the human equivalent dose as follows: Human Equivalent Dose (HED) = animal dose (mg/kg) × [animal weight (kg) ÷ human weight (kg)]$^{0.33}$ (195)

- The Iloprost dose investigated in sheep was 20 µg. The average sheep weight in this experiment was 80 kg, then the dose/kg is 20 µg /80kg = 0,25µg/kg
- Using the average human weight of 70 kg, HED= 0.25 *(80/70)^{0,33} = 0.26 µg/kg

The Iloprost dose will exemplarily be 0,26*70=18,28 µg in a patient with 70 kg body weight. This dose falls within the established IV dose range for the currently marketed indication (from 0.5 ng/kg/min to 2 ng/kg/min bodyweight over 6 hours IV drip, which equals to 12.6-50.4 µg for an average 70 kg patient). Therefore, relying on the established physiological dose of Iloprost was seen as the most reliable strategy.

Patients will be randomized into either one of three groups. All three groups will receive an open reduction and fixation of their PHF with the PHILOS (three arms). The first treatment arm will start treatment with Iloprost 24 hrs post-operatively; patients will receive a single dose of 0.125 ng/kg/min of Iloprost infused over 24 hours. The second treatment arm will start treatment with Iloprost 24hrs post-operatively; patients will receive a single dose of 0.25 ng/kg/min of Iloprost infused over 24 hours. Patients randomized to the third treatment arm will only receive the PHILOS plate, and their outcome will be compared to the other two treatment arms.

3.3.3 Selecting clinically representative endpoints and relevant controls

The planned study is a Phase I/IIa, prospective, mono-center, randomized, open-labeled, controlled study investigating the safety and clinical efficacy of local Iloprost application at the proximal humerus fracture site. The primary endpoint of the planned trail was determined as safety, through the identification of any noxious response or toxicity that has a causal relationship to the treatment. Toxicity shall be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) (196).

Clinical endpoint

The degree of preservation of the summation of the tip-apex distance (TAD) for the humeral head screws of the PHILOS at the 12 weeks postoperative follow-up visit compared to the immediately postoperative TAD. TAD refers to the distance between the tip of the screw and the cortex of the humeral head.

To investigate the therapeutic effect of Iloprost on the study participants, a radiological endpoint, the tip apex distance (TAD) measurement, was determined, which is considered to be directly related to the quality of fracture healing. TAD refers to the distance between the tip of the PHILOS screws and the subchondral outer cortex of the humeral head (fig. 14), which will be measured at 12 weeks postoperatively and compared to the baseline measurements after surgery. This endpoint is an indicator of the progress of fracture healing and the probability of potential complications. Thus, the endpoint reflects both the safety and efficacy of the investigational treatment (197).

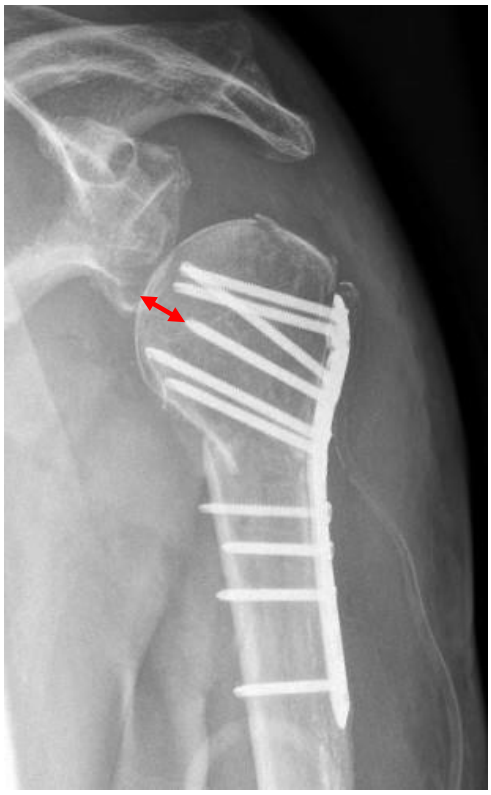

Figure 15: Tip Apex Distance
(TAP) is the distance between the tip of the screw and the humeral cortex (red double arrow) (197)

The degree of TAD preservation shall be classified into one of five arbitrary ranks based on the mean of the TADs of all screws:

- Grade 1: 76%-100% preservation of the original distance (representing the best possible result)
- Grade 2: 51%-75% preservation of the original distance
- Grade 3: 26%-50% preservation of the original distance
- Grade 4: 0%-25% preservation of the original distance

Grade 5: If the patient shows signs of screw protrusion (cut out) through the subchondral bone, this will be graded as 5.

Secondary endpoints

By assessing the following secondary endpoints, further clinical parameters of treatment efficacy could be evaluated:

- Rate of humeral head necrosis
- Change in the humeral head shaft angle (198)
- Pain assessment using the Visual Analogue Scale (VAS) (199)
- Quality of life by applying the questionnaire EuroQol-5 Dimension (EQ-5D) (200)
- Constant-Murley Score (CMS) (201)
- Disabilities of the Arm, Shoulder and Hand score (DASH) (202)

3.3.4 Identifying inclusion and exclusion criteria to assess the suitability of the study population

Patient selection was decided based on stringent criteria that are of critical importance both to protect the patient's rights and to assure valid data on outcomes. Only participants over 60 years of age were included in the study. The complete list of inclusion and exclusion criteria for the planned study is as follows:

a) Inclusion criteria: Patients are eligible to participate in the study if they fulfill the following inclusion criteria:

- Signed written informed consent
- Adult male or female patients 60 to 80 years of age at the time of screening
- Scheduled ORIF with PHILOS (three-hole PHILOS) for proximal humerus fracture Type 3 or 4 according to Neer classification
- Patient with American Society of Anesthesiologists ASA score of ≤ 2
- Single, low energy fracture
- Absence of neurovascular complications at the time of trauma
- Surgery was performed within the first 96 hours of injury

b) Exclusion criteria: Patients are not eligible to participate in this study and cannot be enrolled in the study if one or more of the following exclusion criteria are met:

- Subjects unable to freely give their informed consent (e.g., individuals under legal guardianship)
- Immunosuppression due to illness or medication.

- Subject with malignancy and undergoing treatment including chemotherapy, radiotherapy or immunotherapy
- Known allergies to Iloprost
- Conditions where the effects of Iloprost on platelets might increase the risk of hemorrhage (e.g. active peptic ulcers or intracranial hemorrhage)
- patients with a history of cerebral circulatory disorders
- patients with any symptomatic or treatable heart disease (including stenting), hypertension treated with a β-receptor blocker, calcium agonists, vasodilator or ACE inhibitor at more than moderate doses
- Severe coronary heart disease or unstable angina; myocardial infarction within the last six months; decompensated cardiac failure if not under close medical supervision; severe arrhythmias; suspected pulmonary congestion; cerebrovascular events (e.g., transient ischaemic attack, stroke) within the last three months
- Acute or chronic congestive heart failure (NYHA II-IV)
- Pulmonary hypertension due to venous occlusive disease
- Congenital or acquired valvular defects with clinically relevant myocardial function disorders not related to pulmonary hypertension
- A patient currently enrolled in or has not yet completed at least (a period equal to five times the half-life time of the drug used in the previous trial) since ending other investigational device or drug trial(s)
- Patients dependent on sponsor, investigator or study site
- History of previous proximal humerus surgery on the same side
- History of proximal humerus deformity on the same side
- Pathological or open fracture
- Polytrauma patient
- Any form of substance abuse, psychiatric disorder, or other condition that, in the opinion of the Investigator, may invalidate communication with the Investigator and/or designated study personnel
- Patients committed to an institution by virtue of an order issued by either the judicial or the administrative authorities

3.3.5 Identifying potential harms (Adverse event (AE) and serious AE (SAE))

The known adverse drug reactions (for systemic use) which were reported in the post-marketing surveillance of Iloprost are listed in Table 12 (190), however, due to local infiltration of the Iloprost

at a slower rate than the IV dose rate, the adverse reactions during the planned trial are expected to be lower than the side effects of the systemic use. With this slow rate of local infusion, only mild side effects like dizziness or headache for a short duration can be expected. In such situations, the patient will receive the adequate management, and if the condition does not improve, the infusion of the drug will be stopped, and the patient will continue to receive the required care.

Table 17: Iloprost Adverse reactions reported in clinical trials or during post-marketing surveillance in patients (190)

System Organ Class (MedDRA)	**Very Common ≥ 1/10**	**Common ≥ 1/100 to < 1/10**	**Uncommon ≥ 1/1,000 to < 1/100**	**Rare ≥ 1/10,000 to < 1/1,000**
Blood and lymphatic system disorders			Thrombocytopaenia	
Immune system disorders			Hypersensitivity	
Metabolism and nutrition disorders		Decreased appetite		
Psychiatric disorders		Apathy, Confusional state	Anxiety, Depression, Hallucination	
Nervous system disorders	Headache	Dizziness/ Vertigo, Paraesthesia/ Throbbing sensation/ Hyper-aesthesia/ Burning sensation, Restlessness, Agitation Sedation, Drowsiness	Convulsion*, Syncope, Tremor, Migraine	
Eye disorders			Vision blurred, Eye irritation, Eye pain	
Ear and labyrinth disorders				Vestibular disorder
Cardiac disorders		Tachycardia*, Bradycardia, Angina pectoris*	Myocardial infarction*, Cardiac failure*, Arrhythmia/ Extrasystoles	
Vascular disorders	Flushing	Hypotension*, Blood pressure increased	Cerebrovascular accident*/Cerebral ischaemia,	

			Pulmonary embolism*, Deep vein thrombosis	
Respiratory, thoracic and mediastinal disorders		Dyspnoea*	Asthma*, Pulmonary oedema*	Cough
Gastrointestinal disorders	Nausea, Vomiting	Diarrhoea, Abdominal discomfort/ Abdominal pain	Diarrhoea haemorrhagic, Rectal haemorrhage Dyspepsia, Rectal tenesmus, Constipation, Eructation, Dysphagia, Dry mouth/Dysgeusia	Proctitis
Hepato-biliary disorders			Jaundice	
Skin and subcutaneous tissue disorders	Hyperhidrosis		Pruritus	
Musculoskeletal and connective tissue disorders		Pain in jaw/Trismus, Myalgia/Arthralgia	Tetany/Muscle spasms, Hypertonia	
Renal and urinary disorders			Kidney pain, Vesical tenesmus, Urine abnormality, Dysuria, Urinary tract disorder	
General disorders and administration site conditions		Pain, Pyrexia/Body temperature increased, Feeling hot, Asthenia/Malaise, Chills Fatigue/Tiredness, Thirst, Infusion site reactions (infusion site erythema, infusion site pain, infusion site phlebitis)		

* Life-threatening and/or fatal cases have been reported

3.3.6 Establishing clinical monitoring measures during the infusion of Iloprost

Several clinical monitoring measures were devised and established for the planned study to ensure the safety of the study participants.

a) **Infusion rate**: The planned infusion rate of Iloprost in the clinical trial is four times slower than the data-sheet (190) recommended dose for pulmonary hypertension patients. This slow rate of local infusion is expected to decrease the expected side effects of Iloprost significantly. Moreover, the enrolled patients will be monitored during the time of the infusion, and in the case of occurrence of any serious side effects, the infusion will be discontinued.

b) **Baseline patient characteristics and additional precautions**: The Iloprost data-sheet requires the reduction of the administered dose in patients with renal or hepatic impairment. These patients are excluded from the planned study. Additionally, all contraindications stated in the Iloprost data sheet are included in the exclusion criteria of the planned study. Furthermore, the following precautions will be taken during patient monitoring following infusion:

   a. In patients with low blood pressure, care will be taken to avoid further hypotension. Similar to what is recommended for Iloprost, in the planned study, the drug will not be administered in patients with systolic arterial hypotension less than 85 mmHg. Thereafter, blood pressure and heart rate will be measured 15 min. and 30 min. after the beginning of the infusion, then every two hours during the first six hours, and finally every six hours until the end of the infusion (48 hrs after the surgical procedure).
   b. Care will be taken to avoid any contamination arising from the procedures necessary for the administration of Iloprost.
   c. Oral ingestion and contact with mucous membranes will be avoided. On contact with the skin, Iloprost may evoke erythema, which could be long-lasting but painless. Suitable precautions will be taken to avoid Iloprost contact with the skin. In the event of Iloprost contact, the affected area will be washed immediately with water or saline.
   d. Although highly unlikely, in the event of myocardial ischemia provoked by an overdose of Iloprost, the administration of 125 mg aminophylline IV has been shown to be an effective countermeasure. Iloprost administration will be interrupted, and close monitoring of the affected patient together with symptomatic measures will be carried out.

c) **Oversight during administration**: The investigational drug will be used under strict control at the Center for Musculoskeletal Surgery of the Charité, which is equipped with the appropriate equipment and experienced physicians for continuous monitoring and treatment of patients.

3.3.7 Establishing an overall benefit-risk assessment of the investigational drug

3.3.7.1 Risk assessment

Iloprost has been proven to exhibit an acceptable safety profile and has obtained marketing authorization for pulmonary hypertension in the European market under the marketing authorization number EMEA/H/C/000474 in 2003. Iloprost is also FDA approved and routinely used in clinical practice for pulmonary hypertension. Moreover, Iloprost has also been used for Buerger disease (thromboangitis obliterans), scleroderma, and ischemia (130). Furthermore, Iloprost has previously been successfully used off-label to treat bone marrow edema in early cases of osteonecrosis (132–135, 137). Iloprost is administered via intravenous infusion or inhalation. However, in this study, Iloprost shall be administered via the local application at the fracture site, which is considered a new method of Iloprost application for a new indication. The potential risks that could result from this new application method can be attributed to the following sources:

- Local administration of Iloprost
- Potential systemic adverse events that would stem from the drug reaching the systemic circulation
- Catheter insertion for delivery of Iloprost

a) Local administration of Iloprost

Observations that support the local tolerance of Iloprost were also collected from the *in vivo* experiments, and experiments gathered from the literature. Neither the mouse (100) nor the sheep model (unpublished data) experiments performed by the research group in the (BCRT) revealed any local toxicity, and no negative effect on the cellular composition at and around the fracture gap was observed. Moreover, other research groups have investigated the local application of Iloprost in other tissues. For instance, researchers at Boston University School of Medicine investigated the use of PGI2 analogs such as Iloprost and carbaprostacyclin (cPGI) in the murine corneal model of angiogenesis (203). The corneal tissue is commonly used to examine the potential angiogenic impact of an experimental drug. The experiment revealed that Iloprost and cPGI are able to induce angiogenesis in the murine model, and most importantly, they did not report any signs of local toxicity (203, 204).

The safety and tolerability of local treatment with Iloprost were investigated in patients with Peyronie's disease (progressive fibromatosis characterized by inflammatory plaques on the dorsolateral aspect of the penis, which can cause both pain on erection and penile curvature) in Phase I clinical trial (205). Researchers performed intralesional injections of

an Iloprost dose of 200 ng in 1 mL normal saline for five weeks into the penile tissue to explore the drug's ability to suppress the production of connective tissue growth factor in fibroblasts, for the treatment of Peyronie's disease. All patients tolerated an Iloprost dose of 200 ng well; 19 patients reached a 300 ng dose, and 14 tolerated a 400 ng dose without showing serious side effects. Only mild side effects (burning or pain) at the site of injection were recorded during the treatment. Given the high vascularity of the penile tissue, it is expected that the majority, if not the entire amount of directly injected Iloprost, reaches the systematic circulation of patients. Overall, the local tolerance of Iloprost has been positive, and the drug did not show any significant signs for concern in either preclinical or clinical settings.

b) Potential systemic adverse events that would stem from the drug reaching the systemic circulation

Possible adverse events that could arise from Iloprost reaching the systemic circulation are listed in Table 12. However, the occurrence of these events is most unlikely, and in order to minimize potential toxicity that could occur from Iloprost reaching the systemic circulation, the following measures have been defined to counter the respective risk as also given above:

1) Iloprost infusion rates will be four times slower than the rate of the recommended IV infusion dose for pulmonary hypertension patients. This aims to lower the possibility of any systematic toxicity and increase the exposure time of the fracture to the drug.
2) The exclusion criteria of the trial included all contraindications and precautions of the intravenous use of Iloprost.
3) All study patients will be monitored during and after the infusion of Iloprost for any signs or symptoms of adverse reactions, and appropriate symptomatic treatment will be initiated.

c) Catheter insertion for delivery of Iloprost

It is planned to infuse Iloprost locally to the fracture site through a catheter (InfiltraLong 420, PAJUNK® GmbH, Geisingen / Germany). InfiltraLong 420 is CE certified (no. 51268-16-02) as a pre-assembled kit for wound infiltration/ infiltration analgesia. All precautions will be taken to reduce any potential risks that can be associated with the procedure itself, such as the risk of contamination. The catheter insertion will be performed during the surgical procedure in the operating theatre under strictly aseptic conditions and according to the standard protocol for surgical catheter insertion.

- **The rationale for using the InfiltraLong Catheter**

After fracture fixation with the PHILOS plate, the catheter will be inserted at the end of the surgical procedure just before skin closure. The catheter will be inserted to reach the closest point at the fracture site, enabling the diffusion of Iloprost to the fracture hematoma. The technique of continuous wound infiltration is widely used postoperatively as a postoperative analgesic technique to decrease the need for postoperative systemic analgesia. This technique includes administering local analgesics directly into the surgical wound with a continuous infusion rate through the insertion of a multi holed catheter to the surgical site at the end of the surgical procedure (206). Local wound infiltration of an anesthetic has been widely used in orthopedic surgery effectively, which has been proved to decrease the postoperative systemic analgesia after lumbar disc surgery (207). Furthermore, this technique is considered to be safe and effective in pain management after lumbar laminectomy (208). The local infiltration of analgesia could be considered as a part of the multi analgesic technique for hip and knee replacement surgeries (209, 210). This has been further revised in a systematic review showed that the analgesic local infiltration method is an effective analgesic method in total hip replacement (211). Furthermore, local wound analgesia infiltration has been advised after iliac crest bone grafts as an effective method of analgesia for the known prolonged donor site pain (212).

Therefore, the usage of Iloprost locally through a catheter inserted into the fracture site during the surgical procedure is considered a feasible and straightforward method not only to deliver Iloprost to the fracture site but also to control its dose and infusion rate.

- **Precautions against catheter blockage**

The (InfiltraLong 420) catheter has a flexible helical coil to always keep its lumen open and ten openings in spiral arrangement with precisely chosen hole diameter along 25 mm at the tip to ensure a uniform and homogeneous distribution of the infused fluid along the catheter length (213). The infusion pump will be connected with the catheter in the operating room, and saline infusion of lowest rate (0.1ml/h with a total of 2.4 ml/ day) will start immediately in order to avoid blockage of the catheter before Iloprost infusion starts 24 hours after the procedure. Additionally, close monitoring of the patients will be carried out during the infusion of Iloprost to ensure the integrity of the catheter and the continuous delivery of the drug.

3.3.7.2 Benefit assessment

In recent years, the potential role of Iloprost as an immune-modulatory agent has been observed as promoting an anti-inflammatory and immunosuppressive effect (128, 129). Due to the strong correlation of the immune system and the skeletal system during bone regeneration, Iloprost could

represent a potential and promising agent to further bone fracture healing. Moreover, Iloprost helps to optimize the immune response by avoiding the prolonged and excessive pro-inflammatory reaction that could negatively influence musculoskeletal healing (109). Further investigation of the immune-modulatory effect of Iloprost in the context of bone regeneration has been conducted, where *in vitro* studies confirmed the postulated positive osteogenic effect (100). In a final proof of concept *in vivo* study, the positive impact of a local application of Iloprost into the fracture site during the early phase of bone healing was evidenced in a mouse osteotomy model (100).

Moreover, in the context of bone injuries, Iloprost was previously successfully used off-label to treat bone marrow edema and avascular necrosis via its vasodilator effect and the enhancement of capillary microcirculation, as well as decreasing the oxygen-free radical production and preventing thrombocyte adhesion (131–133, 137, 138).

The established efficacy from the *in vitro* and *in vivo* mouse models coupled with the safety and tolerability profile of the drug seen in the sheep model all together is seen as a strong body of evidence that justifies the need to investigate Iloprost in a target patient population as a next logical step. The local application of Iloprost into fracture sites would represent a new therapeutic opportunity for a significantly large patient population with benefits outweighing potential risk.

Chapter 4 : Discussion

The displaced proximal humeral fracture is one of the unsolved orthopedic problems, especially in elderly patients (214). There are no clear evidence-based guidelines for PHF treatment, which could explain the current conflicting opinions in the scientific community (20). The literature review, which was conducted in the frame of this thesis, confirmed the controversy around treatment options for PHF, which is not only about deciding between conservative or surgical treatment but also in determining the optimal surgical procedure for displaced PHF (17, 215). It has been reported that 70% of 3- and 4-part PHF occur in the patient age group above 60 years old, while most of these patients are advised to be treated surgically to ensure better outcomes (216), as conservative treatment options are no guarantee for a satisfying outcome (22–24). The following interpretations could be deduced from this literature review:

1. **No clear consensus on the surgical approach of choice for surgical fixation of PHF:** The PHF is one of the three most common fractures in elderly patients, mainly after a simple fall (5–7). This number is expected to be tripled in the next three decades due to an aging population (10, 11). Finding the proper treatment for such patients is not easy despite the wide range of treatment varieties, partially due to the lack of specific treatment guidelines for PHF (17, 20). Therefore, a full assessment is necessary for each patient to find the best possible treatment approach. The assessment process should include multiple considerations, such as the patient's general condition and daily activity, in addition to the local evaluation of the fracture and bone quality (31, 49).
2. **Screw cut-out was the most common complication reported after PHF fixation surgery:** Angular stable locking plates such as PHILOS have a potential advantage of preserving the anatomical alignment of the humeral head and save the joint integrity by optimizing the tension of the surrounding structures, which could provide favorable outcomes (88). However, PHILOS has a relatively high and heterogeneously reported complication rate varying from 7% (156) to 60% (177) with an average of 31.3 ± 15.2%. This high variability could be mainly due to the heterogeneity of the study population with different degree of fracture classifications, and finally, the comprehensibility of reporting the complication rate in each study.

   The literature review showed that screw cut out is the most common reported complication (81 out of 207), followed by avascular necrosis of the humeral head (39 out of 207) and loss of reduction (24 out of 207). The cut-out was found in 81 cases out of 471 patients in 15 studies treated with PHILOS (Table 3), representing an average incidence rate of about 17%. Furthermore, loss of reduction and head collapse following avascular necrosis could lead to screw cut-out due to the high mechanical demand transferred mainly through the plate and screw and not through the

bone (88). Therefore, achieving rapid bone healing could help in earlier load transfer through bone and decrease bone cut-out incidences. Similar observations were seen for patients treated with an intramedullary nail where screw cut out was the most common complication (9 out of 33) (Table 4).

Hemiarthroplasty was used in 254 patients in eight studies (Table 5). The most frequently found complication was represented by greater tuberosity healing problems such as loss of reduction with subsequent malunion or nonunion. Achieving proper tuberosity healing could significantly affect the patient outcome after Hemiarthroplasty. Tuberosity malunion and nonunion were previously defined as the most common reasons for hemiarthroplasty failure (29, 182, 187, 188). Therefore, achieving rapid healing could improve the functional outcome of such patients. Finally, Reversed shoulder arthroplasty (RSA) was used in 375 patients in ten studies (table 6). RSA showed the lowest complication rate, with an average of 12.8% ± 9.3%. RSA has the advantage over Hemiarthroplasty in preserving the patient's ability to abduct and elevate the affected arm even in the absence of tuberosity healing. However, nonunion of the tuberosities have been linked to severe complications such as instability, loosening, and even infection (67, 217). Moreover, Boileau P. *et al.* (218) showed that tuberosity healing in RSA could improve external rotation and active forward elevation and, subsequently, patient satisfaction (218).

Additionally, the heterogeneity of clinical outcomes given in the literature makes it challenging to calculate a specific baseline complication rate for PHF. As such, the situation necessitated such retrospective analysis, focusing on patients treated at the Center for Musculoskeletal Surgery at the Charite University Hospital. The retrospective analysis described in this thesis aimed at identifying the patient group with the highest complication rate who underwent surgical treatment following PHF in patients aged 60 years or older. Finding the baseline complication rate of PHF after osteosynthesis is essential to highlight the healing problem in this particular age group as well as to determine the target population that could benefit from potential therapeutic approaches for bone healing improvement.

Retrospective analysis of patients suffering from PHF

Patients with 4-part fractures treated with ORIF showed a higher rate of complications

In this study, 75% (66 out of 88) of PHF were treated with PHILOS plate fixation, while the remaining were treated with arthroplasty. This high percentage of cases treated with the PHILOS plate also reflected in the conclusion of Bell *et al.* (219) that there is a general tendency to treat more patients suffering from PHF with angle stable plate ORIF. This conclusion was based on

studying PHF treatment methods in 306 referral hospitals in the USA, comparing the current number of PHF fixation cases with the number five years earlier. The study showed an increase in the percentage of PHF fixation in the elderly by 29%, assuming that the incidence of PHF had not significantly changed over the five years of the study (219). This means that there is a real increase in the number of elderly patients being treated with surgical fixation. The increase in the PHF treated with surgical fixation could be explained by the growing understanding of the anatomical basis of shoulder function and the development of the angle stable plate systems, together with the cumulatively increased experience of surgeons to fix even the more complex 4-part PHF (219).
The overall detected complications in this study were 27.3% with the overall complication rate of the PHILOS plate irrespective of the fracture classification higher than the overall complication rate of arthroplasty (28.8% vs. 22.7%). When calculating the overall complication rate in relation to the fracture type, complications were more dominant in 4-part fractures (40.5%) than in 3-part fractures (18.2%), which is logically explained by the more complex pattern of the 4-part PHF.
Further analysis of the complication rate recorded for 4-part PHF showed that the PHILOS treated 4-part fractures had more complications than the cases treated with arthroplasty. This relatively low complication rate of arthroplasty, particularly RSA, could be explained by the absence of bone union complications in this type of treatment since, in arthroplasty, the fractured humeral head is replaced with the prosthesis. However, also arthroplasty has its specific complications, such as instability and dislocation.

In this study, among PHILOS treated 4-part fractures, 11 of 16 cases (68.8%) had at least one complication. The most frequent complication that occurred among 4-part fracture patients was the loss of reduction (mainly varus angulation) (Table 10). Identification of loss of reduction as the most common complication following PHILOS comes in line with what was previously reported by Haasters *et al.* (75) and Sproul *et al.* (17). Arthroplasty treated cases in this study were mostly 4-part fractures, with a complication rate of 19% (4 out of 21 cases). The arthroplasty complication rate in this study falls within the wide range previously reported in the literature (14% to 75% (220) and 19% to 68% (221)), and even below the range reported in Westermann *et al.* (222), who described a complication rate of RSA of 27.4%. The regression model in this study showed that 4-part PHF patients treated with ORIF had nine times the odds of suffering from a complication when compared to 4-part PHF patients who were treated with arthroplasty.
However, in this study, the complications in arthroplasty cases were commonly associated with revision surgeries. For example, in 4-part PHF, all 4 arthroplasty cases with complications needed revision surgery, while only five cases out of eleven 4-part PHILOS treated cases with

complications needed revision surgery. This finding shows that complications after angle stable plate osteosynthesis are in their largest part less severe than after arthroplasty. Although the loss of reduction could frequently end with decreased range of motion and unfavorable functional outcome (81), the decreased range of motion in elderly patients could be less critical and could be partially accepted, provided that the patients' daily activity can be accomplished without pain (223).

Nearly all 3-part PHF cases in this study were treated with PHILOS (43 out of 44), which showed fewer postoperative complications (7 out of 43) than PHILOS after 4-part PHF. The most frequent complication reported in the 3-part PHF was screw cut-out followed by infection and loss of reduction with one case reporting avascular necrosis of the humeral head. The frequency of screw cut-out shown in this study as the most common complication come in line with the previous conclusion of Kevin *et al.* (78), which is further confirmed with the study outcome of Plath *et al.* (224). The high rate of screw cut-outs could be explained by the presence of osteoporosis and decreased bone stock in the humeral head. Also, screws cut-out occurrence could be linked to delayed PHF healing as a sequence of loss of reduction, which leads to the projection of the upper screws into the joint (76, 88). Therefore, enhancing bone healing in PHF is strongly needed to reduce the complication rate and its sequences as well as to improve the patient outcome, which in turn is expected to decrease the total treatment costs.

Gender differences and length of hospital stay

The study further yielded several interesting observations and findings. For instance, as reported in the literature, the average female-male incidence for PHF in patients above 60, is around 3:1 (36, 47, 225, 226). However, the overall female-male incidence in this study was 62 females and 25 males, which represents about 2.5:1. The observed mild difference could be explained by the fact that the incidence was calculated for only the surgically operated cases and not for the total PHF cases.

Another critical aspect to investigate was the hospital stay of patients included in the study. Analyzing hospital stay showed a slight difference with no statistical significance between PHILOS plate fixation and arthroplasty group, with a mean length of stay of 10 ± 7.95 days for PHILOS compared to 11 ± 7.32 days for arthroplasty. The duration of hospital stay in this study was quite similar to previously published data showing no significant difference between PHILOS and arthroplasty groups in elderly patients (227). This has been further confirmed in open PHF in the elderly, where no significant difference in the length of stay between PHILOS and arthroplasty treated patients was observed (228).

Revision surgeries were more prevalent in 4-part fractures

The overall revision surgeries in this study were reported in 13 out of the total of 88 PHF, representing about 14.8 %. These numbers are slightly better than the previously reported overall revision rate in the literature, which was 15.6% (229) and 17.6% in reversed shoulder arthroplasty (230). Interestingly, among those 13 cases, the overall revision rates after arthroplasty were higher (23%) than after ORIF with PHILOS (12%). Revision surgery in case of arthroplasty is considered technically demanding and mostly results in a worse outcome than found after primary surgery (231). As stated above, complications after arthroplasty can mainly be classified as serious and frequently need revision surgery. In this study, all five cases of arthroplasty with complications needed revision surgery, compared to only 8 out of 19 in the PHILOS group.

In this study, the revision surgeries were more prevalent in 4-part fractures, with 9 out of all 13 revision surgeries. This high revision rate for the 4-part fracture explains the great importance of proper treatment choice for each patient in order to decrease the complication rate and hence decrease the potential revision rate.

According to the direct results obtained in this study, the choice of arthroplasty to treat 4-part PHF showed a lower short-term complication rate, albeit a higher revision rate in comparison with the PHILOS. Moreover, it has been reported that the long term results of ORIF with the PHILOS showed a better functional outcome than arthroplasty (31). Although arthroplasty has initial potential benefits such as less postoperative joint pain, the range of motion has been reported to be unfavorable, especially in the long-term follow up (18, 37, 71, 232–234). After PHILOS osteosynthesis, a continuous functional improvement over time could be observed, especially over the first postoperative year, which could end with a better outcome in the long term follow-up (149). Therefore, active patients with sufficient bone quality and high functional demand could gain benefits from PHILOS plate fixation, which preserves the natural humeral anatomy and has a better outcome in the long-run (31). In this context, therapeutic approaches to enhance bone healing in elderly patients could overcome the known high complication rate of PHILOS treatment of the 4-part PHF and could ensure better results, which could help in shifting more 4-part fracture patients towards the PHILOS side. Thus, improving the outcome of osteosynthesis for this group of patients would be a significant step forward in trauma care.

Developing and initiating a trial to analyze Iloprost treatment in PHF patients

One of the main goals of this study was to propose a possible therapeutic strategy to improve the outcome for elderly patients undergoing angle stable plate osteosynthesis for PHF. As stated above in detail, complication rates in these patients are tremendously high. A potential treatment strategy

to improve clinical outcomes for these patients is promoting fracture healing in PHILOS treated patients via the utilization of immunomodulatory therapy. Achieving rapid healing with better functional outcomes could help elderly patients to restore their regular daily activity. Moreover, with an approach to improve fracture healing, one could avoid possible revision surgery in PHILOS treated patients, which in turn could decrease both direct and indirect treatment costs of PHF. Such an immunomodulatory therapy strategy is expected to promote healing by controlling the initial inflammatory phase in the bone healing process. This phase is known to be of high amplitude and long duration in the elderly due to an over-reactive immune response (101, 120, 127). Local Iloprost application to the fracture site was successfully evaluated as a potential immunomodulatory agent in preclinical animal models showing a positive bone healing effect (100). Therefore, translating this preclinical data into the clinic is considered to be a beneficial treatment option for PHF patients.

Clinical development strategy

Choice of the inclusion and exclusion criteria

The designed clinical trial is a Phase I/IIa; therefore, safety is a significant concern as a primary endpoint; the study has been designed with precautions to ensure the participants' safety. The inclusion criteria have been chosen to select the specific targeted population with PHF, which frequently suffers from bone healing complications. The selected participants' age is from 60 to 80 years old. Patients in this age range are prone to an unfavorable immune response, which can lead to delayed or nonunion (100, 107, 126). Therefore, these patients exhibit a high medical need for a biological solution and are a suitable patient cohort for the application of this immunomodulatory intervention. Further inclusion criteria have also been chosen based on discussions with the BfArM to ensure participants' safety, such as including healthy participants (score I) or participants with only mild systemic disease (score II) according to the criteria of the American Society of Anesthesiologists score (ASA).

Similarly, the exclusion criteria of the designed study have been chosen cautiously to ensure a high-quality study protocol. The exclusion criteria can be divided into three main categories; firstly, safety exclusion criteria, where the exclusion criteria have been extended to involve any contraindication and precaution to Iloprost usage. Therefore, any patient who may be at risk from Iloprost usage will be excluded from the trial. Secondly, exclusion criteria that protect participant rights and dignity according to the European Parliament and council and the declaration of Helsinki (140, 141) are respected. Lastly, the exclusion criteria to assure valid outcome data aiming to minimize any confounders for the outcome parameter, such as previous humerus fracture,

deformity, or surgery have been included. The patients with previous humerus fracture, deformity, or surgery will be excluded from the study because these pathologies would have an influence and bias with respect to the functional outcome scores, which are secondary endpoints.

Determining the dose regimen (duration of treatment, formulation, and method of delivery)

The main aim of the planned clinical trial was to evaluate both the safety and efficacy of a local application of Iloprost at the site of PHF to promote bone healing. In the preclinical work performed on the local application of Iloprost in fractures, Iloprost was applied in a fibrin delivery system to delay the release of the drug locally (100). This delayed release of Iloprost was essential so as not to compromise the initial inflammatory phase of bone healing that has a significant role in the healing process by initiating the healing cascade (106). Normally, this initial inflammatory phase has been seen to reach its peak within the first 24 hours following fracture and then decline (107). However, at the same time, a prolonged or high amplitude inflammatory phase is harmful and inhibits bone healing (100). Not only the timing of applying Iloprost to the fracture site is of relevance, but also the mode of local release has a significant influence on the benefit profile of the drug. The preclinical data provided strong evidence of the benefits of administering Iloprost in a delayed-release system (100).

However, mixing Iloprost with fibrin outside the body is considered a manufacturing step that could create a new drug with new properties. According to § 13 AMG (144) and § 5 of the GCP-V (143), new manufacturing permission, new labeling, and full quality control documents for the new drug would thus be needed. This manufacturing permission process is costly, time-consuming, and complicated.

Therefore, another delivery method for Iloprost was developed. Iloprost will be administered via a catheter that will be inserted at the end of the surgical procedure. The application of Iloprost locally via a catheter inserted into the fracture site during the surgical procedure is considered a feasible and straightforward method not only to deliver Iloprost directly to the fracture site but also to control its dose and infusion rate. Iloprost will be infused through the inserted catheter over 24-hours starting after the first day (24 ± 2 hours) postoperatively after ORIF. The reason for waiting for the first 24-hours postoperatively before starting the infusion is to maintain the initial inflammatory phase of bone healing, which represents a significant role in initiating the bone healing cascade (105).

Target population age choice

The age group in the planned trial includes patients from 60 to 80, who have reported good health status. Iloprost is expected to be beneficial in elderly patients as this group is known to have a high

risk of delayed healing due to their experienced immune system (100, 107, 126). Therefore, these patients exhibit a high medical need for a biological solution and are a suitable patient cohort for the application of this immunomodulatory intervention.

The link between the immune system in the elderly and the bone healing process has been previously shown (100, 101, 103). Both bone cells and immune cells originate from common bone marrow progenitor cells and share common cell receptors; furthermore, immune cells can differentiate into osteoclasts (235). Osteoblasts have the ability to control osteoclasts and influence various immune cells as well as hematopoietic stem cells through their ability to release receptor activator of nuclear factor κB (RANK) ligand and various mediators (236–238). It has been established that the immune system affects the turnover of bone through specific mediator receptor interaction (239–241). This effect has been established on physiological bone turnover as well as in pathological conditions as in the case of fragility fractures (104).

Cytotoxic T cells such as Terminally Differentiated Effector Memory CD8+T ($_{TEMRA}$) cells have proven to play a crucial role in controlling bone cells through specific cytokines that control the osteoclasts via specific RANK on the cell surface (118). These cells release RANK-ligand that is capable of stimulating osteoclasts and hence increasing bone resorption, which, as a result, delays the healing process (119). The link between CD8+T (T_{EMRA}) cells and the delayed union has also been further proved through the finding of a high population of CD8+T (T_{EMRA}) cells in the delayed bone healing fracture site (109, 120). Similarly, fractures in an animal model with a low population CD8+ show enhancement of bone healing process (101). Moreover, CD8+T (T_{EMRA}) cells were enriched in fracture hematoma; these cells were the major producers of interferon ɣ/tumor necrosis factor α, which inhibit osteogenic differentiation and survival of human mesenchymal stromal cells (101). Alternatively, CD4+T cells, especially the T regulatory (T_{reg}) subtype, showed a positive impact on both wound and bone healing (121–125). Moreover, an animal model with a high population of (T_{reg}) showed a higher bone density with decreased bone resorption and improved bone healing capacity (121–125).

In aged patients, the CD8/ CD4 ratio is unbalanced in favor of the CD8+ T cells (242), which reveals them as potential candidates for a delayed and insufficient healing of musculoskeletal injuries. These patient groups are prone to an advert early immune response after a musculoskeletal injury and particularly long healing times after standard care surgical procedures. The cellular difference between the young and old population strengthens this assumption, as with aging and the continuous exposure to pathogens, the memory T cell population increases. The negative effect

of the aged immune system has been proven by improving the bone healing after a rejuvenation of the aged immune system (100, 105, 243).

Iloprost is expected to reduce the risk of delayed bone healing in fracture patients with a potential dysregulation of the immune reaction and altered immune cell compositions. Moreover, Iloprost reduces the TNF-α and IFN- γ secretion of T cells and further supports macrophage polarization towards an anti-inflammatory type. In other words, Iloprost downregulates the initial inflammatory phase that is known to be of high amplitude and long duration in this specific age group due to an overreactive immune response (101). As such, the product is expected to exert an immunomodulatory effect in enhancing bone regeneration in elderly patients with bone fracture.

The clinical trial approval process

In order to obtain the necessary approvals for the clinical trial, an application was prepared and submitted to both the competent regulatory authority (BfArM) and the ethics committee (LAGeSo) in Germany. The entire approval process for obtaining the regulatory permissions and approval for the Iloprost study was completed as part of this study. The process included establishing the study protocol, preparing and completing all of the required documents as well as addressing the raised questions by the authorities in a scientific discussion. The clinical trial application was submitted to the ethics committee in April 2019, and the (BfArM) in May 2019.

The ethics committee comments were focused on the ethical aspect of conducting the clinical trial and are related to either formal issues such as completeness of the study documents according to the AMG/GCP-V(143, 144) or concerns over the content of the patient information sheet, informed consent, or study insurance. The second aimed at warranting that the content of these documents is simple and understandable for all patients with all potential risks and side effects mentioned in detail. Another critical point was to ensure that the patients´ data protection and patient rights to withdraw their consent without any consequences were described in the consent form.

The BfArM role is to inspect the clinical trial design aspects and the relevance of the design parameters to the therapeutic concept being investigated. The authority raised concerns over the technique used for the local application of the Iloprost, in particular, whether the use of intraoperative catheters would bring additional risks for the patients. Moreover, the authority asked for additional safety considerations related to the suitability of the calculated dose, local tolerance of the investigational drug, and the timing of its application to the fracture site. Another concern was to ensure that all the potential contraindications to the drug were listed as exclusion criteria while ensuring the reporting of any possible potential side effects or toxicity with a causal relationship to the investigational product.

All comments and questions raised by the authorities, either the ethics committee or BfArM, were addressed adequately, depending on the previously conducted preclinical studies and through deep literature research to show the safety and the efficacy of the investigational drug in the planned study. Moreover, the positive risk-benefit assessment of using the investigational drug in this new clinical indication was shown. The trial design has since been revised, and additional data has been provided to address the outlined concerns with greatest attention to details, while maintaining patient safety as the first and utmost priority. The clinical trial approvals were obtained in November 2019.

Concluding Remarks and Future perspectives

The choice of the proper treatment method for PHF in the elderly is a difficult task, and a clear consensus on the treatment of choice is currently lacking (67–69). The Cochrane review in 2015 concluded that the evidence is insufficient to decide the proper intervention for PHF (70). Studies that attempted to solve this ambiguity rather collected further evidence of uncertainty (71). In this retrospective study, we aimed at providing important clues that could help the decision-making process of choosing the most suitable treatment option for this complex fracture. In our cohort about 75% of the surgically treated PHF patients aged 60 or above were treated with PHILOS. The overall complication rate of the PHILOS irrespective of the fracture classification was higher than the overall complication rate of arthroplasty (28.8% vs. 22.7%). Patients undergoing fixation would clearly benefit from potential therapeutic approaches to improve their outcome. We used these observations to select the target patient population for a prospective pilot clinical study to investigate the role of local application of the immunomodulator Iloprost at the fracture site to improve bone healing. Further efforts by the scientific and clinical community could be directed toward establishing a comprehensive treatment guidance for patients suffering from PHF.

Study limitations

This study has several limitations, such as being a retrospective study done in a tertiary academic hospital that receives more complicated cases referred from other hospitals. Moreover, the functional outcome assessment has not been included in this retrospective search. In addition, the follow-up time was relatively short. Data on all surgically treated PHF patients were collected from the digital hospital information system (SAP, Walldorf, Germany). The patient's files were searched for all relevant data before and after surgery and at follow-up visits. However, functional outcome measures were not continuously documented among all patients, while detailed information on the patient's secondary diagnoses (the complete set of comorbidities) was

somewhat deficient. As already described in the chapter Materials and Methods, patients with missing data relevant to the outcome measures were excluded.

Chapter 5 : Summary

Traumatic bone fracture is one of the most common injuries worldwide. In Germany, about 1.6 million bone fractures have been reported per year. Proximal humerus fracture (PHF) is considered to be one of the most common traumatic bone fractures in the elderly population (>65 years old) and is ranked third after hip and distal radial fracture. The incidence of PHF is expected to triple in the next three decades because of the cumulative aging of the world population. This high incidence in old age is linked to osteoporosis and is more common in females, who represent 75% of the cases. Because of poor bone quality and high incidence of complications in the postoperative follow-up, the treatment of PHF is expected to be a significant challenge in the near future.

PHF is mainly classified according to the Neer classification into four main classes, which are derived from the involvement of the four main anatomical parts of the proximal humerus: head, humeral shaft, greater tuberosity, and lesser tuberosity. Fractures are considered displaced if there is a fragmental displacement of one cm or angulation of more than 45 degrees. The more the advancement of the fracture class from one to four, the more complex is the fracture pattern.

The treatment of PHF remains one of the unsolved orthopedic problems, also mainly due to the absence of clear evidence-based guidelines for treatment. The recommended treatment of displaced patients suffering from 3-part and 4-part PHF is mainly surgery. Currently, surgeons rely on a compilation of factors when deciding on the most suitable management strategy, which is, apart from the classification, the degree of fracture comminution, patient bone quality (osteoporosis), patient age, physical capacity, and functional demand of the patient. The main available surgical options used in the treatment of PHF are angle stable plate osteosynthesis (ORIF, e.g., with the PHILOS, Synthes, Switzerland) and arthroplasty. Unfortunately, the outcome after PHF surgery exhibits a high complication rate that differs from one study to another and ranges from 9.7% to 57% after ORIF and 14% to 68% in arthroplasty treated patients. The current treatment strategy for PHF does not involve pharmacologic treatments since no drugs exist that could stimulate bone healing in fractured patients sufficiently, especially compromised elderly patients.

Using an immunomodulatory therapy as a novel strategy could be of potentially beneficial therapeutic value as a new strategy for treating elderly PHF patients with an experienced immune system, which has been shown to compromise bone healing. Such therapy is expected to reduce the risk of delayed bone healing in fracture patients with a potential dysregulation of the immune reaction and altered immune cell compositions at the fracture site via downregulating CD8+ cytotoxic T cells, which have a potentially unfavorable effect on bone healing. Moreover, an

immunomodulatory therapy could reduce the cytokine secretion of T cells and further support macrophage polarization towards an anti-inflammatory M2 type.

The primary study question of this thesis was which group of patients in the elderly population with PHF have the least favorable clinical outcomes after surgical intervention. In addition, the study aimed at proposing a potentially beneficial drug that may enhance bone healing in patients treated with ORIF. The thesis was divided into two parts. The first focused on a literature review and retrospective medical record analysis. This medical record analysis was conducted to measure the outcome of surgical management strategies, including complication and revision rates, of the two most commonly performed surgical procedures (angle stable plate ORIF and arthroplasty) in elderly patients with PHF. The complications included in this study were all of Grade 2 or more according to the surgical complication classification described by Dindo *et al.*, which includes any complication that may need medical treatment (except simple medications such as antipyretic or analgesic) or prolonged hospital stay. It also includes all complications that are considered major and need active management and/or further surgical intervention. A retrospective medical record review analysis was performed at the Center for Musculoskeletal Surgery of the Charité - Universitätsmedizin Berlin for all primary treatments of PHF between March 2017 and June 2018. All surgically treated patients aged 60 years old or older, who were operated with either ORIF (PHILOS) or arthroplasty with a follow-up period of at least six months were included. This enabled us to identify the group of ORIF patients with the highest complication rate that would benefit the most from novel therapeutic approaches. The second part focused on developing a scientifically sound clinical and translational strategy for a novel immunomodulatory approach that may improve the healing outcomes for the identified group of patients.

A clinical trial was designed to investigate the local application of Iloprost as an immunomodulatory agent for PHF healing. The study aims at investigating the safety of the drug and its ability to improve healing outcomes for these patients by reducing a prolonged and excessive pro-inflammatory reaction. The clinical testing of such a novel therapeutic approach requires the translation of generated pre-clinical knowledge into a sound clinical strategy. The work performed within the framework of this thesis focused on the utilization of previous nonclinical studies of the BCRT group to determine the clinical testing strategy in PHF patients.

In the retrospective analysis, 105 surgically treated PHF patients who underwent operative treatment were screened and 88 PHF in 87 patients with a mean age of 72.9 years included. The study population had an expected higher proportion of females (70.4%). According to the Neer classification, 50% of the patients suffered from 3-part fracture, while 42% suffered from 4-part fractures. The majority of the study patients (75%) were treated with ORIF. The overall

complication rate was 27.3%. As expected, the incidence of complications increased with more displaced fracture fragments (higher fracture type according to the Neer classification). In 3-part PHF, patients treated with the PHILOS had a complication rate of about 16.3%, while ORIF treated patients with 4-part PHF exhibited a complication rate of about 68%. The 22 patients treated with arthroplasty had an overall complication rate of about 19%. It should be mentioned that all arthroplasty prostheses used in this study were reversed shoulder arthroplasty, with the exception of only three cases, which were of the hemiarthroplasty prosthesis type. All of these three hemiarthroplasty cases were used to treat 4-part PHF and ended with complications, revised later on with total shoulder arthroplasty. The complications seen in the hemiarthroplasty cases were instability, dislocation, and infection.

The logistic regression model showed that 4-part PHF patients had about seven times the odds of suffering from a complication compared to patients with 3-part PHF, regardless of the received surgical intervention (PHILOS or arthroplasty). Treating 4-part PHF patients with angular stable ORIF had an odds rate of about nine times when compared to the odds rate of arthroplasty. These results showed that 4-part PHF had the highest complication rate, particularly when treated with ORIF. This relatively low complication rate of arthroplasty, especially RSA, could be explained by the absence of bone union complications in this type of treatment since, in arthroplasty, the fractured humeral head is replaced with the prosthesis. Whereas arthroplasty also has its specific complications, such as instability and dislocation, in this study, the complications in arthroplasty cases were more frequently associated with the need for revision surgery. For example, in 4-part PHF, all four arthroplasty cases with complications needed revision surgery, while only five cases out of eleven 4-part PHILOS treated cases with complications needed revision surgery.

Moreover, revision surgery in the case of arthroplasty is considered technically demanding and mostly results in an even less satisfactory outcome than found after primary surgery. Unlike shoulder arthroplasty, the treatment of complex PHF in the elderly with angle stable plate osteosynthesis has the advantage of continuous functional improvement over time, especially in the first postoperative year, which has been shown to be accompanied by a better outcome in the long-term follow-up. In this context, providing a specific treatment that could improve bone healing and enhance the overall outcome of PHF undergoing ORIF with an angular stable plate fixation would be of great value for PHF patients. Therefore, Iloprost as an immunomodulatory therapeutic agent has been identified, and a trial was designed based on its properties and previous results. The trial design development and the process for obtaining all regulatory permissions and approvals for the Iloprost study were completed as part of this research. The complete clinical trial application was submitted in May 2019 to the Federal Institute for Drugs and Medical Devices

(BfArM). After responding to several questions raised by the authorities in a scientific discussion in two iterations, the final approval for the clinical trial was obtained in November 2019. The trial is expected to start in 2021.

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Statutory Declaration

"I, Hisham Elazaly, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic (Complication rate analysis of proximal humerus fracture surgery in elderly patients – Guiding the benefit-risk assessment for an immunomodulatory therapy) [Komplikationsratenanalyse der proximalen Humerusfraktur bei älteren Patienten - Anleitung zur Nutzen-Risiko-Bewertung für eine immunmodulatorische Therapie], independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

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