

DOCTORAL (Ph.D.) THESIS

Epidemiological and experimental assessment of surgical complications in elderly following femoral neck fractures

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Introduction

Senile osteoporosis is a common problem of the elderly affecting the skeletal system. Bones are fragile due to symptomless bone loss. Its most severe complication is osteoporotic fracture with 9 million cases registered worldwide in 2000. Fractures typically arise with minimal trauma, like a minor fall or during routine activities. Most common fracture sites are the hip with 1.6 million cases, the wrist, and forearm with 1.7 million cases and the spine with 1.4 million cases.

The significance of osteoporotic hip fractures is immense since it is the cause of admission in 35-50% of patients receiving inpatient treatments in traumatology. These patients require extensive care and suffer from the highest rates of complication along with consequent increased mortality.

As our populations in industrialized countries face the challenges of ageing the number of femoral neck fractures is rising causing an increase in number of surgeries and inpatient treatments in my area of expertise, traumatology. Evaluation of epidemiological data may generate new, clinically valuable prognostic factors to aid care-related decision-making and improve patient outcome. The management of femoral neck fractures has its challenges, as well as complications. Operative treatment is favoured to avoid displacement and possible avascular necrosis of the femoral head. During arthroplasty bone and cartilage are removed. To investigate the potential reutilization of this biological waste we studied the structural properties and the impact of cryopreservation on extracted human cartilage using calorimetric analysis.

Study I: Non-operative predictors for subsequent interventions after intracapsular femoral neck fractures in elderly

Objectives

Our objective was to investigate the correlation between non-operative prognostic factors and non-prosthetic fracture-related treatments following internal fixation of intracapsular femoral neck fractures in elderly patients.

1. To evaluate the potential effects of different risk factors on the non-prosthetic treatments (NPT) occurring after intracapsular femoral neck fractures treated with reduction and internal fixation (RIF).
2. To examine if non-surgical prognostic factors influence the incidence of NPT following primary osteosynthesis of femoral neck fractures in the Hungarian population aged 60 years or over.
3. To focus on the common outcome of different complications regardless of the type of the complication.

Materials and methods

The data for our retrospective, nationwide observational co-hort study were collected using the database of the Hungarian National Health Insurance Fund Administration (NHIFA), selecting cases with code group of S7200 (femoral neck fractures), based on the 10th revised edition of International Classification of Diseases (ICD).

The retrospective analyses included data collected from patients over the age of 60, who underwent reduction and internal fixation (RIF) after intracapsular femoral neck fracture caused by mono-trauma; treated and discharged during the year of 2000 at the institutes providing in-patient health care services. Total of 69 hospitals were involved in the primary care. Data were validated and complemented with the help of the hospitals that provided the primary surgical treatment; they checked and confirmed the data from the NHIFA and provided additional information on surgical delay and the exact types of fractures. The follow-up period lasted by the end of the 8th postoperative year with the latest date of 31st of

December 2008. During this time-period, the secondary, non-prosthetic surgical interventions were recorded. Patients were followed until the definitive non-prosthetic further surgical intervention, death or the completion of the study.

Of the prognostic factors, age, gender, type of fracture, time spent to the operation, co-morbidities and the season and the day of the primary surgery were selected for further analyses. On the other hand, we did not aim to evaluate the any further effects of different type of femoral neck fractures or to assess the details of surgical techniques (choice or positioning of implant, quality of reduction, etc.) in our current study. The age reflects to the age of the patient when the femoral neck fracture occurred; the age-related analyses were performed for every age, starting of 60. The femoral neck fractures were classified as intracapsular non-displaced (Garden stage I or II) or intracapsular displaced (Garden stage III or IV) types of fractures. The season of primary surgery (winter, spring, summer and fall) was also registered and further analysed as a possible prognostic factor. Hungary lies in the northern temperate zone between the northern latitudes of 45°45' and 48°35' and it has a continental climate where the four seasons are well separated. In regard of the day of surgery, primary surgery performed on working days and weekends were distinguished.

Based on the time elapsed between the injury and primary operation, the following time-intervals were formed: surgery performed in the first 6 h, surgery performed 6-12h, 12- 24h or longer, than 24 h after injury. Regardless of the type of co-morbidity, the effect of the presence (or absence) of any kind of existing co-morbidity was evaluated. The co-morbidities existing at the time of the admission or developed during the hospital stay were classified based on the system of ICD-10 diagnostic groups.

All statistical analyses were performed using IBM SPSS 19 software. The collected data were analysed using multivariate Cox regression models. The prognostic value of the different factors was calculated and described with hazard ratios (HR) and 95% confidence intervals (CI). The statistically significant differences were defined as $p < 0.05$.

Results

Based on the NHIFA database, the records of 5404 patients with ICD code S7200, discharged in the year of 2000 were sent back to the hospitals for validation and for getting detailed information. After revalidation and completion of the NHIFA data, records of 2895 elderly patients underwent internal fixation were found suitable for further evaluation. The average age was 77.96 years (SD: 8.54; median: 78). Approximately 75% of the intracapsular femoral neck fractures occurred in females. Almost 74% of the fractures were classified as intracapsular, displaced type. The seasonal-based distribution of the primary surgeries had the similar weight. 77.2% of the primary operations was performed on workdays and in about 50% of the cases the primary operation was within the first 12h after injury. However, absence of co-morbidities was found in only 8.4% of all patients (Table 1).

Prognostic factors	Primary RIF patients (number, distribution-%)	Secondary NPT patients (number, distribution-%, ratio-%)	
Patients	2895 (100.0%)	265 (100.0%)	9.2 %
Age at time of primary trauma			
Mean ± SD	77.96 ± 8.54	75.51 ± 8.24	
Median	78	75	
Range (years)	60-102	60-96	
Gender			
Male	716 (24.7%)	61 (23.0%)	8.5 %
Female	2179 (75.3%)	204 (77.0%)	9.4 %
Type of femoral neck fracture			
Intracapsular non-displaced	760 (26.3%)	61 (23.0%)	8.0 %
Intracapsular displaced	2135 (73.7%)	204 (77.0%)	9.6 %
Season of surgical intervention			
Winter	751 (25.9%)	67 (25.3%)	8.9%
Spring	734 (25.4%)	67 (25.3%)	9.1%
Summer	695 (24.0%)	79 (29.8 %)	11.4 %
Fall	715 (24.7%)	52 (19.6%)	7.3%
Day of surgical intervention			
Workday	2236 (77.2%)	197 (74.3 %)	8.8 %
Weekend	659 (22.8%)	68 (25.7%)	10.3%
Surgical delay			
0-6 h	849 (29.3%)	69 (26.0%)	8.1 %
6-12 h	608 (21.0%)	52 (19.6%)	8.6%
12-24 h	483 (16.7%)	53 (20.0%)	11.0 %

24 h +	950 (32.8%)	91 (34.3 %)	9.6%
N.A.	5 (0.2%)	0 (0%)	0 %
Accompanying diseases			
Presence	2651 (91.6%)	243 (91.7%)	9.2 %
Absence	244 (8.4%)	22 (8.3%)	9.0 %

N.A.: data not available.

Table 1: Patients characteristics and prognostic factors for secondary, non-prosthetic treatment (NPT).

The mortality rate following femoral neck fractures was 30.9% during the first post-operative year, 9.1% in the second year, 8.9% in the third year, 6.6% in the fourth year, 5.9% in the fifth year, 4.9% in the sixth year, 4.3% in the seventh year and 3.8% in the eighth year. The cumulated mortality rate was total of 74.5% during the eight years of follow-up period. The cohort of patients was observed for a total of 10,077.8 person-years (mean: 3.48 ± 3.15 years per patient, median: 2.53 years per patient).

Patients with non-prosthetic treatments

During the 8 years of follow-up period, in 265 (9.2%) of 2895 patients were NPT performed, 1977 (68.3%) patients died and 653 (22.5%) survived without the need of further surgical interventions. The median of the time elapsed between the primary surgery and the definitive NPT was 3.5 months (average: 9.2 months, SD: 13.4, range: 0.1-84). The average age of patients who required further treatments was 75.51 years (5D: 8.24; median: 75) at the time of the primary care. Of the 265 patients who underwent NPT, according to the distribution analyses, 77% was female, 77% had intracapsular displaced fracture. 29.8% of trauma happened during summer, 74.3% occurred on workdays and 54.3% were treated with at least 12 h or longer delay (Table 1).

The definitive, non-prosthetic surgical procedure was the removal of metalwork in 4.21% (122 patients) of all cases, wound revision in 1.55% (45 patients), implant replacement in 2.59% (75 patients), resection arthroplasty in 0.73% (21 patients) and in 0.07% (2 patients) other intervention, performed during the follow-up period.

Of the NPT patients, the definitive treatment was received in 6.87% (199 patients) during the first, in 1.38% (40 patients) in the second, in 0.41 (12 patients) in the third, 0.28% (8 patients)

in the fourth, in 0.1% (3 patients) in the fifth, in 0.03% (1 patients) in the sixth and in 0.07% (2 patients) during the seventh postoperative year. Meanwhile, no patient was found with the need of further treatment during the eighth year, after the primary surgery.

Based on the distribution of the different types of NPT procedures, the removal of metalwork showed a continuous decreasing tendency by the seventh year, meanwhile, implant replacements were done during the first two postoperative years and wound revisions, resection arthroplasties and other interventions were performed during the first year following primary care.

Risk factors	HR	95% CI	p
Age	0.977	0.962- 0.991	0.002
Gender			
Female / <u>Male</u>	1.055	0.789-1.410	0.718
Type of femoral neck fracture			
Intracapsular displaced / Intracapsular non-displaced	1.340	1.004- 1.789	0.047
Season of primary surgical intervention			
Fali / <u>Summer</u>	0.636	0.448- 0.904	0.012
Winter / <u>Summer</u>	0.794	0.573- 1.101	0.167
Spring / <u>Summer</u>	0.808	0.583-1.120	0.202
Day of primary surgical intervention			
Weekend / <u>Workday</u> Surgical delay	1.193	0.902- 1.568	0.220
6-12 h / <u>0- 6 h</u>	1.081	0.753- 1.550	0.674
12-24 h / <u>0-6 h</u>	1.518	1.058-2.177	0.023
24 h+ / <u>0-6 h</u>	1.372	1.001- 1.881	0.050
N.A. / 0- 6 h	Non applicable		
Accompanying diseases			
Abscence / <u>Presence</u>	1.251	0.805-1.944	0.320

Table 2: Multivariate Cox regression analysis of all variables for non-prosthetic fractures-related further treatments.

According to the multivariate Cox regression analyses, the hazard of occurrence of NPT decreases by 0.977 ($p=0.002$) in every year (Table 2). In regard of surgical delay and incidence of NPT, we did not find significant differences, comparing the time intervals of 6-12 h vs first 6 h ($p=0.674$). However, delay of the primary treatment with 12-24 h and over 24 h results in

a significant increase in the risk of NPT, compared to the 0-6 h delay (HR: 1.518, $p=0.023$ and HR: 1.372, $p=0.050$, respectively).

In regard of the seasonal distribution of the primary osteosyntheses and the consequential reoperations, primary surgeries performed in the fall meant 0.636 times ($p=0.012$) decreased risk, compared to operations performed during summer. Meanwhile, surgeries during winter season or springtime showed 0.794 times and 0.808 times decrease in the risk of NPT; however, this result was not statistically significant ($p=0.167$ and $p=0.202$, respectively).

The risk of reoperation was 1.340 times higher after intracapsular displaced ($p=0.037$), compared to the intracapsular non-displaced fracture types. Additionally, there were no significant correlation revealed between the incidence of non-prosthetic treatment and gender ($p=0.718$), weekend or workday of primary surgical intervention ($p=0.220$) and absence or presence of accompanying diseases ($p=0.320$) (Table 2).

Discussion

According to the literature, the fracture healing complications after femoral neck fractures treated with internal fixation and their prognostic factors have widely been investigated recently, with special interest of the avascular femoral neck necrosis (AVN) and non-union (NU). In our current study, we evaluated the non-surgical prognostic factors of NPT following osteosynthesis of femoral neck fractures during the postoperative 8 years follow-up period, regardless of the type of complications.

Previously, description of the prognostic effects of age and gender in relation to different complications were controversial in the literature. Parker et al. showed an increased risk for intracapsular hip fractures developing NU at older age and in females. Min et al. have demonstrated that patients' gender, age and the time interval between the injury and surgery were not statistically associated with the development of avascular necrosis after intracapsular femoral neck fractures. In our previous study, we did not find any significant correlation between the age (<50 years vs. >50 years) or gender and incidence of further surgeries. On the contrary, in our current study, we showed that the incidence of NPT decreases with age. This could be explained by the fact that elderly patients are less likely to

consent to further operations, the surgical intervention is contraindicated due to the patients' medical conditions or a number of patients could not live long enough for the development of complications and the need for NPT.

Furthermore, the literature was also controversial in regard of the analysis of the time elapsed between the injury and primary surgery, the development of fracture-healing complications (AVN, NU) and the number of consequential reoperations. Smektala et al. found that surgical treatment over 48 h resulted in a significantly higher risk of surgical complications. Moreover, Hoelsbrekken et al. also emphasized the need to perform the primary surgery within 48 hours. However, Upadhyay et al. have demonstrated that the time elapsed between the injury and the primary operations (longer or shorter than 48 h) had no effect on the development of NU and AVN in young adults with displaced intracapsular fractures of the neck of the femur. Meanwhile, Yeranorian et al. showed that delay of treatment beyond 24 h was indeed associated with a higher incidence of AVN. On the other hand, Razik et al. did not find the time delay to internal fixation to be significant predictor of the development of osteonecrosis in patients younger than 60 years, after intracapsular femoral neck fractures. In contrast to other studies, Damany et al. found no significant correlations between the time delay to primary surgery (less or more than 12 h) and development of AVN and NU. Other publications emphasize the fact that a primary surgery performed within first 6 h results in significantly lower incidence of complications and mortality rate. However, in our study we have proved that the delay of primary surgery over 12 h was associated with a significantly increased risk of NPT.

Only a limited number of studies have been published in relation to the femoral neck fractures and the prognostic effect of primary surgery performed on workdays or on weekends. Based on the study of Smektala et al., primary surgeries performed on weekends are associated with higher rates of surgical complications due to delayed operations and issues with the hospital staff. Our previous study showed that primary surgeries performed during weekends significantly increased the incidence of NPT in the population below age of 60, meanwhile we could not find any significant effect for the time of surgery among patients over 60 years.

Only a limited number of publications evaluated the effects of existing co-morbidities on the incidence of additional surgical interventions. Duckworth et al. showed that excessive alcohol consumption, certain kidney and respiratory diseases could be predictive factors for the development of complication and occurrence of failures, after osteosynthesis of intracapsular femoral neck fracture in young patients. Based on our previous results on the evaluation of different co-morbidities, presence of nervous system-related diseases and general infections were associated with an increased risk in the population below the age of 60 years. However, in our current study we did not find any significant effect with the overall analyses of comorbidities on the incidence of NPT among patients over 60 years.

Notably, only a few studies have been published in regard of evaluation of seasonal effects on the development of complications following the osteosyntheses of femoral neck fractures. Our previous study has clearly demonstrated that wintertime primary osteosynthesis almost doubles the risk for the need for conversions to arthroplasty. The results raise the possibility of a correlation between the seasonal changes in the levels of vitamin D and the impaired fracture healing of femoral neck fracture.

Here we demonstrated for the first time that primary surgeries performed during fall, winter and springtime were associated with a significantly decreased risk of NPT (30-40%), compared to osteosynthesis during the summertime. These findings could be explained by the initiating effect of increased summer temperature on wound healing and septic complications; however, the reduced number of hospital staff due to summer vacations can also be a cause of higher summer rates. Nevertheless, interpretation of these results requires further investigations. According to Foss et al., staff reduction during holiday periods in acute surgical units may adversely influence postoperative outcome.

The displacement of fractures was analysed in relation to development of AVN and NU, generally. According to the literature, most of authors demonstrated a significant relationship between the fracture displacement and the development of AVN or NU. However, Toh et al. found no statistical differences between displaced and non-displaced fractures. While we could not demonstrate a significant relationship between the displacement of intracapsular femoral neck fractures and rate of NPT in patients below the age of 60. In our current study,

we found that the displacement in case of intracapsular and extracapsular fractures resulted in a significantly higher risk for NPT, compared to the non-displaced intracapsular fractures.

A potential limitation of the study is that in case of NPT patients requiring multiple surgical treatments, only the selected, definitive type of care was analysed in relation to the time elapsed from the primary surgery. In addition, the partial or total removal of metal implants were uniformly analysed, independently from the length of time elapsed from the primary care since the subtype of these surgical interventions cannot be differentiated in the NHIFA database. Another limitation of study is that the different kind of indications for the removal of metal implant, i.e. screw cut-out or femoral neck shortening, were not evaluated in more detail. However, in the setting of public health surveillance, our primary goal was to evaluate the potential effect of different contributing factors leading to further surgical interventions in general, regardless of the type of surgeries.

Study II: Effects of deep-freezing and storage time on human femoral cartilage

Introduction

Articular cartilage is generally considered an avascular, aneural and alymphatic structure. Once damaged or injured, the cartilage does not heal well and may give rise to secondary osteoarthritis. This type of injury is relatively common and mostly affects the young, physically active population. Therefore, the athlete is taken out of the game and practice leading to inefficient and decreased level of activity on the field. The injury can also progress into osteoarthritis. Lifestyle, psychosocial-mental behaviour, and well-being are strongly affected in short and long term as well. From the physicians' perspective maintaining pain free function is an absolute priority. Articular cartilage lesions are most frequently treated with allograft transplantation and autologous osteochondral transplantation methods, including osteochondral mosaicplasty after Hangody, chondrocyte transplantation, periosteal and perichondrial resurfacement. The latter category is the safest and most reliable method based on infection and tissue reactivity rates, but we cannot eliminate the high rate of intervention-

related morbidity. The use of allografts can help solve this problem once the risk of chronic or unknown, emerging infections (slow virus, prion, etc) can be tackled. Allografts are collected from a cadaveric donor, therefore, the graft has to be sterilized and prepared in laboratory settings as well as tested for any possible disease transmission. Despite the well-described protocols, preservation of the collected cartilage grafts could be challenging, since both the cryopreservation on -80°C and the length of the storage can impact the biomechanical parameters and the integrity of collagen structure. Surgical techniques including new, possible resources to repair injured joints and damaged cartilage are still evolving. The exact effects of cryopreservation on the collected cartilage samples require accurate determination prior to utilization.

Objectives

The aim of our study was to analyse the impact of cryopreservation at -80°C on the structural properties of the human cartilage. The effects of storage time were also evaluated in conjunction with optimal utilization.

1. To analyse the impact of cryopreservation at -80°C on the structural properties of human cartilage and if these changes correlate with length of storage time.
2. To determine if differential scanning calorimetry (DSC) is a reliable method for the evaluation of the structural changes of cartilage samples.
3. To evaluate the possibilities of optimal reutilization of biological waste materials of arthroplasty.

Materials and methods

The human samples serving as a basis for research were derived from tissue fragments taken during operations and considered to be waste material. Such were the femoral head pieces removed during hip hemi-prosthesis implantations in the cases of fresh Garden III and IV type femoral neck fractures. The donors of this group taken into our study were all females between the age of 65 and 75, without symptoms of osteoarthritis. The hyaline cartilage samples were removed from the non-weight bearing part of the femoral headpieces, and the samples were fresh frozen and stored at -80°C . Cryopreservation times were: 0, 1, 3, 6, and

12 weeks. We used thermal analysis to assess the biological and structural properties of the frozen human cartilage. Thermal analysis applies a controlled temperature program allowing quantification of a change in a material's properties with change in temperature. Out of the 3 main types of thermoanalytical techniques, we performed differential scanning calorimetry (DSC), in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature.

Thermoanalytical techniques:

- **DTA and DSC: differential thermoanalysis and differential scanning calorimetry**
- TGA: thermogravimetric analysis
- TMA: thermomechanical analysis

DSC measurements

The freshly collected 'control' human cartilage samples have been prepared and measured within 6 h of removal, kept on 4 °C during the whole process. The samples stored on -80 °C for different durations (1, 3, 6, or 12 weeks) were thawed up and processed the same way than the 'controls.' The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 Kmin⁻¹. Conventional Hastelloy batch vessels were used during the denaturation experiments. Typical sample wet masses for calorimetric experiments were in between 100 and 200 mg. Sterile physiologic saline solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ± 0.1 mg, and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. OriginPro 7.5 did the data treatment after ASCII conversion.

Results

The results of DSC scans clearly demonstrate differences between the groups of cartilage samples processed after different length of storage time. The shape of DSC scans of the

different groups also showed a marked difference. It is important to note that neither the freezing, nor the duration of storage changed significantly the DSC curves during the first 3 weeks, but both the denaturation temperature and thermal enthalpy showed an increase, compared to the control, freshly collected cartilage samples (Figure 1, Table 3); therefore, we can conclude that the original structure of the cartilage is still maintained independent of storage time at -80°C during the early time of cryopreservation.

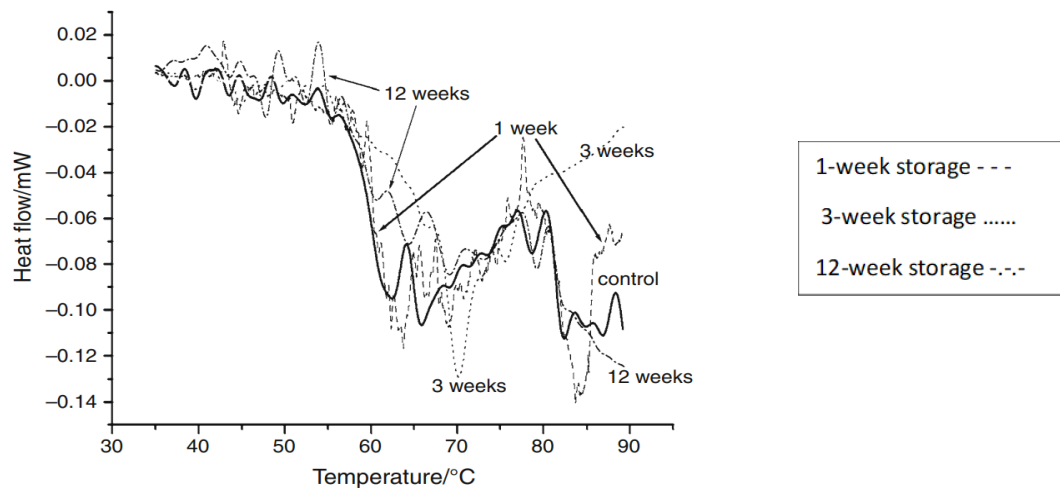


Figure 1. Thermal denaturation scans of human cartilage samples following different length of cryopreservation. The endotherm processes are directed downward. The DSC curves represent significant alterations of the structure after 3-week storage (dash line 1-week storage, dot line 3-week storage, dash dot line 12-week storage)

At the sixth week, the calorimetric enthalpy and the T_m indicated a slight decrease, compared to the control samples (data are not shown). However, at the 12th week, both the thermal enthalpy and the transition temperature showed a significant decrease, compared to the control, freshly processed samples [$[\Delta H_{cal} (\text{Jg}^{-1})=1.60$ vs 1.44 , $T_{m1} (^{\circ}\text{C})=61.73$ vs 60.85 , $T_{m2} (^{\circ}\text{C})=67.20$ vs 64.20 , $T_{m3} (^{\circ}\text{C})=77.98$ vs 69.40]. These later data could be explained by the decrease in the bound water, and with the consequence of the frozen storage-related degeneration in the cartilage.

Samples	$T_{m1}/^{\circ}\text{C}$	$T_{m2}/^{\circ}\text{C}$	$T_{m3}/^{\circ}\text{C}$	$\Delta H_{\text{cal}} \text{ J g}^{-1}$
Control (freshly collected) $n = 4$	61.73	67.20	77.98	1.60
1-week storage $n = 4$	63.81	71.32	86.71	2.12
3-week storage $n = 4$	63.64	75.44	83.54	2.49
12-week storage $n = 4$	60.85	64.20	69.40	1.44

Table 3. Measured thermal parameters of the denaturation of human cartilage samples after different time of storage

Summary of novel findings

Study I.

We have demonstrated that wintertime surgery significantly increases the risk of conversion to hip arthroplasty after internal fixation of femoral neck fractures.

Based on our results, we strongly emphasize the need to perform the primary surgery within 12 h after the injury and use of surgical methods allowing immediate full weight bearing. In addition to the evaluation of regular, well-known risk factors of complications, such as blood supply of the femoral head, trochanteric and fracture shortening, Garden stage/grading, posterior comminution, poor reduction technique and improper positioning of the screws and early reduction and stabilization; it would be worthwhile to analyse and consider the seasonal effects. During the summertime months, intensified cautiousness is needed by hospital staff and patients in the perioperative and postoperative period to reduce the initiating effect of increased summer temperature on the wound healing and septic complications.

The summertime primary surgical intervention, delay of surgery longer than 12 h and type of femoral neck fracture are independent predictors of non-prosthetic reoperations of femoral neck fractures in elderly patients. Our data could help to build a prevention strategy to decrease the number of complications of primary fracture treatment of the hip.

Study II.

In the past decades, there have been a growing number of publications released about the different type of cartilage grafts, parallel to the increased need for joint resurfacing surgeries. Pearsall et al. have evaluated the biomechanical properties and the extracellular matrix integrity of the freshly collected hyaline cartilage. Their data indicated that the biomechanical integrity of cartilage samples could only be preserved for up to 28 h. Meanwhile, the number of viable chondrocytes has been shown to decrease significantly over the time. Numerous studies reported that the different periods of cryopreservation on -80°C affects the viability and biomechanical aspects of cartilage allografts. Interestingly, the frozen samples may have the advantages of reduced immunogenicity and decreased disease transmission, although it also indicates lower chondrocyte viability.

Overall, the observed structural changes in human cartilage due to deep-freezing at -80°C generally correlate with longer storage time. It is important to note that the lower mass samples have a higher noise on DSC curves. In our results, the thermal parameters of denaturation have demonstrated an increasing thermal stability until the third week, indicated by the higher T_m and calorimetric enthalpy values. Meanwhile, starting of the sixth week, the cartilage structure becomes significantly sleazier, indicated by the decreasing T_m (data are not shown). Therefore, the authors suggest that the use of harvested cartilage for surgical surface repair, stored for six or more weeks, should be reconsidered prior to application. Recently, several publications have shown that the differential scanning calorimetry (DSC) is a useful method in the diagnosis of different musculoskeletal diseases, including the degenerative and inflammatory changes of human cartilage. Previous studies have found that the differences of thermo-analytical parameters reflect to the integrity of collagens structure and extracellular matrix of the cartilage tissue. Based on our experiences, differential scanning calorimetry (DSC) is a reliable method for the evaluation of the structural changes of cartilage samples. Since cryopreserved samples need quality assessments, utilization of DSC scans has a clinical relevance. However, the use of DSC scans in the clinical settings requires further studies.

Our data suggest the following:

- Deep-freezing at -80°C affects the structure of human cartilage and these structural changes strongly correlate with the length of storage time.
- Assessment of cartilage grafts is recommended after a longer-term cryopreservation prior to human use.
- Results of the DSC scans (thermo-analytical parameters and curves) can demonstrate and characterise the structural differences related to the duration of cryopreservation.
- Due to the reproducibility, specificity and sensitivity of DSC, it could also be a reliable method in the clinical practice, for the quality assessment of human cartilage samples following a longer preservation time.
- Based on DSC measurements the optimal threshold for storage time to prevent storage-related degeneration in the cartilage was 6 weeks.
- The predictive value of DSC-based markers could help the clinical utilization of cartilage grafts. Further research is needed concerning the following factors: technical parameters of deep freezing and variety of liquids used for storage.

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List of publications

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Publications related to the PhD thesis

1. Patczai B, Juhász K, Bűcs G, Nöt LG, Wiegand N, Sebestyén A: Nonoperative predictors for subsequent interventions after intracapsular femoral neck fractures in elderly: A comprehensive analysis of the Hungarian nationwide health insurance database. INJURY 2020; S0020-1383(20)30130-3. doi: 10.1016/j.injury.2020.02.055. PMID: 32067768 **IF2019: 2.106**
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