Cryopreservation of autologous bone flaps following decompressive craniectomy: A new method reduced positive cultures without increase in post-cranioplasty infection rate

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A R T I C L E   I N F O

Keywords:
- Bacterial culture
- Cryopreservation
- Decompressive craniectomy
- Cranioplasty
- Pulsed lavage

A B S T R A C T

Introduction: Cranioplasty (CP) after decompressive craniectomy (DC) is a common neurosurgical procedure. Implementation of European Union (EU) directives recommending bacterial cultures before cryopreservation, lead to increased number of autologous bone flaps being discarded due to positive cultures. A new method for handling bone flaps prior to cryopreservation, including the use of pulsed lavage, was developed.

Research question: The aim was to evaluate the effect of a new method on proportion of positive bacterial cultures and surgical site infection (SSI) following CP surgery.

Material and methods: Sixty-one bone flaps from 53 consecutive DC surgery patients were retrospectively included and the study period was divided into before and after method implementation. Patient demographics, laboratory and culture results, type of CP and occurrence of SSI were analyzed.

Results: Twenty-six and 18 bone flaps were available for analysis during the first and second period, respectively. The proportion of positive bacterial cultures was higher in the first period compared to the second (n = 9 (35%) vs 0 (0%); p = 0.001), and thus the use of custom made implants was considerably higher in the first study period (p = 0.001). There was no difference in the frequency of post-cranioplasty SSI between the first and second study period (n = 3 (11.5%) vs 1 (4.8%), p = 0.408).

Discussion and conclusion: The new method for handling bone flaps resulted in a lower frequency of positive bacterial cultures, without increased frequency of post-cranioplasty SSI, thus demonstrating it is safe to use, allows compliance with the EU-directives, and may reduce unnecessary discarding of bone flaps.

1. Introduction

Decompressive craniectomy (DC) is a neurosurgical life-saving procedure for treating elevated intracranial pressure (ICP) that is refractory to medical therapy. Common causes of elevated ICP are brain edema due to traumatic brain injury (TBI), malignant middle cerebral artery (MCA) infarction, or aneurysmal subarachnoid haemorrhage (SAH), where DC has shown to be efficient in decreasing mortality rates and improving neurological outcomes (Ban et al., 2010; Diedler et al., 2009; Honeybul et al., 2014; Juttler et al., 2007; Sahuquillo and Arikam, 2006; Schirmer and Ackil, 2008; Smith et al., 2002; Unterberg and Juettler, 2007; Vahedi et al., 2007). After the cerebral swelling has resolved, cranioplasty (CP) is performed for cosmetic reconstruction of the skull defect and to restore brain hemodynamics to help optimize neurologic recovery (Sakamoto et al., 2006; Winkler et al., 2000; Kuo et al., 2004; Erdogan et al., 2003; Agner et al., 2002).

Various materials have been used for CP, of which autologous bone is preferred due to its low cost and perfect match to the bone defect, although it carries risk of bone flap resorption (BFR). In cases when the autologous bone flap option is unavailable, custom made implants may...
be used. Regardless of material used, surgical site infection (SSI) after CP is a severe complication with incidence rate exceeding 20% (Cheng et al., 2008; Gooch et al., 2009; Broughton et al., 2014; Martin et al., 2014; Coulter et al., 2014; Kim et al., 2015; Sundseth et al., 2014; Brommeland et al., 2015; Shibahashi et al., 2017). Despite several different methods of sterilization and preservation of bone flaps have been studied over the last decades, there is no standardised best practice guidelines (Mirabet et al., 2021). The most commonly used techniques involve cryopreservation or subcutaneous implantation in the abdominal pocket.

In order to be compliant to the European directives 2004/23/EC, 2006/86/EC and 2015/565/EC the standard protocol in EU now includes obtaining a culture swab of the extracted autologous bone flap prior to its cryopreservation. This screening for microbial contamination has resulted in positive cultures of unknown significance. However, our department protocol only allows for reimplantation of bone flaps with negative cultures, leading to increased amounts of discarded bone flaps and thus increasing the use of custom made implants. To address this problem, the authors developed a new method for handling cryopreserved bone flaps including pulsed lavage of the bone flap preceding culture swab. Pulsed lavage is standard in orthopaedic and general surgery procedures to reduce the rate of SSIs (Bath et al., 2021), and has synergistic effects combined with antimicrobial therapy in an in-vitro study (Poilvache et al., 2020). The objective of the present study was to retrospectively compare the rates of positive cultures and SSI in patients who underwent CP before and after the new method was implemented.

2. Materials and methods

2.1. Patients

The study protocol was approved by the Ethics Review Authority in Sweden (Ref.no 2021–05372), which waived the need for written informed consent from the patients as this was a retrospective study. Consecutive patients undergoing decompressive craniectomy (DC) and bone flap cryopreservation in the Neurosurgical Department, University hospital in Linköping, Sweden, with a catchment area of 1 million people were included. Clinical and demographic parameters, laboratory results, microbiological culture results and clinical evaluation at twelve months and at last available follow up date were retrieved from patient medical records. Post-cranioplasty infection was defined as either having gone through revision surgery for infection or aseptic bone resorption with subsequent positive perioperative bacterial cultures.

The study period was divided into two periods were the first period (September 2017 to September 2019) was prior to the change in routine handling of bone flaps and the second period (October 2019 through to April 2021) was after the change in routine handling of bone flaps.

The routine perioperative antibiotic given was cefazolin 2 g intravenously 30 min before incision. This was not changed between the two study periods. Furthermore, there were no changes made during the entire study period in the preoperative preparations for surgery, routine preoperative showers, perioperative sterile draping, suture material, surgical instruments or post-operative wound dressing. No changes between the periods were made in routine blood tests including tests for blood borne infectious diseases which were taken at the time of surgery.

2.2. Old method of handling bone flaps

After removal from the patient the bone flap was stored on the scrub nurse instrumentation table wrapped in surgical swabs drenched in saline solution in a sterile cup. At the end of surgery, the bone flap was cleansed from soft tissue and subsequently a piece of bone for deep tissue culture was secured. The piece of bone was incubated in a broth culture. Instructions were to use uncontaminated instruments, not used previously during the surgery, to procure this piece of bone. No swab culture was taken. Subsequently the bone flap was packaged in several layers of surgical swabs, a sterile surgical glove, sterile bags and identified clearly with patient ID. This entire procedure was done by the scrub nurse. Following the end of surgery, the packaged bone flap was delivered to the hospital bone freezer which is used strictly for autologous and allogenous bone transplantation tissue.

2.3. New method of handling bone flaps

After removal from the patient the bone flap was stored on the scrub nurse instrumentation table wrapped in surgical swabs drenched in saline solution in a sterile cup. At the end of DC surgery the bone flap was cleansed from any soft tissue. Surgical gloves were changed and the bone flap was then rinsed using pulse lavage (Pulsavac Plus, Zimmer Biomet, Zürich, Switzerland) with saline solution. Plastic covers were arranged around a bowl in an effort to avoid the rinse water splashing back up at the bone flap. A culture swab (Eswhab standard, Unilabs, Skövde, Sweden) was then taken from the surface of the bone flap and sent to the microbiology laboratory. The bone flap was packaged in surgical swabs, a sterile surgical glove, and sterile bags in the same manner as previously, identified clearly and subsequently delivered to the bone freezer. This entire procedure was carried out by the scrub nurse (Fig. 2).

2.4. Bacterial cultures

All cultures were delivered directly to the laboratory of Clinical Microbiology if possible. If immediate delivery was not possible deep cultures were stored in a heat cupboard (35 °C), and Eswab cultures in refrigerator (8 °C) until delivery to the laboratory.

Deep cultures taken from pieces of bone were cultured in MH-broth (MH Difco Broth, BD, USA) for seven days. If there was no visible bacterial growth in the vial a control culture was performed. The broth was cultured on a hematin plate (CG agar (EO labs, Scotland) with hemoglobin (Bovine Freeze dried, BBL Dickinson, France)) incubated in carbon dioxide for two days, and an anaerobic plate without antibiotics (Fastidious Anaerobe Agar Neogen with horse blood, United Kingdom), incubated for four days in an anaerobic environment. If there was growth in the vial these bacteria were cultured on a hematin plate, an anaerobic plate without antibiotics, UTI-agar plate (Brillance TM UTI medium Oxoid, United Kingdom), and a blood agar plate (Columbia agar base with horse blood, Difco, Neogen, USA) all of which were incubated in regular air for two days. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) was used for microbial identification and antibiotic susceptibility was determined using disc diffusion or E-test on agar plate (Millnerhinton II agar, BBL TM, USA or Muller Hinton NAD, BBL TM, USA) with NAD-solution for disc diffusion in beta-hemolytic streptococci and other streptococci. The culture method was not changed during the entire study period.

Eswab cultures taken from the bone surface were cultured on Hematin agar, Staf-chromar agar, UTI agar and streptococcus agar plate. All plates were incubated for two days in 35 °C. The hematin plate in carbon dioxide, streptococcus plate in an anaerobic environment and the UTI and staf-chrom plate in regular air.

2.5. Statistical method

Normally distributed data are presented as mean and standard deviation. Non-normally distributed ordinal data are presented as median and interquartile range. Nominal data are presented as numbers and percentages. Normally distributed data were analyzed using students t-test, ordinal data using Mann Whitney U test and nominal data using $\chi^2$-test. All statistical analyses were performed using IBM SPSS 27.0 (IBM, Kista, Sweden).

3. Results

Sixty-one bone flaps from 53 consecutive patients undergoing decompressive craniectomy (DC) were included in the study, 34 in the
first study period (September 2017–September 2019) and 27 in the second study period (October 2019–April 2021; Fig. 1). Eight bone flaps in the first period and five in the second period were lost to follow-up due to either the patient passing away before cranioplasty (CP) (n = 7), no access to medical records of CP surgery (n = 3), or opting out of CP (n = 4). Thus 26 bone flaps from 22 patients and 21 bone flaps from 19 patients were analyzed from the first and second study period, respectively (Fig. 1).

There was no difference in mean age at time of DC (40.8 (±16.0) years vs. 46.8 (±21.2) years, p = 0.270), proportion male patients (17 (65%) vs. 12 (57%); p = 0.563), median [IQR] Glasgow coma scale motor score (GCS-m; 6 [3-6] vs 5 [4-6], median [IQR] score according to American Society of Anesthesiologists (ASA) Physical Status (2 [1-3] vs 2 [2-3], p = 0.798), mean body mass index (BMI; 29.9 (±15.4) vs 28.3 (±8.0), p = 0.798), between the first and second study period (Table 1). There was no difference in levels of hemoglobin (Hb), or C-reactive protein (CRP) between the groups (Table 1), whereas leukocyte count was higher in the first period compared to the second period (16.1 (±6.4) vs 10.3 (±2.8) ×10⁹/L, p < 0.001, Table 1). The proportion of patients with one or more comorbidities was lower in the first study period compared to the second (n = 7 (28%) vs n = 13 (62%); p = 0.021; Table 1).

There was no difference between the two periods in mean time between the DC and CP surgery (8.7 (±6.0) vs. 6.6 (±3.6) months, p = 0.153), proportion of patients receiving antibiotics within 45 min of start of surgery at DC (n = 13 (81%) vs. n = 12 (92%), p = 0.390, 38% missing data) and CP surgery, respectively (n = 15 (83%) vs n = 13 (81%), p = 0.874; 28% missing data; Table 2) or proportion of patients receiving broad spectrum antibiotics at the time of DC surgery in the first study period compared to the second (n = 8 (35%) vs n = 3 (16%), respectively, p = 0.163; Table 2). There was significantly fewer bone flaps in several pieces in the first study period compared to the second (n = 5 (19%) vs n = 10 (48%), p = 0.038; Table 2). Mean follow-up time was longer in the first period compared to the second period as all patients were followed to last available date (27.6 (11) vs 12.8 (6) months, respectively, p < 0.001).

There was no difference in the frequency of post-cranioplasty infection of bone flaps (3 (11.5%) vs 1 (4.8%; p = 0.408) between the first and second study period. Post-cranioplasty infection was diagnosed at 17, 38 and 556 days post-surgery in the first group and after 30 days in the second group. The proportion of positive cultures was significantly higher in the first period compared to the second (9 (35%) vs 0 (0%); p < 0.001), as was the proportion of missing cultures (8 (31%) vs 1 (5%); p < 0.001). The use of custom-made flaps instead of autologous bone flaps was considerably higher in the first study period compared to the second (13 (50%) vs 1 (5%); p = 0.001). One patient in the second study period, a 6-month-old boy, had his bone flap removed due to bone flap resorption.

Bacterial species in positive cultures consisted of *Streptococcus anginosus* (n = 1), *Cutibacterium acnes* (n = 4), methicillin-susceptible *Staphylococcus aureus* (MSSA; n = 2), and oxacillin-susceptible *Staphylococcus epidermidis* (n = 2).

There was a difference in the number of patients with primary CNS infections in the two study periods, with five patients undergoing DC due to meningitis or intracranial abscess during the first period, and none in the second period. Of the positive bacterial cultures in the first study period (n = 9), however, only two came from patients with primary CNS-infections (*Streptococcus anginosus* and *Staphylococcus epidermidis*).

**4. Discussion**

The new method of using pulsed lavage prior to culture swab and cryopreservation resulted in a significant reduction in positive cultures without any increase in frequency of post-cranioplasty infections, thus reducing the number of bone flaps being unnecessarily discarded.

In the current study, overall SSI rate was 8.5%, which is similar to what has previously been reported (Cheng et al., 2008; Chang et al., 2010; Huang et al., 2013; Kim et al., 2013). The proportion of positive cultures was 35% in the first study period compared to 0% in the second period. The number of primary CNS infection as a cause for decompressive craniectomy (DC) was higher in the first study period (n = 5) compared to the second period (n = 0) which may contribute to the great

![Fig. 1. Flow chart of included patients.](image-url)

Sixty-one bone flaps from 53 consecutive patients were included in the study in total, with 34 and 27 bone flaps in the first and second study period, respectively. In the first study period eight bone flaps were lost to follow-up in the first period due to death prior to cranioplasty (CP; n = 3), no access to records of CP (n = 3) or deliberate choice not to have CP surgery (n = 2). In the second period five bone flaps were lost to follow-up due to patient death prior to cranioplasty (n = 4), or patients deliberate choice not to have CP surgery (n = 1). This resulted in 26 bone flaps included in the first study period and 21 bone flaps in the second study period.
The frequency of SSI did not increase after reimplantation of bone (Morton et al., 2016). Similarly, in a study of 377 cranioplasty procedures caused by organisms that differed from those in the original culture swab not predict post-cranioplasty infection, and SSI that occurred were infection at the time of the initial DC. However, the culture results could operations and found 20% positive cultures taken in absence of clinical changes implemented. A recent study investigated 754 cranioplasty (CP) bone fluids, whereas a swab from the surface of the bone was cultured in the second study period. Therefore, it is not determined if the reduction in cultured whereas a swab from the surface of the bone was cultured in the second study period is the difference in culture sampling, or a combination of the two factors.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First study period (N = 26 bone flaps)</th>
<th>Second study period (N = 21 bone flaps)</th>
<th>p-value</th>
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<td><strong>DC surgery</strong></td>
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<tr>
<td>Age (years), mean (SD)</td>
<td>40.8 (16.0)</td>
<td>46.8 (21.2)</td>
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<tr>
<td>BMI at DC, mean (SD)</td>
<td>29.9 (15.4)</td>
<td>28.3 (8.0)</td>
<td>0.798</td>
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<td>Male, n (%)</td>
<td>17 (65)</td>
<td>12 (57)</td>
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<td>One or more comorbidities, n (%)</td>
<td>7 (28)</td>
<td>13 (62)</td>
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<td>Immunosuppressive therapy incl.</td>
<td>2 (8)</td>
<td>1 (6)</td>
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<td>cortisone, n (%)</td>
<td>24</td>
<td>18</td>
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<tr>
<td>ASA group, median [IQR]</td>
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<td>2 [2-3]</td>
<td>0.798</td>
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<tr>
<td>GCS-m, median [IQR]</td>
<td>6 [3-6]</td>
<td>5 [4-6]</td>
<td>0.674</td>
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<td>Indication DC, n (%)</td>
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<tr>
<td>Trauma</td>
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<td>9 (43)</td>
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<tr>
<td>Stroke</td>
<td>9 (35)</td>
<td>12 (57)</td>
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<tr>
<td>Infection</td>
<td>7 (27) +</td>
<td>0 (0) +</td>
<td></td>
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<tr>
<td>Perioperative edema</td>
<td>1 (4) +</td>
<td>0 (0) +</td>
<td></td>
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<td>Right side, n (%)</td>
<td>14 (54)</td>
<td>13 (62)</td>
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<td>Hemoglobin (g/L), mean (SD)</td>
<td>128 (17)</td>
<td>119 (17)</td>
<td>0.093</td>
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<tr>
<td>CRP (mg/L), mean (SD)</td>
<td>57 (78)</td>
<td>62 (66)</td>
<td>0.803</td>
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<td>Leukocyte count (x10^9/L), mean (SD)</td>
<td>16.1 (6.4)</td>
<td>10.3 (2.8)</td>
<td>0.000</td>
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<tr>
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<tr>
<td><strong>CP surgery</strong></td>
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<tr>
<td>BMI at CP, mean (SD)</td>
<td>26.8 (6)</td>
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<td>3 [2-3]</td>
<td>3 [2-3]</td>
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<td>135 (13)</td>
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<td>CRP (mg/L), mean (SD)</td>
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<td>11 (14)</td>
<td>0.588</td>
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<td>Leukocyte count (x10^9/L), mean (SD)</td>
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</table>

Abbreviations: DC = decompressive craniectomy, CP = cranioplasty, BMI = body mass index; ASA = American Society of Anesthesiologists; CRP = C-reactive protein; incl. = including; GCS-m = Glasgow Coma Scale motor score.

Majority of patients were followed more than twelve months also in the second group, the shorter follow-up time could lead to low-virulent infections being underreported in the second study period. However, a previous study of cranioplasty cases showed median time of SSI infection at 31 days, thus the duration of follow-up of 12.8 months in the second study period in the current study should be sufficient (Morton et al., 2016).

Fig. 2. Illustration of the new method of bone flap handling
A) A decompressive craniectomy is performed and the bone flap is removed from the patient. B) The bone flap is cleansed from any soft tissue and rinsed with saline solution. C) The bone flap is rinsed using pulsed lavage with saline solution. Plastic covers are arranged around a bowl in order to avoid the rinse water splashing back and re-contaminating the bone flap. D) A culture swab is taken from the surface of the bone flap and sent to the laboratory. The bone flap is packaged in surgical swabs, a sterile surgical glove, and a sterile bag. The bag is then tagged with identification data and delivered to the bone freezer for cryopreservation.
in the operating field, including surgical instruments (Davis et al., 1999). This contamination of the operating field results in a high risk of positive cultures with unclear significance. Although the pathogens colonizing the bone flap may be less likely to cause SSI, it is essential that the bone flap is thoroughly cleansed prior to culture swab to avoid unnecessary positive cultures and subsequent discarding of bone flaps. Different types of irrigation and pulsed lavage has historically been used in orthopedics and abdominal surgery in order to reduce SSI rates (Bath et al., 2021). A strong relationship has previously been demonstrated between bacterial load in the wound and risk of SSI, where pressurized irrigation of contaminated wounds has been found to significantly reduce bacterial colonization (Owen and Stoessell, 2008; Rodeheaver et al., 1975). The high pressure irrigation method has, however, also been associated with increased tissue trauma (Wheeler et al., 1975). In the current study, the risk of harm is minimal since the bone flap is extracted and isolated from the patient whilst cleansed. However, the role of pulsed lavage in neurosurgery is limited to extracranial usage for this particular reason.

Bone flap resorption (BFR) is a known long-term complication of CP with autologous bone flap. In this study BFR occurred in one patient, a 6-months-old boy, consistently with previous reports of BFR being associated with young age (Martin et al., 2014; Macek et al., 2015; Schwarz et al., 2016). BFR can occur long time after CP, thus this study could underrepresent the frequency of BFR. However, the aim of the study was to investigate the frequency of SSI following the implementation of the new method, not BFR. BFR is, nevertheless a problematic issue which may occur after CP with autologous bone flaps. Longer term follow-up is encouraged to evaluate the frequency of BFR.

This is a single-center study, with a relatively small number of patients. However, numbers are large enough to draw conclusions with statistical certainty and this clinically relevant problem which affects many neurosurgical centers around Europe is pressing. Further clinical studies with larger study samples and longer follow-up are encouraged.

5. Conclusion

The new method of handling bone flaps at our center resulted in a drastically lower frequency of positive bacterial cultures, without any increase in frequency of post-cranioplasty infections, thus allowing compliance with the EU-directives, without leading to unnecessary discarding of bone flaps.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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