Periprosthetic Joint Infection after Primary Total Knee Arthroplasty With and Without Sinus Tract: Treatment Outcomes

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Abstract

Background. Sinus tract appears up to 20% of patients with periprosthetic joint infection (PJI) after primary total joint arthroplasty (TJA). The adverse effect of different patient related risk factors is well known, but the role of the sinus tract in PJI recurrence wasn’t properly investigated. The aim of the study was to analyze the influence of a sinus tract on the effectiveness of two-stage reimplantation in patients with PJI. Materials and Methods. In order to reduce the influence of known risk factors on effectiveness of infection eradication several including criteria were introduced: patients with PJI after primary total knee arthroplasty (TKA), without surgeries before TKA and after infection manifestation and without attempts of antibiotic suppression. Finally, 119 of 475 patients with PJI after primary TKA, treated with two-stage protocol, were retrospectively analyzed: 33 patients with presence of the sinus tract, 86 — without sinus tract. Pathogen type, comorbidities, PJI type, bone defects, duration of surgery, intraoperative blood loss and the effectiveness of two-stage reimplantation were analyzed. Results. Pathogen structure in analyzed groups was comparable. Staphylococci were the leading cause of PJI in compared groups: 64.4% of patients without sinus tract and 59.1% of patients with sinus tract. Wherein S. aureus was more frequently isolated in case of active sinus tract while S. epidermidis played the leading role in compared group. Polymicrobial PJI was more likely to develop in patients with sinus tract (p = 0.09). Massive bone defects of femur and tibia (type 3 according AORI classification) were identified only in patients with sinus tract PJI, as well as significantly longer duration of the spacer implantation and higher intraoperative blood loss at this stage (p<0.05). Infection eradication after spacer implantation stage was achieved in 98.8% (n = 79) of patients without sinus tract while only 81% (n = 17) of patients with sinus tract PJI successfully passed this stage of surgical treatment (p<0.05), effectiveness of revision knee arthroplasty was 98.7% (n = 78) and 76.5% (n = 13) respectively (p<0.05). Finally, complex effectiveness of two-stage reimplantation in patients with sinus tract was significantly lower (61.9%) than in patients of compared group (97.5%), p<0.05. Conclusion. Presence of the sinus tract in patients with PJI after primary TKA adversely effects on the effectiveness of two-stage reimplantation, this fact could be explained by aggressive development of infection, caused by more virulent pathogens and higher frequency of polymicrobial cases among this cohort of patients. The established patterns require further research to develop tactics for managing this category of patients in order to increase the effectiveness of two-stage reimplantation.

Keywords: periprosthetic joint infection, sinus tract, total knee arthroplasty, two-stage reimplantation.

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The number of total knee arthroplasty (TKA) surgeries is steadily increasing all over the world. According to national registries, over the past 15 years, the number of both primary and revision TKA increased [1]. Periprosthetic joint infection (PJI) is one of the most frequent and most serious complications after the primary TKA (KA) with a rate from 0.4 to 2% [2]. In some cases, after modular endoprosthesis implantation in the patients with cancer, this number reaches 9% [3]. According to the Australian Arthroplasty Registry, PJI is the most common cause of revision surgery in the elderly patients and accounts for 35.2% of all revisions in the 80 to 89 age group and 61.5% in the ≥90 age group [4].

The course of PJI is associated with severe pain syndrome, prolonged hospital stay, the need for a comprehensive multidisciplinary approach to treatment, multiple surgeries and disability [5]. The results of revision arthroplasty complicated by PJI showed a five-fold increase in mortality compared with the revision arthroplasty for aseptic components instability [6].

A sinus tract formed during the PJI was identified by S. Xu et al. in 21.3% of patients with knee and hip PJI. The sinus tract penetrated into the joint cavity in 51.2% of all cases [7]. Such type of a penetration is currently considered as definite sign of PJI [8, 9].

Currently, there are number of studies analyzing the influence of risk factors for PJI development and recurrence, after the both stages of surgical treatment [10, 11, 12]. At the same time, there is lack of information about the influence of the sinus tract about the treatment outcome.

The aim of the study was to analyze the influence of a sinus tract on the effectiveness of staged reimplantation in patients with PJI.

**Materials and Methods**

**The study design – a retrospective trial**

We retrospectively analyzed 475 patients with confirmed PJI who undergone staged revision arthroplasty in Vreden National Medical Research Center of Traumatology and Orthopedics, St. Petersburg, Russia, from 2014 to 2018.

In order to achieve the homogeneity of the compared groups, the inclusion criteria were formulated: PJI development after primary TKA in the patients without previous surgical interventions on this segment, history of DAIR procedures and attempts of antibiotic supression therapy until admission to our clinic.

As a result, 119 patients were included in the study, divided into two compared groups depending on the presence of the sinus tract. The 1st group (33 patients) included the patients with a sinus tract (actively functioning on admission stage). The 2nd group (86 patients) included the patients without a sinus tract, both – in past and on the admission stage (Fig. 1). The average follow-up was 35.6 months (13 to 72).

**Figure 1.** Research flowchart

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**Total 474 patients with knee PJI**
- after primary TKA
- without history of surgical interventions on this segment
- without history of antibiotic supression therapy attempts

**119 patients with knee PJI**

**33 patients with knee PJI with sinus tract (1st group)**

**86 patients with knee PJI without sinus tract (2nd group)**
**Assessment methods**

The following parameters were analyzed in full cohort using the medical records and the PJI registry data:

- comorbidities;
- PJI type according the time of infection manifestation after primary TKA: early (<3 months after TKA delayed (3 to 12 months after TKA and late (> 12 months after TKA [13]);
- pathogen type;
- type of bone defects according to the AORI classification after components removal [14];
- the duration of spacer implantation in minutes;
- intraoperative blood loss in ml;
- rate of intraoperative blood loss in ml/min;
- inpatient period in days;
- total protein and albumin serum blood level (g/L) before spacer implantation.

Absence of clinical and laboratory signs of PJI recurrence at the time of the patient’s admission for the 2nd stage of surgical treatment was taken as a successful outcome of the 1st stage. The need for spacer reimplantation between the stages due to the PJI recurrence was regarded as a failure. The effectiveness of the 2nd stage revision arthroplasty was assessed based on the results of the follow-up or remote questioning.

PJI diagnostics was carried out according to the International Consensus Meeting (ICM) criteria (2013) and confirmed in presence of 1 of 2 major diagnostic criteria or 3 of 5 minor criteria [8]. At preoperative stage, all patients underwent clinical and laboratory examination.

During physical examination, presence of a sinus tract was determined with its further fistulography to visualize the tract communication with knee cavity (Fig. 2).

Diagnostic aspiration of the operated knee with further bacteriological examination was performed.

**Surgical technique**

All patients included in the study underwent the 1st stage of surgical treatment: arthrotomy, components and cement mantle removal, debridement of all infect tissues, massive joint cavity irrigation with lavasept solution (at least 5 L) and further antimicrobial cement spacer implantation: articulating (n = 100; 84%) or static (n = 19; 16%). For articulating spacer resterilized femoral components and sterile inserts were used.

Components fixation of articulating or static spacers was carried out using gentamicin-containing bone cement (Refobacin bone cement/DePuy CMW 3 bone cement) with additional impregnation with vancomycin (4 g for every 40 g of cement). Removed components were sent to the microbiological laboratory for sonication and subsequent bacteriological examination of sonicated fluid. Also, 5 tissue biopsy specimens were taken intraoperatively for bacteriological examination.

**Postoperative patient management**

All patients underwent thromboprophylaxis and a course of parenteral antibiotic therapy in the postoperative period. The initial scheme of empirical antibiotic therapy provided a wide spectrum of antimicrobial activi-
ty due to the combination of vancomycin with beta-lactam antibiotics or fluoroquinolones, or as an alternative – beta-lactam antibiotics with fluoroquinolones. After receiving the final results of intraoperative samples bacteriological examination antibiotic therapy correction was carried out, if necessary. After 12-14 days, of intravenous antibiotic therapy was switched to oral regimen for 6-8 weeks. The patient’s operated joint was immobilized for 2 weeks after spacer implantation. In the case of massive bone defects, unsatisfactory soft tissues or extensor mechanism condition, knee immobilization and walking with additional support (crunches) was recommended until the 2nd stage of surgical treatment.

**Statistical analysis**

The clinical results obtained in the course of this work were analyzed using the STATISTIKA 10 software system. Categorical data is presented as proportions (gender, type of PJI, comorbidity, type of spacer and outcome) was carried out using nonparametric methods: $\chi^2$, $\chi^2$ Pearson, Fisher’s test. The comparison of quantitative parameters (age, length of stay, operative time and blood loss volume) was carried out using the Mann-Whitney criteria. The median was used as the central characteristic, and the lower Q (1) and upper (Q3) quartiles (25 to 75% interquartile range) were used as the dispersion measures. Reported P values are two-tailed. The P value below 0.05 was considered significant.

**Results**

The gender analysis revealed a significant predominance of male patients in the 2nd group, p = 0.04 (Fig. 3). The median age of the 2nd group patients (without sinus tract) on the admission stage was 64 years (38 to 81), 61 years (27 to 80) and in the 1st group (with sinus tract).

Patients with hematogenous type of infection predominated in both groups. Delayed PJI occurred 2 times more often in the 2nd group, p <0.05 (Tab. 1).

Bone defect data was available for 52 patients: 37 patients without sinus tract and 15 patients with it. The 2nd type of the bone defects prevailed in the patients of both groups.

The proportion of the patients with minimal defect of femoral condyles was significantly higher in the group without sinus tract (p <0.05). F3 type massive bone defects did not occur in this group at all (Fig. 4).

**Table 1**

<table>
<thead>
<tr>
<th>PJI type</th>
<th>Group without sinus tract (%)</th>
<th>Group with sinus tract (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>22 (25.6)</td>
<td>10 (30.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Delayed</td>
<td>26 (30.2)</td>
<td>5 (15.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Late</td>
<td>38 (44.2)</td>
<td>18 (54.6)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td>86 (100)</td>
<td>33 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Gender distribution in the compared groups, here and further * means — p <0.05
Tibial defects structure was similar in both groups, however, massive bone tissue destruction was also found only in patients with sinus tract (Fig. 5).

Comparative analysis did not reveal any significant difference in comorbidity structure in both groups (Tab. 2).

Attention is drawn to the fact that over 80% of the patients in the studied groups were overweight, more than 70% had pathology of the cardiovascular system and gastrointestinal tract. 30% of the patients without sinus tract and 45% of patients with it had PJI associated anemia at the moment of admission.

The body mass index (BMI) in the investigated groups was comparable. BMI value corresponding to normal body weight was only in 13 (17.1%) patients in the group without sinus tract and in 4 (12.1%) patients with it (Fig. 6).

Spacer implantation in patients with sinus tract required a significantly longer time (192.4 minutes) than in compared group (151.6 minutes), p = 0.0005, and was also accompanied by significantly greater blood loss, 956.1 ml and 611.1 ml, respectively (p = 0.0002). Although intraoperative blood loss rate in both groups was comparable, 5 ml/min in the 1st group and 4 ml/min in the 2nd group.

### Table 2

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Group without sinus tract, n (%)</th>
<th>Group with sinus tract, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight and obesity</td>
<td>71 (82.6)</td>
<td>29 (87.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>73 (84.9)</td>
<td>27 (81.8)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>67 (77.9)</td>
<td>26 (78.8)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>52 (60.5)</td>
<td>21 (63.6)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gastrointestinal tract diseases</td>
<td>64 (74.4)</td>
<td>23 (69.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Preoperative anemia</td>
<td>26 (30.2)</td>
<td>15 (45.4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9 (10.5)</td>
<td>5 (15.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Kidney and urinary tract diseases</td>
<td>9 (10.5)</td>
<td>5 (15.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Liver and biliary tract diseases</td>
<td>11 (12.8)</td>
<td>4 (12.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>13 (15.1)</td>
<td>3 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>7 (8.1)</td>
<td>4 (12.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Respiratory tract diseases</td>
<td>8 (9.3)</td>
<td>3 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Systemic diseases</td>
<td>7 (8.1)</td>
<td>3 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Cardiac arhythmias</td>
<td>7 (8.1)</td>
<td>3 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Peripheral vascular diseases</td>
<td>3 (3.5)</td>
<td>3 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Malignant neoplasms</td>
<td>1 (1.2)</td>
<td>0 (0)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*Figure 4. Femoral defects structure according AORI classification in compared groups

*Figure 5. Tibial defects structure according AORI classification in compared groups*
There was no significant difference between the groups in the pre- and postoperative periods for total protein and albumin serum blood level. Table 3 presents the median values of these indicators.

Pathogen structure in the both groups was comparable. Staphylococci were the most common pathogen accounting for 64.4% and 59.1% in the group without sinus tract and with it, respectively. The most common causative agent in the 2nd group as Staphylococcus epidermidis, and in the 1st group – Staphylococcus aureus. Moreover, in the group with sinus tract the strains of anaerobic microorganisms were isolated 1.5 times more often (p>0.05) and the strains of Enterococcus sp. – 2 times less often (p>0.05) than in patients of compared group Table 4.

The proportion of methicillin-resistant strains of Staphylococcus epidermidis (MRSE) was also comparable in both groups and amounted 70% (n = 21) in the group without sinus tract and 60% (n = 6) in the group with it. The methicillin-resistant strains of Staphylococcus aureus (MRSA) were found only in the group with sinus tract (7.1% of the cases).

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**Figure 6.** Distribution of the patients in the study groups by BMI

**Table 3**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Period</th>
<th>Group without sinus tract, g/L</th>
<th>Group with sinus tract, g/L</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>preoperative</td>
<td>39.3</td>
<td>37.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>postoperative</td>
<td>39.2</td>
<td>34.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total protein</td>
<td>preoperative</td>
<td>72.0</td>
<td>74.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>postoperative</td>
<td>62.5</td>
<td>64.3</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Table 4**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Group without sinus tract, n (%)</th>
<th>Group with sinus tract, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All staphylococci</td>
<td>58 (64.4)</td>
<td>26 (59.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>30 (32.3)</td>
<td>10 (22.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. aureus</td>
<td>23 (24.7)</td>
<td>14 (31.8)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>4 (4.3)</td>
<td>3 (6.8)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>10 (10.8)</td>
<td>7 (15.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>10 (10.8)</td>
<td>2 (4.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gram (–)</td>
<td>10 (10.8)</td>
<td>4 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Others</td>
<td>6 (6.5)</td>
<td>4 (9.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td>93 (67.9)</td>
<td>44 (32.1)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
Polymicrobial infection was 1.8 times more frequent in the 1st group with sinus tract, 27.3% compared to the same indicator in the 2nd 15.1%, (p = 0.09). The associations with Gram (-) microorganisms were characteristic only for the patients without sinus tract, accounting for 30.8% of all polymicrobial infection cases.

108 of 119 patients were available for the outpatient follow up - 85 patients from 2nd group and 23 patients from 1st group. It was found that infection eradication after the spacer implantation was achieved in 98.8% (n = 79) of the patients without sinus tract and in 81% (n = 17) of patients with it (p <0.05). The mortality rate not related to PJI was 5.9% (n = 5) in the 2nd group and 8.7% (n = 2) in the 1st (Fig. 7).

The effectiveness of the 2nd stage of surgical treatment in the group without sinus tract was 98.7%. One patient after PJI recurrence underwent knee arthrodesis. The same indicator in the group with sinus tract was significantly lower - 76.5% (p <0.05). Three patients underwent knee arthrodesis after PJI recurrence One patient after reinfection was successfully re-treated with two-stage PJI treatment protocol (Fig. 7). As a result, overall efficacy of the two-stage treatment in the patients with sinus tract was significantly lower than in compared group - 61.9% and 97.5%, respectively, (p <0.05).

Thus, the presence of a sinus tract increased the risk of PJI recurrence: both at the 1st and the 2nd stage surgical treatment.

![Figure 7. Treatment outcomes in the compared groups](image-url)
Discussion

There are few studies investigating predictors of sinus tract formation after primary total knee arthroplasty. C. Xu et al. performed the comparative analysis of two-stage revision arthroplasty effectiveness in patients with PJI both with and without sinus tract. They identified the following factors that increased the risk of sinus tract formation: DAIR procedures after primary total knee arthroplasty, hypoalbuminemia, hypothyroidism and smoking. Taking into account the negative impact of the DAIR procedures after primary total knee arthroplasty on the sinus tract formation and on effectiveness of two-stage revision arthroplasty, we excluded this category of patients from our study. The average level of albumin and total protein in blood serum was normal in the compared groups. Proportion of smokers was also comparable. The effectiveness of infection eradication using two-stage protocol was significantly lower in the group of patients with sinus tract, 61.9% vs 97.5%. Actively functioning sinus tract in patients with PJI was a significant risk factor for PJI recurrence, influencing both on the effectiveness of antimicrobial spacer implantation, OR 4.5 (2.4 to 8.4), and on revision knee arthroplasty, OR 5.6 (2.9 to 10.9) [7].

The analysis of concomitant pathology revealed the higher rate of anemia among patients with sinus tract (45.5% vs 30.2%). This could be due to endogenous intoxication caused by PJI. Presence of anemia in patients with sinus tract increased the risk of PJI recurrence from 15.5% to 37.5% and could be considered not only as a known risk factor of PJI development, but also as a pathology that worsens the prognosis of treatment outcome, (OR 3.9 (1.0 to 15.4)). At the same time presence of anemia did not affect on the effectiveness of surgical treatment in patients without sinus tract. We can’t exclude that the reason of this consistent pattern was longer surgery duration, greater volume of intraoperative blood loss in PJI patients with sinus tract [16, 17].

Male gender is a known risk factor for PJI development confirmed by S.K. Kunutsor et al. meta-analysis [18]. Our analysis also showed the prevalence of men in the full cohort of investigated patients (p <0.05). And this was despite the fact that the proportion of women undergoing in the primary TKA is usually significantly higher [19]. However, no correlation between the sinus tract formation and the gender was found. There were 15 men (45.5%), 18 women (54.5%) out of 33 patients in the group of patients with sinus tract.

PJI etiology analysis did not reveal any statistically significant differences in pathogen structure depending on the presence of a sinus tract. On the one hand, this could be explained by a similar pathogenesis, since the late hematogenous PJI prevailed in the patients of both groups. On the other hand, it could be due to a small number of observations in 1st group. Generally the obtained data correspond to the trend of the last decade, which demonstrates the widespread increase of S. epidermidis resistance and its role in the development of PJI [20, 21]. Extremely high frequency of S. epidermidis strains resistant to methicillin was confirmed: 60% and 70%, respectively, in the 1st and 2nd investigated groups, compared with 7.1% of MRSA of isolated only in patients with sinus tract. At the same time S. aureus (the most virulent among all Staphylococci species) was the leading cause of PJI development in patients with sinus tract [22].

The microbial associations in our study were detected 1.8 times more often (p = 0.09) in patients with a sinus tract. The similar data was obtained by C. Xu et al. They found in their retrospective study on of more than 1200 patients with PJI that polymicrobial infection was more characteristic for the patients with sinus tract (OR 2.10; 95% CI: 1.29 to 3.42). Taking into consideration negative impact of polymicrobial etiology on the effectiveness of infection eradication, one can assume, that this was due to more frequent PJI recurrence in patients with sinus tract PJI
This hypothesis is supported by the results of T.L. Tan et al., who showed that a significant risk factor for PJI relapse in treatment of polymicrobial PJI is the presence of a functioning sinus tract (OR 2.20; 95% CI: 1.39 to 3.47; p = 0.001) [24]. Despite increasing popularity of one-stage revision arthroplasty in patients with PJI due to its high effectiveness, according to various authors from 91% to 100% [25, 26], the attempts to apply it in patients with sinus tract could lead to a significant increase in relapse. J.Y. Jenny et al. reported an increase in the recurrence rate from 16% to 27% (6 patients out of 22) while performing one-stage revision arthroplasty in this category of patients [27].

The analysis of bone loss at the spacer implantation stage showed that massive loss of femoral and tibial condyles (AORI type 3 defects) was characteristic only for the patients with sinus tract reflecting the severity of PJI in presence of sinus tract. Despite the fact that presence of the sinus tract along with massive bone defects are considered only as a relative contraindication for one-stage revision arthroplasty, our data, as well as the results of scientific publications, shows the advisability of a two-stage reimplantation use in treatment of the patients with sinus tract PJI [28].

The study limitations

Limitations of the study include its retrospective nature and the small number of the patients in both groups. Although, this disadvantage we tried to compensate by strict inclusion criteria and adequate statistical methods for processing the obtained data.

Conclusion

Presence of the sinus tract in patients with PJI after primary TKA adversely effects on the effectiveness of two-stage reimplantation, this may be associated with aggressive development of infection, caused by more virulent pathogens and higher frequency of polymicrobial cases, massive femoral and tibial metaphyseal bone loss, longer operation time, and increased intraoperative blood loss among this cohort of patients. The established patterns require further research to develop tactics for managing this category of patients in order to improve the effectiveness of two-stage reimplantation.

Ethics approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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References


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Authors’ contributions:

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S. A. Bozhkova – research conception and design, text preparation and editing, data interpretation.

A. N. Panteleev – data collection and interpretation, text preparation.

R. M. Tikhilov – the substantial contribution to the research conception and design, text editing.

A. V. Kazemirsky – research conception and design, text editing.

All authors made a significant contribution to the research and preparation of the article and read and approved the final version before its publication. They agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests: the authors declare no conflict of interest.