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Case Report | General Adult Cardiac

Composite reverse shoulder arthroplasty for metastatic disease with 5-year follow-up

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Abstract

We present a 67-old patient with pathologic fracture of the proximal humerus 5 monts after nephrectomy for the treatment of renal cell carcinoma. The patient was treated by resection of the proximal humerus and composite reverse shoulder artroplasty with humeral allograft. Satisfactory range of motion and good functional result (Constant score 74, 8 points less than the contralateral shoulder, DASH score 10) were achieved. In the case-report we present the surgical technique, possible complications and postoperative rehabilitation protocol, as well as literature review, comparing the different options for the treatment of large metaststic and primary bone tumors of the proximal humerus.

Keywords

RSA, composite aloplasty, allograft, metastaic disease, renal-cell carcinoma

Introduction

Extensive bone defects resulting from trauma, tumors, or prosthetic loosening with osteolysis present a significant challenge in orthopedic surgery. The modern treatment is aimed at functional recovery of the extremity. A variety of treatment options are available, including tumor endoprostheses, custom-made endoprostheses, anatomic hemiarthroplasty, frozen osteoarticular allograft ("hemi-joint"-type) from cadaveric donor – both with and without soft-tissue attachments, iliac crest bone grafting, arthrodesis, allograft composite arthroplasty, etc. [1–5]

The allograft composite arthroplasty was introduced as a reconstructive procedure for the surgery of tumors of the proximal femur and pelvis. The earliest well-documented publications are from the 70s and 80s. The prerequisites for the emergence of this procedure were the progress in oncology on one hand, and in endoprosthetic orthopedic surgery on the other. The resection of the proximal femur or periacetabular region due to tumor invasion requires the reconstruction with large allograft in combination with arthroplasty [6, 7]. Bone cement is usually used for the fixation of the endoprosthesis. The advantages of this procedure include better primary stability and lower risk for dislocation due to the feasibility of muscle repair, the restitution of favorable biologic environment, superior long-term survival of the endoprosthesis, and cost-efficiency [8]. Ideally, full incorporation of the allograft with revascularization can be achieved. This method is well recognized and with good outcomes, especially in composite arthroplasty of the proximal femur. Mean 10-year survival rate of 80% has been reported in the literature [7, 8]. But, despite the good results, a high rate of complications such as deep infection, cancer recurrence requiring removal of the composite, and amputation have been reported.

In the last couple of decades, the reconstructive surgery of the upper extremity developed significantly [9]. Reverse shoulder arthroplasty became a common procedure [9–11]. It restores joint stability and function while only partially depending on a preserved function of the rotator cuff. Due to this fact, in the last twenty years reverse arthroplasty

in elderly patients has practically taken over anatomical shoulder arthroplasty [12]. Allograft composite with reverse shoulder arthroplasty in upper extremity reconstruction after resection of bone tumors has been favorized in a number of recent publications [4, 13, 14]. The composite allows for restoration of the length of the humerus, which in return preserves the tensile-compression forces of the deltoid muscle and ensures joint stability. This effect is known as "wrapping technique" [10]. It also allows for repair of the rotator cuff, deltoid and pectoral muscles, and secure fit of the prosthetic stem.

The etiology of the tumors is variable [11]. The proximal humerus is a target for a wide range of primary and metastatic tumors, most commonly chondro- and osteosarcoma, lymphoma and Ewing sarcoma [15, 16]. Bone metastases commonly result from cancer of the lung, breast, prostate, kidney, rectum, etc [16, 17].

This case-report presents the treatment of a single metastatic lesion, located in the right proximal humerus, originating from renal cell carcinoma. The chosen surgical method involved the use of composite allograft arthroplasty in combination with reverse shoulder arthroplasty. Our aim is to present the preoperative planning, the surgical technique, the follow-up protocol, the rehabilitation and the functional results. To our best knowledge, results from the use of the method for this type of pathologic conditions have never been published in Bulgarian journals.

Patient history

A 67-year-old patient had nephrectomy on 27.09.2018 for the treatment of renal cell carcinoma. A targeted oral therapy was performed. A couple of months after the procedure, the patient complained of pain in the right shoulder, and a localized malignant formation in the shoulder region was diagnosed. The metastasis affected the dominant extremity of the patient. In January 2019 thin-needle biopsy was performed at a different hospital. The histology showed metastatic renal cell carcinoma. A month later the patient felt sudden pain during routine movement of the shoulder, with loss of shoulder function. On 11.02.2019 he was admitted to our clinic with pathologic fracture of the proximal humerus. The neurologic and vascular function of the extremity remained intact. The axillary nerve showed no motor function impairment.

Diagnostic imaging

The right shoulder X-ray showed multifragmentary fracture of the proximal third of the humerus, osteolytic zone, bone deformity and peri-osseous and metaphyseal expansion of the formation (Fig. 1a). The computed tomography (Fig. 1b) showed an osteolytic tumor formation, engaging the humeral head, metaphysis and the upper third of the diaphysis. The long diameter of the formation was 8 cm.

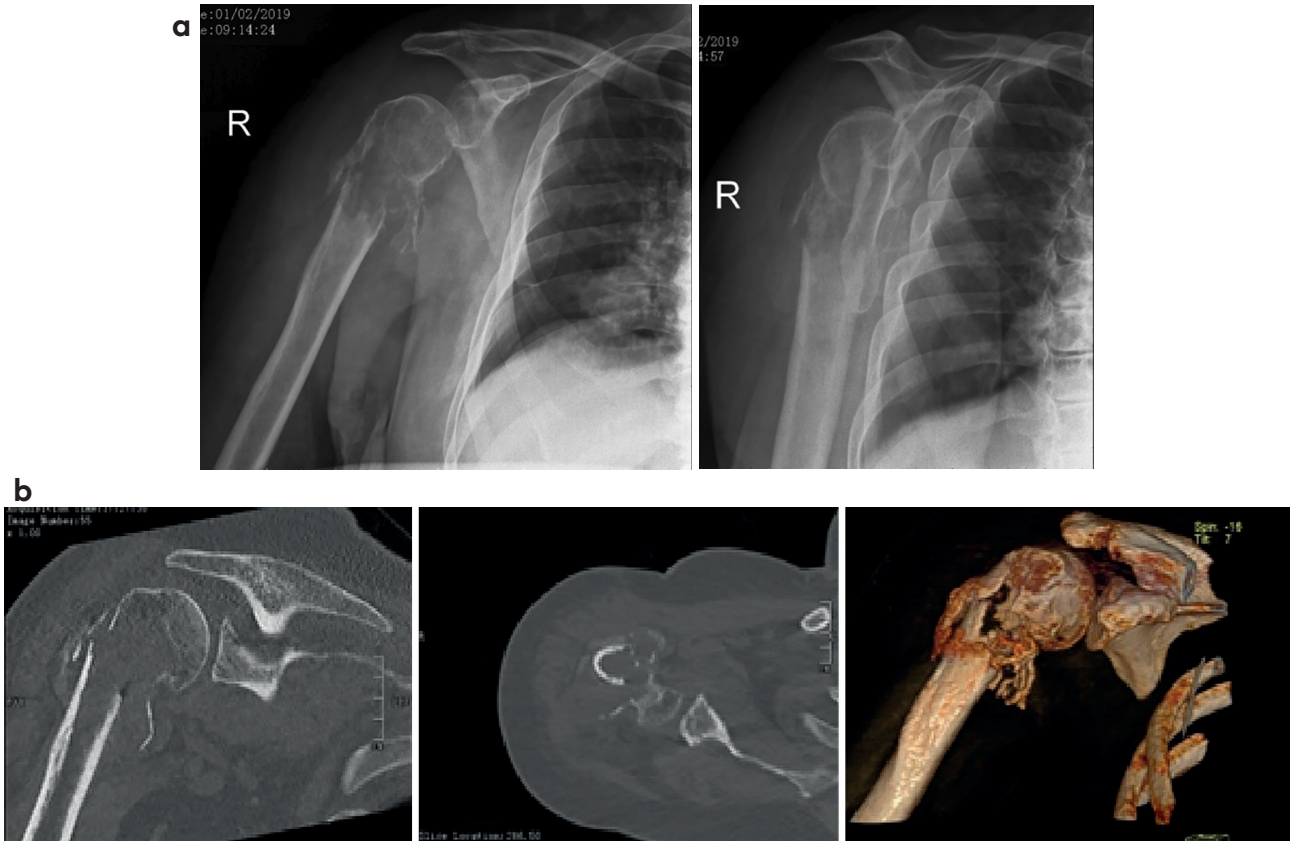

Figure 1. a) X-ray – multifragmentary fracture of the proximal third of the humerus, bone deformity and peri-osseous and metaphyseal expansion of the formation. **b)** CT – osteolytic tumor formation, engaging the humeral head, metaphysis and the upper third of the diaphysis. Formation size – 8 cm.

Preoperative planning and therapeutic embolization

In order to decrease the risk for tumor spread and intraoperative bleeding, an angiography was performed. The upper right angiography showed a big caliber a. circumflexa humeri posterior, providing blood supply for a large capillary bed. Coil-embolization of a. circumflexa humeri ant. et posterior was performed with complete isolation of the pathologic blood vessels (Fig. 2).

A frozen proximal humerus allograft with an appropriate length and diameter was prepared (the length was 2 cm greater than the defect and a bigger diameter than the patient's humerus).

Surgical technique

The patient was positioned in a standard beach chair position. Skin preparation and draping were in accordance with the current guidelines. Deltopectoral approach, incorporating the approach for the previous biopsy, was used for exposure of the right proximal humerus. The bone and adjacent soft tissues had visible signs of pathologic changes. The next step was resection and removal of the proximal humerus with a healthy tissue margin. The pathologic tissue was sent for histologic examination. The resection was type S3S4a according to the Musculoskeletal Tumor Society (MSTS) classification (Fig. 3).

Partial excision of the rotator cuff and m. pectoralis major was performed. The insertion of the deltoid muscle was preserved, as it showed no signs of tumor invasion. A meticulous debridement of the soft tissues, followed by lavage with antiseptic solutions was performed.

The glenoid fossa was prepared using automatic reamers. A press-fit metaglene DELTA XTEND was introduced. The metaglene was secured by three screws (diameter 4,5 mm, lengths 24, 42 and 42 mm). A standard glenosphere DELTA XTEND with a 42 mm diameter was inserted. The positioning of the glenosphere was distal to the equator with an inclination in slight varus (>90°) , whilst covering the lower border of the glenoid in order to prevent notching and adduction impingement.

A frozen proximal humerus allograft with a length of 10 cm was prepared. The allograft was shaped to match the size of the resected area and was reamed manually. Twenty grams of gentamicin-loaded PMMA was introduced intramedullary in the graft. Next the humeral component of the endoprosthesis, consisting of a monoblock humeral stem (size 2, 12 mm diameter, humeral cup 42 mm / + 9 mm) was inserted in the graft. Ethibond 5 sutures were introduced through bone tunnels in the greater and lesser tubercles (Fig. 4).

In the next step the medullary canal of the humerus was gradually prepared using manual reamers until a size 12 reamer could be fitted. Thorough lavage was performed. Cement restrictor was introduced in the medullary canal. Forty grams of gentamicin-loaded PMMA were introduced using a bone cement gun. The next step was the introduction of the composite endoprosthesis. The diaphysis of the humerus was fixed to the allograft using an 11-hole 1/3-tubular steel plate, while simultaneously applying the cement. The distal screws were placed in a monocortical fashion. Proximally the plate was shaped like a blade and buried in the proximal part of the allograft. Thanks to the compression applied, the cement was prevented from getting trapped between the diaphysis and the allograft, and stable fixation, neutralizing the rotational forces, acting on the allograft, was achieved. The joint was reduced. The rotator cuff and m. pectoralis major were reinserted. The range of motion and stability of the joint were tested intraoperatively. Thorough lavage with antiseptic solution followed. Number 12 Redon drain was placed subfascially. Closure with Vicryl 1 sutures. Dry sterile dressing was applied. Radiographic imaging. The extremity was immobilized in an abduction orthosis in 30°.

Postoperative care

Postoperatively the extremity was immobilized for 6 weeks. The rehabilitation of the shoulder joint began immediately after removal of the abduction orthosis. The patient began

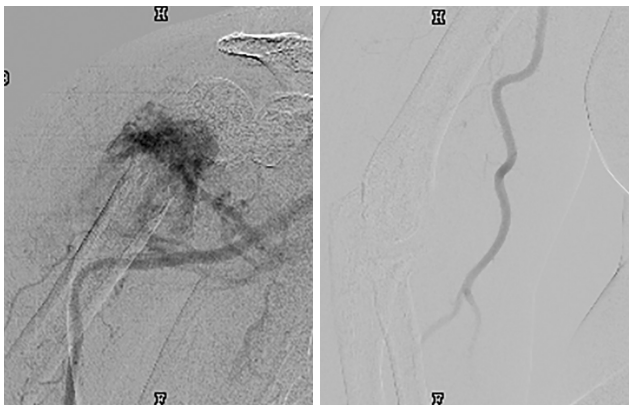

Figure 2. The upper right angiography showed a big caliber a. circumflexa humeri posterior, providing blood supply for a large capillary bed. Coil-embolization of a. circumflexa humeri ant. et post. was performed.

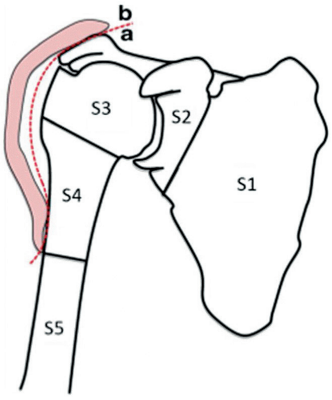

Figure 3. Musculoskeletal Tumor Society (MSTS) classification. a) With preservation of the abductor muscles b) Without preservation of the abductor muscles.

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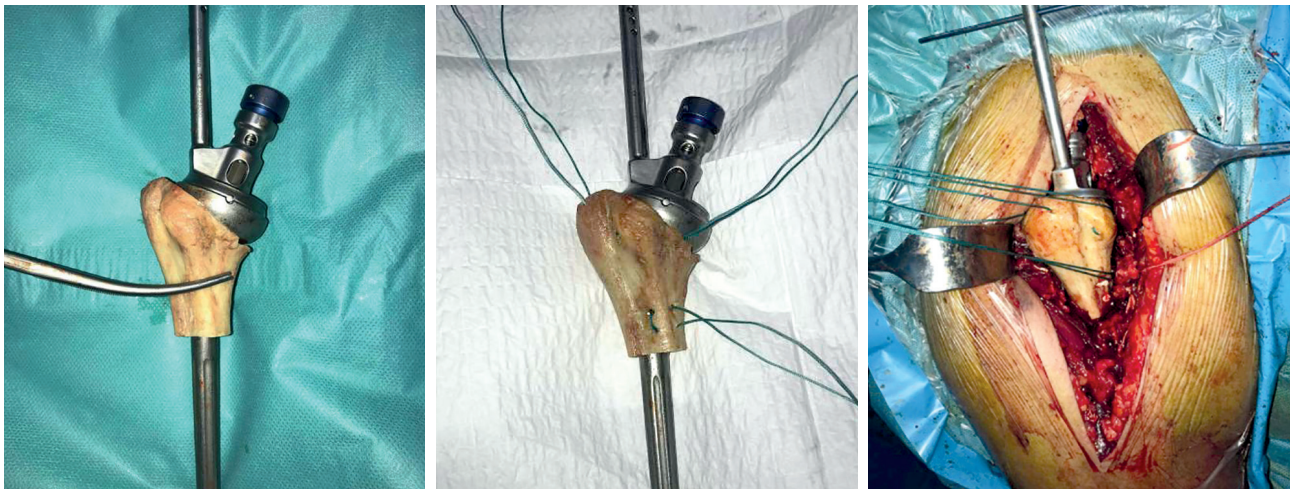

Figure 4. Shaping the allograft. The allograft was fixated to the prosthesis using bone cement. Bone tunnels and Ethibond 5 suture insertion through the tubercles.

active movement against resistance 6 months after the surgery. Follow-up examinations were routinely conducted. The assessments were performed on the 3rd and 6th month and 1 year postoperatively. An additional examination was done every year after. The patient was followed until the 5th year after the surgery.

Functional results

The DASH and Constant Shoulder Scores were used to evaluate the functional results. The scoring evaluation was performed on the second postoperative year. The DASH score was 10 points, which classifies as a good result. The patient was unable to participate in contact sports, which put load on the upper extremity, or use heavy instruments like hammer or chisel. The Constant Shoulder Score for the healthy shoulder was 82 points, and for the operated shoulder – 74, i.e. the score for the operated shoulder was 90.2% of the score for the healthy one. This is a good functional result according to the CSS scoring system. The range of motion of the shoulder joint was measured with a goniometer. On the second year the ROM was 160° forward elevation, 155° abduction, full external rotation and internal rotation to L3. The patient reported no pain while active or during sleep (Fig. 5).

Radiographic follow-up

The osteointegration of the allograft, allograft resorption and periimplant osteolysis according to the Levigne's [22] modification of Sperling's [21] classification were assessed on AP X-ray in internal and external rotation. On the third month after the procedure, we noticed allograft resorption in Levigne zones 1 and 7. Osteointegration of the allograft according to Vander Griend's criteria [23] was found on the 7th postoperative month (Fig. 6 a, b).

On the 29th month we noticed resorption of the allograft in Levigne zone 2. There were no signs of resorption or periimplant osteolysis in the remaining zones. 56 months after the procedure the prosthesis was well

Figure 5. At 2 years the patient had 160° forward elevation, 155° abduction, full external rotation and internal rotation to L3. The patient reported no pain while active or during sleep.

incorporated, however tumor recurrence in zones 5 and 6 were found (Fig. 7a, b). Three months later the patient died due to progression of his condition.

Ultrasonography

Two years after the surgery, shear-wave elastography of both deltoid muscles was performed. The acromial and spinate segments of the deltoid muscle on the operated side showed faster conduction of the ultrasound wave, which could be associated with fatty degeneration, but the muscle showed good function.

Discussion

The aims of the orthopedic treatment of tumor diseases of the humeroscapular joint have changed drastically in recent years. This is primarily due to advances in the adjuvant treatment of oncological diseases. Operative interventions such as amputation, arthrodesis and interscapulothoracic resection after Tikoff-Linberg became rarities [5, 17]. At present we aim at reconstructive surgeries, preserving the shoulder joint function. The allograft-prosthetic composite (APC) alloplasty is one of these options. The allograft enables the preservation of the length of the limb, biologic restoration of the soft-tissue envelope through muscle reinsertion, and increases the stability of the endoprosthesis [13]. This approach gives improved functional results [5, 10, 13, 14, 24, 25].

On the other side, the use of reverse shoulder arthroplasty (RSA) without an allograft in cases with severe bone deficiencies has shown high complication rate. Due to the lack of the greater tubercle, the main fixation is to the stem, which in turn leads to significant rotational stress, resulting in loosening or disassembly of the modular implants [10, 26, 27]. Still, the resorption of the allograft, the high infection risk, instability, tumor recurrence and the long-term survival are significant challenges [26, 28].

The allograft-prosthetic composite with hemiarthroplasty or anatomic arthroplasty are considered as less

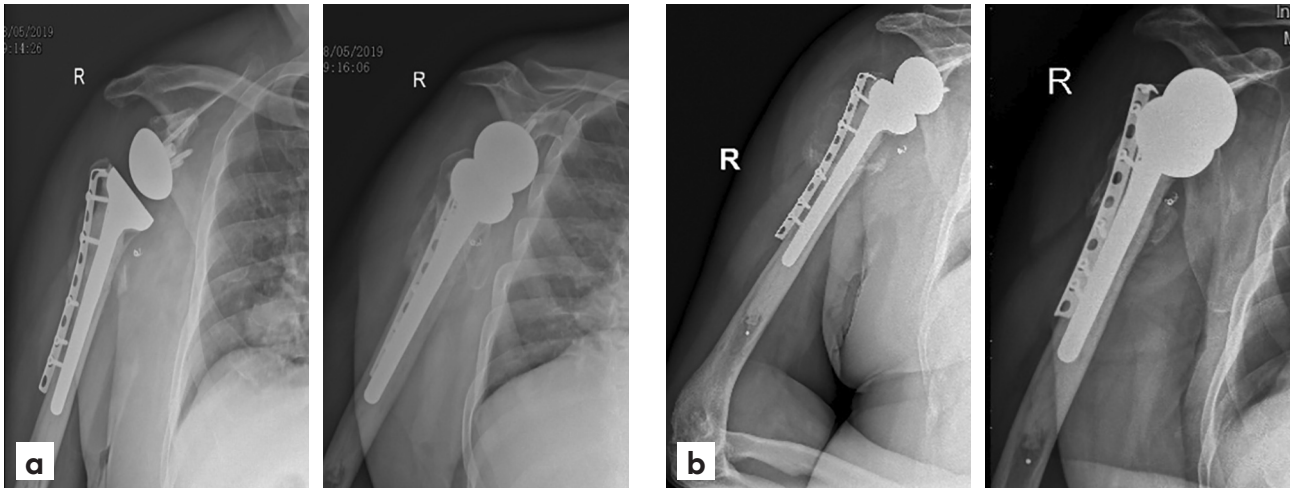

Figure 6. a) On the third month after the procedure, we noticed allograft resorption in Levigne zones 1 and 7. **b)** allograft osteointegration to the diaphysis was found on the 7th postoperative month.

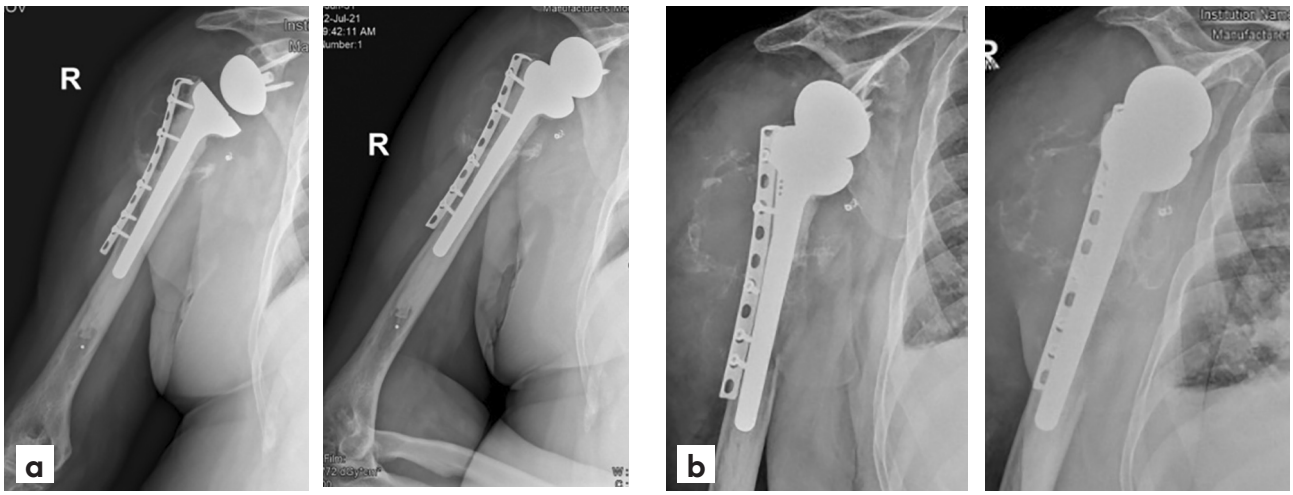

Figure 7. a) Resorption of the allograft in Levigne zone 2, 29 months after the operation. **b)** Local tumor recurrence in Levigne zones 5 and 6, 56 months after the surgery.

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reliable options for the treatment of tumors of the proximal humerus [29].

The main problem is the loss of the soft-tissue stabilizers of the joint, which in most cases must be resected. This causes instability and inferior functional results [14, 16]. Poorer functional results have been reported even in cases, when reattachment of the rotator cuff to the allograft (or to the tendinous insertions of the allograft) was possible [4, 5].

This method is viable for well-selected group of younger patients [5]. Another option is reconstruction with a tumor megaprosthesis [17]. The main advantage of this procedure is that it enables a radical resection of the tumor. Besides this, there are no other benefits in comparison to APC with anatomic endoprosthesis. The principal problem is the non-biologic repair of important soft-tissue stabilizers of the shoulder joint. Over time, the instability and proximal migration compromise the functional results [5].

Generally speaking, the unpredictable functional outcomes of the anatomic shoulder arthroplasty has led to the popularization of the reverse shoulder arthroplasty, which relies only on the intact deltoid muscle. RSA is established as the principal option for functional restoration of the shoulder in patients with advanced arthritis, compromised rotator cuff, including due to fracture-dislocations, and as revision for failed hemiarthroplasty [10, 12, 30–33]. A variety of authors prefer APC-RSA in tumor invasions of the proximal humerus and revision arthroplasty situations where the bone defect exceeds 5 cm [5, 13–15].

The first and most important condition for planning RSA is functioning axillary nerve. Functional impairment of the nerve is the principal contraindication for RSA [11].

In preparation for the procedure a wide range of diagnostics is mandatory, including X-rays, CT and MRI scans; additionally, angiography with embolization are needed in tumor or metastatic lesions with good blood supply. Embolization reduces the risk for tumor dissemination and bleeding. CT- and MRI scans give a detailed picture of the tumor infiltration and sizing of the bone. An important aspect is choosing an appropriately sized allograft, at least 2–3 cm longer than the affected segment and with 2-3 mm greater diameter than the patient's proximal humerus [5]. This allows for good fit of the allograft to the humerus and stable fixation of the endoprosthesis. The frozen allograft we used had a length of 10 cm. The planned length of the distal end of the stem, fixed to the patient's humerus, should be more than twice the diameter of the diaphysis. In our case the part of the stem, lodged in the diaphysis, has length of 11cm. The operative technique has a couple of important stages [33]. First and foremost – an adequate and sufficient resection of the tumor mass according to established oncosurgical criteria. The first step is the incorporation of the previous biopsy approach in the resected tissue. Resection into healthy tissue was assessed macroscopically. A partial resection of the rotator cuff and m. pectoralis major was performed. The insertion of the deltoid muscle was preserved, as it showed no signs of tumor invasion. This is an important prerequisite for the postoperative rehabilitation. After the preparation of the allograft, we used Ethibond 5 sutures to attach the important soft tissue structures to it. Besides reinserting the soft tissue joint stabilizers, we also reattached the pectoralis muscle, despite fear of loss of range of motion (as described by some authors) [28]. The joint capsule was removed completely. The tendon of the long head of the biceps was resected and sutured to the aponeurosis of the short head. We used a conventional technique to fix the metaglene. The prosthesis was inserted in 10° inclination and was locked in subequatorial position with 4 screws, whilst caring for minimal bone removal from the glenoid. In accordance with established surgical criteria, we cemented the allograft in 30° internal rotation. The follow-up was clinical and radiographic. The limb was immobilized in an orthosis in 30° abduction for 6 weeks. We allowed for active loading of the extremity 6 months after the surgery in compliance with the expected incorporation of the allograft.

The osteointegration of the allograft to the humerus is a topic of controversy. In a publication by Sanchez-Sotello [32] regarding revision arthroplasty, bone consolidation is reported on average 7 months after the procedure. A similar time frame was reported in the treatment of oncological diseases [13]. In other cases, the most common complication was the lack of osteointegration of the allograft [26, 34]. Of pivotal role are the techniques, employed for securing the allograft to the diaphysis [14]. In our case we found osteointegration 7 months after the surgery. Viable options for securing the allograft to the diaphysis include chevron osteotomy, fixation with cerclage and/or conventional compression plate/ LCP-plate [5, 31, 32]. Good contact and compressive osteosynthesis eliminate the rotational forces on the allograft, which allows for primary consolidation and limits the risk of entrapment of cement between the allograft and the patient's humerus. We approximated the two fragments through the use of a transverse osteotomy, giving good fit. As a next step we cemented the allograft to the proximal end of the endoprosthesis. The medullary canal of the host humerus was reamed, and the endoprosthesis was inserted after gentamicin-loaded PMMA was introduced in the distal segment. The diaphysis of the humerus was fixed to the allograft using an 11 hole, 3,5 mm 1/3 tubular steel plate while simultaneously applying the cement. Conventional plating allows for free choice of the direction of the screws. Just as other authors do, we consider this a predictable technique.

Reports regarding the rate and location of resorption of the allograft are ambiguous. In their series of 11 patients, in 64% of the cases Gallamand [13] et al. observed osteolysis in the epi/metaphyseal segment of the allograft (Levigne zones 1–7, 7 cases). Signs of osteolysis were found on average 10 months after the procedure. They also found osteolysis in zone 2 in 80% of cases. According to the authors, this is due to the implementation of the „Episcopo" muscle transfer technique (m. teres major and m. latissimus dorsi), which increases the range of motion. Regardless of the high percentage of osteolysis, loosening of the endoprosthesis was not recorded. In another study by Boileau et al. [10], bone resorption in Levigne zones 1-7 was reported in 2/3

of the cases. The authors argue that this is due to shear forces, produced by reattaching the muscle tissue with non-absorbable sutures. Osteointegration of the allograft was observed in 3/4 of the cases. Bonnevialle et al. [28] think that although APC-RSA restores the bone defect, length and stability of the joint, this method is viable only in the short term. They maintain that in the long term, severe resorption of the allograft will take place, leaving the humeral stem to "hover" beneath the cement mantle.

We discovered minimal resorption rate in Levigne zones 1 and 7 three months after the intervention. 29 months postoperatively we discovered resorption in Levigne zone 2 as well. Nevertheless, the endoprosthesis didn't loosen until the patient's death.

Our patient's functional outcome is comparable to the ones reported in the literature. On the second year the DASH and Constant Shoulder Scores showed good result. During the whole follow-up (until the recurrence was found), the patient evaluated his shoulder function and quality of life as good. Then range of motion that was achieved was 160° of forward elevation, 155° of abduction, full external rotation and internal rotation to L3. The patient had no pain during activity or sleeping.

The structure and function of the deltoid muscle were preserved, and this was verified by shear wave ultrasound elastography [35, 36]. Partial fatty degeneration of the upper part of the deltoid muscle was found (Fig. 8a, b) These good results are partly attributed to the fact that we were able to preserve the deltoid muscle insertion, as it was spared by tumor infiltration. Gallamand et al's series [13] of 11 patients with APC with an allograft with a mean length of 10 cm and a mean follow-up of 30 months, reported mean Constant Shoulder Score of 49 points (24–75), and the CSS compared to the healthy limb was 59%. The mean elevation reported was 105°, external rotation with the elbow fixed to the torso of 23°, external rotation in abducted shoulder of 21°, and internal rotation of 4 from a maximum of 10 points. In 5 of the cases a muscle transfer to augment the muscle function was performed. Another study on 14 patients with bone tumors treated with APC-RSA observed mean active abduction of 157°. The deltoid muscle was detached in only 2 cases [24, 25].

Bonnevialle et al. [28] reported on 10 cases with tumors of the proximal humerus, 2 of which were treated by APC-RSA, the rest with cemented RSA. The functional outcomes were as follows: a mean elevation of 122°, external rotation of -2°, internal rotation up to L4 and a comparative Constant Shoulder Score of 61%. Streitbuerger A et al. [11] reported a series of 18 patients with tumors of the proximal humerus, treated by reversed shoulder tumor prosthesis. Their results are influenced by the inevitable in some cases resection of the axillary nerve, the large tumor resections of a mean of 15.1 cm, and the removal of the insertion of the deltoid muscle. The results achieved were a mean abduction of 80° (40–180°), active forward elevation of 84° (30–160), internal rotation of 35° and external rotation of 45°. The results were significantly worse in 4 of the cases, in which preservation of the axillary nerve was not possible. The authors conclude that tumor RSA delivers promising results, however in cases when the axillary nerve could not be preserved, RSA has no advantages. Other studies of 8 patients treated with a RSA tumor prosthesis with a 3 year follow-up reported active abduction of 62°, forward elevation of 71°, external and internal rotation of 50° [3].

Regardless of the good results after APC-RSA, there are literature reports of instability, although with a significantly lower rate compared to anatomic composite arthroplasty – of Gallamand's 11 cases, treated by APC-RSA, there was only one case of early dislocation. The insertion of the deltoid was removed, and the bone defect after the resection of the tumor (chondrosarcoma) was 17 cm long. The solution of the authors was to revise the implant, lengthen the humerus with a 9 mm spacer, and insert a 9 mm bigger inlay [13]. In another series of 6 patients treated by APC-RSA, there was one case of a secondary dislocation [15].

Bonnevialle et al. [28] described 3 instances of dislocation in a series of 10 patients (6 with primary tumors, 4 with bone metastasis), 8 of which were treated by RSA only and 2 – by APC-RSA. One of them – with anterior dislocation, was treated by open reduction without component revision.

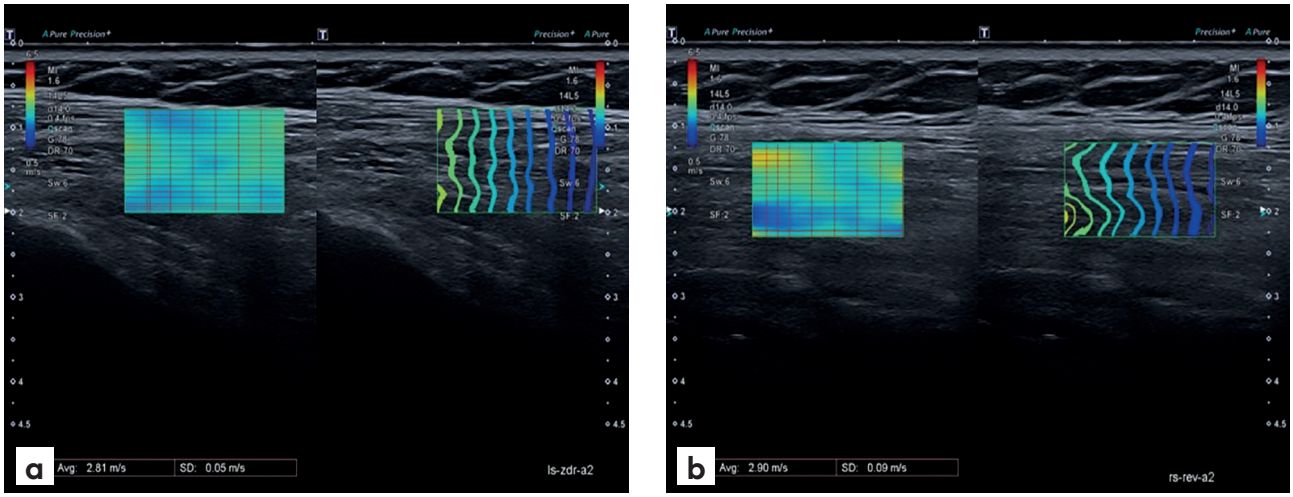

Figure 8. Ultrasonography a) Segment a [2], non-operated shoulder b) Segment a [2], operated shoulder.

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Another – with inferior dislocation, was successfully treated with a brace. In a third case – also with anterior dislocation, implant revision was performed, but it was unsuccessful. In two of the patients the deltoid insertion was resected.

The evaluation of the survival rate of patients with primary tumors and metastases in the proximal humerus based on literature data is difficult. On one hand the publications are relatively scarce, on the other a wide variety of tumor diseases are included in such studies. Additionally, a range of factors influence the survival rate. The modern complex treatment including adjuvant therapy and reconstructive surgery delivers promising, but still unsatisfactory results. The main objectives remains the preservation of the patient's life, achieving permanent cure and the elimination of the risk for local recurrence [5]. The main principles of the oncological surgery – radical resection and removal of the lesions, are based on the aforementioned objectives [37]. In cases where this is not possible, an alternative is to perform intralesional resection in order to prolong the patient's life. Even after total humeral resection and replacement with tumor endoprosthesis, the result are comparable to the ones after APC-RSA or APC with HA [5]. The long-term prognosis is worse in metastatic tumors of the proximal humerus combined with a pathologic fracture [38].

In a series by Asavamongkolkul et al. of 59 patients with different oncological diseases [16], 6 had metastatic carcinoma and were treated by intralesional resection and arthroplasty. All received palliative radiotherapy. Three of these patients had pathologic fractures of the humerus. In this treatment group there were no local tumor recurrences recorded. In the same series, of the total of 59 patients, 6 had local recurrence and 4 of them had pathologic fractures at the beginning of treatment. The type of tumors were 3 cases of chondrosarcoma, 2 of osteosarcoma and 1 of histiocytoma. All 10 patients with pathologic fractures were in advanced stages. Five died shortly after the surgery from cancer progression and one was still alive at the time of publication, but with lungs metastases. In a large series of 20 patients treated with total resection of the humerus and tumor prosthesis, 6 had metastatic renal cell carcinoma. Local recurrence was observed in one patient, and all patients received adjuvant chemotherapy. The mean follow-up was 42.9 months (1–172). Nine patients died and 11 were still alive. The reported 10-year survival rate was 70.1%, and the implant survival rate was 65.3%.

The survival rate in our case was 5 years. The patient didn't receive adjuvant chemotherapy after the APC-RSA. Chemotherapy was started 2 months after local tumor recurrence was diagnosed (58 weeks after the surgery) and was continued for a month.

Conclusion

APC combined with RSA in patients with primary and metastatic tumors of the proximal humerus is a feasible therapeutic method, comparable to the conventional allograft prosthetic composite and tumor megaprosthesis in terms of survival rate, but with better prognosis regarding the functional recovery of the shoulder joint.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

Informed consent from the humans, donors or donors' representatives: "N. I. Pirogov" Emergency Hospital, Sofia, Bulgaria

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

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Author contributions

Writing – original draft: OM. Writing – review and editing: BT.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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