*Short Research Article*

**The impact of sarcopenia on morbi-mortality in patients who underwent radical cystectomy for bladder tumor.**

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ABSTRACT

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| --- |
| Sarcopenia is a physiological alteration defined as a loss of skeletal muscle mass as a result of fragility due to aging or neoplastic pathology.**Aims: T**he aim of this work was to evaluate whether sarcopenia was predictive of morbidity and mortality in patients undergoing radical cystectomy for bladder tumor.**Study design:** Monocentric prospective study**Place and Duration of Study:** Department of Urology and Department of Radiology, at the Ibn Rochd University Hospital, between January 2021 and June 2022.**Methodology:** A single-center prospective study was conducted, including patients treated by radical cystectomy between January 2021 and June 2022. Sarcopenia was retained according to Martin's definition, which takes into account striated muscle area on a CT cross-section passing through the 3rd lumbar vertebra. Short- and long-term post-operative follow-up was performed, and complications were classified according to the Clavien Dindo classification adapted to radical cystectomy.**Results:** Of the 85 patients listed in our study, 74 were selected for inclusion. The average age of our patients was 68.70 years. Sarcopenic patients accounted for 48.6%. No association was observed between sarcopenia and the occurrence of complications in the immediate and 1-month operative follow-up. However, a strong association was noted at the 3-month and 1-year follow-up. Furthermore, sarcopenia was correlated with mortality in our sample (p<0.05).**Conclusion:** Sarcopenia was identified as an independent preoperative prognostic factor for morbidity and mortality. |

*Keywords: Radical cystectomy, sarcopenia, bladder cancer, prognosis, mortality.*

1. INTRODUCTION

For localized bladder tumors (BT), radical cystectomy (RC) remains the treatment of choice (can be specific that muscle invasive and high risk non muscle invasive). However, the morbidity and mortality associated with this procedure remain high, with a complication rate ranging from 40% to 60%, and a mortality rate of 9% within the first three months (1,2). For best post-operative results, several parameters will influence the choice of cystectomy, such as the patient's age, performance status (PS score), general condition and, of course, tumor status (3,4). Recently, sarcopenia has been reported as a new predictor of prognosis and risk of postoperative complications (1,5). A correlation between sarcopenia and surgical outcome has been reported for malignant melanoma, breast cancer and hepatocellular carcinoma. In patients with BT, several studies have suggested that sarcopenia correlates with a worse prognosis than in non-sarcopenic patients (1,5-7).

Sarcopenia is a physiological alteration underlying frailty that can occur as a result of aging or malignant disease (8), it is defined as severe loss of skeletal muscle mass and has received considerable attention as a new objective preoperative prognostic factor; it is associated with significantly poorer survival in patients with various types of cancer (9-12). Classification is based on the skeletal muscle index measured by computed tomography (CT), which is widely available and accurate.

The aim of this work was to assess whether sarcopenia was predictive of morbidity and mortality during the therapeutic management of patients with localized BT (who undergo cystectomy) and whether other factors were associated with sarcopenia.

Recommendation to authors: Introduction could be bulked by adding on robotic / open cystectomy rather than sticking to cystectomy in general

2. material and methods

**Patients:**

We prospectively included patients treated with RC between January 2021 and June 2022 at the Ibn Rochd university hospital center in Casablanca, for the management of a urothelial or non-urothelial bladder tumor requiring RC. The diagnosis of bladder tumor was confirmed by anatomopathological (kindly check if anatamo is needed ; rather pathological diagnosis is a better term) examination after endoscopic resection. Patients whose CT scan was unavailable or whose bladder was unremovable intraoperatively were excluded from the study.

**The clinical scores and parameters used are:**

* The American Society of Anesthesiologists (ASA) score
* Charlson comorbidity index (CCI)
* Performance Status (PS) scale
* Mini Nutritional Assessment (MNA-SF) scale
* Body Mass Index (BMI)

In addition, other variables were collected from patients, including their profession, the presence or absence of comorbidity, the number of resections and their anatomopathological study (kindly check if anatamo is needed ; rather pathological diagnosis is a better term) , the notion of endovesical instillation and the occurrence of acute obstructive renal failure (AORF) prior to RC. In addition to the parameters required to establish the above-mentioned scores, the clinical examination collected abdominal circumference, the presence of lower- extremity oedema and pelvic touch findings.

**Paraclinical data:**

A basic biological workup was performed prior to RC to assess and collect hemoglobin levels, as well as albuminemia (albumin levels), calcemia (serum calcium) and renal function. Histopathological data were collected after RC from the surgical specimens to assess grade, pTN stage, the presence of vascular emboli, in situ carcinoma (ISC) and the quality of surgical excision. (Seems the authors have used AI here and the conventional terms like CIS, Serum calcium are not seen here. Strongly suggest the authors to rephrase this)

**Measurement of scannographic indicators:**

Scannographic mass indicators were assessed by morphometric measurements from axial sections taken from pre-operative scans performed within 3 months prior to RC. A scan section passing through the 3rd lumbar vertebra when the 2 transverse processes are visible was used to record :

* Skeletal mass index (SMI): represents the area of the 2 psoas and other striated muscles visible on the cross-section, notably the rectus abdominis, transversus abdominis, internal and external obliques, squared lumbar and spinal erector muscles. A radiologist with no knowledge of the treatment performed calculated the area of these muscles, which was then divided by the height squared to give the SMI (cm²/m²).
* Total Psoas Area (TPA): Same as SMI, but takes only the area of the 2 psoas passing through the same CT section, and is not divided by height squared (cm²).
* Psoas Muscle Index: TPA divided by height squared (cm²/m²).
* Total adipose area: on the same scan section passing through L3, we calculate the surface area of all adipose tissue present in the viscera, muscles and subcutaneous tissues. Their density was between -190 HU and -30 HU (cm²).

**Definition of sarcopenia:**

Martin's definition (13) of sarcopenia takes into account SMI as well as gender and body mass index was used. The cut-off points defining sarcopenia were as follows:

- SMI < 43 cm2/m2 in men with BMI < 25 kg/m2 ;

- SMI < 53 cm2/m2 in men with BMI ≥ 25 kg/m2 ;

- SMI < 41 cm2/m2 in women regardless of BMI.

**Modalities of cystectomy and follow-up:**

All RCs were performed at the same hospital. The decision to perform a RC was taken in a multidisciplinary concertation meeting. Cysto-prostatectomy was performed in men and anterior pelvectomy (anterior pelvic exenteration) in women. Bilateral pelvic curage was systematically performed. A bilateral cutaneous ureterostomy or transileal ureterostomy (Bricker) was performed, depending on the patient's wishes and the surgical feasibility. Immediate postoperative follow-up was based on the RC-specific Clavien Dindo score, and basic data such as transit recovery, 1st rise and number of days in hospital were also collected. Patients were reevaluated at 1 month, 3 months and 1 year. Functional status was scored according to the Instrumental Activities of Daily Living (IADL) score, which assesses patient’s degree of autonomy. Patients were classified into 3 categories: 0-4: dependent; 5-10: partially dependent; 11-14: independent.

**Statistical analysis:**

Statistical analyses were performed using R software version 4.3.3. Quantitative variables were expressed as mean or median, depending on the distribution of the data. A Chi2 test was used to compare qualitative variables. A p < 0.05 was considered significant. Univariate analysis was performed using logistic regression.

3. results :

**Patient characteristics:**

Eighty-five patients were enrolled in our study, after exclusion of 11 patients, 4 of whom had an unremovable tumor discovered intraoperatively and 7 of whom had a CT scan unavailable and therefore impossible to interpret. The median age was 66.5 years, with a mean of 64.7 and a standard deviation of 7.86. 48.6% of patients were sarcopenic. Males predominated in our study, accounting for 89.2% of patients (advisable to mention n=?), and 82.4% were smokers. The clinico-pathological characteristics of the patients are summarized in Table 1.

Biologically, sarcopenic patients had mean (+/- standard deviation) haemoglobin, albumin and blood calcium levels of 12.19 (+/-1.53) g/dL, 35.48 (+/- 4.98) g/L and 84.11 (+/- 7.34) mg/L respectively. Whereas non-sarcopenic patients had mean (+/- standard deviation) hemoglobin, albumin and blood calcium levels of 11.55 (+/- 1.64) g/dL, 35.58 (+/- 4.77) g/L and 86.79 (+/- 4.81) mg/L respectively. The p-values of the 3 variables studied were 0.089, 0.931 and 0.07 respectively, so there was no apparent correlation with sarcopenia.

What percentage of study population were sarcopenic? Its an important factor worth mentioning here though its on the table.

**Histopathological and scannographic features:**

Measurement of the mean of scannographic indicators, in sarcopenic and non-sarcopenic patients respectively, had shown: a mean of TAA at 116.80 cm² and 210.44 cm² (p < 0.001), a mean of PMI at 5.74 cm²/m² and 6.47 cm²/m² (p = 0.07) and a mean of TPA at 16.17 cm² and 17.91cm² (p = 0.091). Histopathological features, including tumor grade, pathological T stage, lymph node metastases, presence of vascular emboli and presence of concomitant in situ carcinoma (ISC) are summarized in Table 2; there was no association between sarcopenia and tumor histological type.

**Mortality, immediate and long-term complications:**

There was no correlation between the immediate post-operative course and sarcopenia. In sarcopenic and non-sarcopenic patients, bowel transit recovery (p=0.887) was 3.69 days and 3.66 days respectively, and ICU stay (p=0.086) was 1.89 days and 1.29 days respectively. There was no association with sarcopenia in the immediate postoperative period or at 1 month (Table 3). However, a strong association was found between sarcopenia and the degree of autonomy according to the IADL score, and the occurrence of complications at 3 months and 1 year. The most frequent complications in our study were wall infections and deep vein thrombosis. Mortality in our cohort was 30.5% in sarcopenic patients and 5.3% in non-sarcopenic patients (p<0.05).

Its worth to mention the mean follow up as we are talking about mortality that’s huge in sarcopenic group around 30%. Also if the cause of death in this group be sub analyzed it would be valuable in make clinical decisions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Total population (n=74) | Sarcopenic patients (n=36)(48,6%) | Non sarcopenic patients (n=38)(51,4%) | Total (n=74) | P value |
| Mean age (standard deviation) | 66 (7,53) | 63,55 (8,07) | 68,70 (7,78)  | 0,183 |
| Gender* Male
* Female
 | 31 (86,1%)5 (13,9%) | 35 (92,1%)3 (7,9%) | 65 (89,2%)8 (10,8%) | 0,649 |
| BMI* <18,5
* 18,5-24,9
* 25,0-29,9
* >29,9
 | 5 (13,9%)13 (36,1%)17 (47,2%)1 (2,8%) | 2 (5,3%)31 (81,6%)2 (5,3%)3 (7,9%) | 7 (9,5%)44 (59,5%)19 (25,7%)4 (5,5%) | NR |
| PS score* 0
* 1
* 2
 | 16 (44,4%)17 (47,2%)3 (8,3%) | 13 (34,2%)21 (55,3%)4 (10,5%) | 29 (39,2%)38 (51,4%)8 (9,5%) | 0,663 |
| ASA score* 1-2
* 3-4
 | 36 (100%)0 (0%) | 36 (94,7%)2 (5,3%) | 72 (97,3%)2 (2,7%) | NR |
| CCI * < 3
* 3-6
* > 6
 | 0 (0%)29 (80,5%)7 (19,5%) | 2 (5,3%)32 (84,2%)4 (10,5%) | 2 (2,7%)61 (82,4%)11 (14,9%) | NR |
| MNA-SF score* 0-7
* 8-11
* 12-14
 | 6 (16,7%)19 (52,8%)11 (30,5%) | 2 (5,3%)23 (60,5%)13 (34,2%) | 8 (10,8%)42 (56,8%)24 (32,4%) | 0,274 |
| Notion of AORF | 6 (16,7%) | 13 (34,2%) | 19 (25,7%) | 0,84 |

**Table 1**: Comparative table of clinical data between sarcopenic and non-sarcopenic patients

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Total population (n=74) | Sarcopenic patients (n=36)(48,6%) | Non sarcopenic patients (n=38)(51,4%) | Total (n=74) | P value |
| Histopathologic grade* Low
* High
 | 1 (2,8%)35 (97,2%) | 6 (15,8%)32 (84,2%) | 7 (9,5%)67 (90,5%)  | 0,130 |
| Pathological T stage* ≤T1
* T2
* T3
* T4
 | 11 (30,6%)18 (50%)5 (13,9%)2 (5,6%) | 10 (26,4%)26 (68,4%)2 (5,3%)0 (0%) | 21 (28,4%)44 (59,5%)7 (9,5%)2 (2,7%) | NR |
| Lymph node metastasis * Positive
* Negative
 | 11 (30,6%)25 (69,4%) | 17 (44,7%)21 (55,3%) | 28 (37,8%)46 (62,2%) | 0,209 |
| Vascular emboli * Yes
* No
 | 22 (61,1%)14 (38,9%) | 21 (55,3%)17 (44,7%) | 43 (58,1%)31 (41,9%) | 0,610 |
| Concomitant ISC* Yes
* No
 | 8 (22,2%)28 (77,8%) | 6 (15,8%)32 (84,2%) | 14 (18,9%)60 (81,1%) | 0,480 |

**Table 2**: Comparative table of histopathological data between sarcopenic and non-sarcopenic patients

|  |  |  |  |
| --- | --- | --- | --- |
| Total population (n=74) | Sarcopenic patients (n=36)(48,6%) | Non sarcopenic patients (n=38)(51,4%) | P Value |
| Immediate post-operative follow-up: * Bowel transit recovery
* First rise
* Drain removal
* ICU stay
* Hospitalization days
* Albumin transfusion
* Red blood cell transfusion

Clavien Dindo : * 0
* 1
* 2
* 3a
* 3b
 | **3,69****4,44****10,75****1,89****11,89****13 (36,1%)****19 (52,8%)****6 (16,7%)****3 (8,3%)****22 (61,1%)****5 (13,9%)****0 (0%)** | **3,66****5,29****9,53****1,29****12,32****12 (31,6%)****14 (36,9%)****12 (31,6%)****4 (10,5%)****16 (42,1%)****4 (10,5%)****2 (5,3%)** | **0,887****0,440****0,396****0,086****0,771****0,910****NR****NR** |
| 1 month follow-up : IADL : * 11-14
* 0-4
* 5-10

RehospitalizationClavien Dindo * 0
* 1
* 2
* 3a
* 3b
* 5
 | **17 (47,2%)****9 (25%)****10 (27,8%)****6 (16,77%)****19 (52,8%)****2 (5,6%)****3 (8,3%)****4 (11,1%)****2 (5,6%)****6 (16,7%)** | **26 (68,4%)****7 (18,4%)****5 (13,2%)****4 (10,5%)****29 (76,3%)****3 (7,9%)** **2 (5,3%)****0 (0%)****4 (10,5%)****0 (0%)** | **0,153****0,666****NR** |
| 3 month follow-up : IADL : * 11-14
* 0-4
* 5-10

RehospitalizationAdjuvant chemotherapyClavien Dindo * 0
* 1
* 2
* 3a
* 3b
* 5
 | **11 (30,6%)****6 (16,7%)****13 (36,1%)****5 (13,9%)****17 (47,2%)****16 (44,4%)****0 (0%)****3 (8,3%)****3 (8,3%)****3 (8,3%)****2 (5,6%)** | **16 (42,1%)****10 (26,3%)****12 (31,6%)****2 (5,3%)****26 (68,4%)****30 (78,9%)****3 (8,3%)****6 (15,8%)****2 (5,3%)****0 (0%)****0 (0%)** | **0,017****0,003****0,006****<0,001** |
| 1 year follow-up : IADL : * 11-14
* 0-4
* 5-10

Clavien Dindo * 0
* 1
* 2
* 3a
* 5
 | **5 (13,9%)****6 (16,7%)****17 (47,2%)****8 (22,2%)****6 (16,7%)****7 (19,4%)****4 (11,1%)****3 (8,3%)** | **15 (39,5%)****5 (13,2%)****18 (47,4%)****19 (50%)****5 (13,2%)****9 (23,7%)****3 (7,9%)****2 (5,3%)** | **0,001****0,006** |

**Table 3**: Comparative table of immediate follow-up between sarcopenic and non-sarcopenic patients and at 1 month, 3 months and 1 year.

**Abbreviations :**

BMI : Body Mass Index

PS : Performance Status

ASA : American Society of Anesthesiologists

CCI : Charlson Comorbidity Index

MNA-SF : Mini Nutritional Assessment

AORF : Acute Obstructive Renal Failure

ISC : In-Situ Carcinoma

IADL : Instrumental Activities of Daily Living

ICU : Intensive Care Unit

NR : Not Relevant

 **4. discussion**

Every surgical procedure carries intraoperative and postoperative risks that vary considerably from one individual to another. In the case of highly complex surgeries such as CR, which is particularly challenging for urologists and is associated with increased morbidity and mortality rates, it is crucial to identify preoperative factors that predict individual outcomes of this surgery. Sarcopenia, identified by measures of muscle wasting, has emerged as an objective and exhaustive preoperative risk factor known to predict survival in various types of cancer (9-12,14,15). – please avoid redundancy here as the same sentence seems to be mentioned in introduction.

Advanced age seems to have a constant influence on surgical outcome, especially as sarcopenia most often develops as a result of aging or malignant pathology. In our study, age and sex were not correlated with sarcopenia, while the absence of correlation was observed in the Fraisse et al. study (16), other studies have concluded to the opposite (6,17).

No correlation was found between sarcopenia and BMI. Miyake et al. have shown that a low BMI is associated with sarcopenia (18). However, in patients with a BMI in excess of 40 kg/m², the risk of cancer-related mortality is elevated. Indeed, studies have shown a strong link between obesity and increased perioperative complications during RC, posing significant challenges for surgeons performing this procedure (19). Obesity is correlated with longer operative times, greater blood loss and a greater likelihood of perioperative complications such as wound infections, deep-vein thromboembolism and cardiopulmonary dysfunction (20-23).

In our study, there was no correlation between sarcopenia-related mortality and the CCI, PS score, ASA score and MNA-SF score. However, a retrospective study showed that the CCI was an independent factor in 3-month mortality after multivariate analysis (17), while another study concluded that the ASA 3-4 score was a significant predictor of 3-month mortality (24).

No correlation was found, in our study, between sarcopenia and histopathological features: grade, T stage, presence of lymph node metastasis, presence of vascular emboli or concomitant ISC. Saitoh et al. conducted a prospective study of 78 patients, and came to the same conclusion as in our study (6). However, in other studies with larger numbers, sarcopenia has been shown to be associated with advanced clinical T stage, the presence of lymph node metastases and tumour histological type (18,24,25). Similar results to these studies have been reported previously in gastric cancer patients (26), with a higher rate of T3 and T4 in patients with a positive sarcopenia status pre-operatively. A possible explanation for this difference in results is the need for a larger population sample to investigate this factor.

In recent years, reduced skeletal muscle mass has been widely studied as an indicator of fragility and impaired physiological reserve. In addition, it has been included as a new tool for risk stratification in trauma and surgical candidates in various surgical fields (27). However, previous studies have used different approaches to describe sarcopenia. These studies use a quantitative measure of muscle only, probably due to their retrospective design. In the specific setting of bladder tumor patients undergoing RC, most studies used SMI measured at L3 (1,18,24,25), others SMI measured at L4/L5 (28), and still others TPA measured at L3 (7,29) or TPA measured at L4 (30) to assess significant changes in the muscle compartment. Studies have used TPA normalized to size: PMI (6,31), and concluded that it is a promising potential prognostic biomarker that can be easily assessed in routine imaging studies of cancer patients, a low PMI was associated with longer hospital stays and more frequent complications.

In our study, a strong correlation was found with TAA, which measures the adipose tissue present in a scan section through L3. This corresponds to a presumed relationship between sarcopenia and obesity, although these two conditions appear to be opposites - one involving a loss of muscle mass and the other an accumulation of adipose tissue - they can coexist in some people. For example, an elderly person may present both a loss of muscle mass due to sarcopenia and an excess of body fat leading to obesity, a condition sometimes referred to as “ sarcopenic obesity”. There are hypotheses that obesity may contribute to the development of sarcopenia, notably through chronic inflammation, insulin resistance and mitochondrial dysfunction. Similarly, sarcopenia may compound the adverse effects of obesity by reducing functional capacity and increasing the risk of obesity-related complications such as joint problems and cardiovascular disease. In summary, although there is no direct correlation between sarcopenia and obesity, these two conditions can coexist and reinforce each other (32). The discussion part so far is well compared with the existing and relevant literature

In our investigation, we used Martin's definition of sarcopenia as it offers a strong and well-established approach, taking into account factors such as gender and BMI, and having been validated in different patient populations, making it a valuable tool for assessing this condition in the clinical setting (13,24,25).

The most impressive finding of the current study is the independent association between sarcopenia and elevated risks of postoperative cancer-specific survival and all-cause mortality. Notably, the trend towards increased mortality risk in the sarcopenic patients in the study appeared as early as 90 days after RC, underlining the potential importance of using SMI for risk stratification prior to surgery. Smith et al. published a study including 200 patients evaluated for total psoas surface area in 2012, where they were able to demonstrate that sarcopenia (OR 2.25; 95% CI 1.11-4.56; p=0.02) was an independent predictor of major complications (7). Mayr et al.'s retrospective study of 327 patients showed that sarcopenia was a significant predictor of 90-day survival after radical cystectomy (p=0.013), and that sarcopenic patients experienced significantly more severe Clavien Dindo 4a-5 complications (p=0.003) than non-sarcopenic patients (24). Other studies support our findings, Psutka et al. reported that, compared with non-sarcopenic patients, sarcopenic patients had significantly lower 5-year cancer-specific survival (49% vs. 72%; p = 0.003) and OS (39% vs. 70%; p = 0.003) (1); Wan et al. revealed that a low SMI was frequently observed in bladder tumor patients undergoing radical cystectomy, and that this index was strongly associated with early complications after surgery (28). Another study using PMI as a marker of sarcopenia found that in male patients (63 of 78), the low PMI group had a significantly higher rate of complications than the high PMI group (82.9% vs. 31.8%, p < 0.001), and patients in the low PMI group experienced severe complications (Clavien Dindo grade ≥ 3, 19.5%) (6).

In line with these data, our results showed that low SMI was associated with poor survival and more severe postoperative complications after RC, suggesting that sarcopenia could be a reliable factor in predicting morbimortality in RC patients. Furthermore, postoperative volume loss of the psoas major muscle has been identified as a strong prognostic factor among patients with and without sarcopenia (18,33). Consequently, it has been suggested that bladder tumor patients who are candidates for RC should be checked for the presence or absence of sarcopenia during follow-up. Nutritional support and prevention of cachexia may be necessary in some patients with BT who have undergone RC.

Early rehabilitation before surgery protocols are beginning to be increasingly integrated into daily clinical practice (34). The underlying idea is that corrective measures for sarcopenia, such as prescribing dietary supplements and exercise before surgery, could have a beneficial impact on postoperative morbidity and mortality. However, a prospective study by Ritch et al. comparing the effect of oral supplementation before CR on sarcopenia and its impact on postoperative morbidity and mortality revealed no significant difference between the two groups with regard to the occurrence of complications and rehospitalization. Despite this, oral supplementation significantly corrected sarcopenia (35).

To this day, combination therapy, including transurethral resection, systemic chemotherapy and radiotherapy, has been considered the most effective treatment for bladder preservation, with a 5-year survival rate of around 50-60%. In patients over 70 years of age, systemic chemotherapy combined with radiotherapy has been shown to be associated with a more favorable prognosis than RC. Similarly, if RC is inappropriate due to advanced age, sarcopenic patients may also be good candidates for bladder preservation (2,36).

This study has both strengths and limitations that are worth mentioning. The strengths include the prospective design of this study. The limitations of our study must be taken into account when interpreting our results. These include data from a single institution, potential selection bias, analysis with a relatively small sample and short follow-up time. The study was also limited by the relatively small number of patients in the female cohort. In addition, changes in postoperative SMI and weight were not recorded, and their impact could not be assessed.

4. Conclusion

Finally, the study shows that sarcopenia emerges as a new independent preoperative prognostic factor in patients undergoing RC for BT. This association has already been observed in various types of cancer, including melanoma, lung, pancreatic, breast, colorectal, renal and hepatobiliary. Interventional management of these pathophysiological effects of sarcopenia could potentially reduce the risk of complications, readmissions and death following radical cystectomy. In the specific cases of people who have undergone RC, targeted nutritional support and strategies to prevent sarcopenia should be offered.

Consent (where ever applicable)

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical approval (where ever applicable)

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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