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Original Article

Does Apoptotic Index Predict the Response to Neoadjuvant Chemotherapy in Patients with Breast Carcinoma?

Apoptotik İndeks Meme Kanserli Hastalarda Neoadjuvan Kemoterapiye Yanıtı Predikte Eder mi?

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ABSTRACT

Objective: Neoadjuvant chemotherapy (NACT) plays a major role in the treatment of patients with locally advanced breast carcinoma. Although most patients have benefited from NACT, the rate of residual tumors is still high after treatment (AT). An increase in apoptosis is expected in tru-cut biopsy (TCB) during treatment or AT as the mechanism of NACT is inducing apoptosis. This study aimed to investigate whether evaluating the apoptotic index (AI) from TCB can predict the response before treatment (TC-BT) and whether there is a correlation between AI and clinicopathologic parameters.

Methods: Seventy cases of breast carcinomas were included. The AI was evaluated BT and AT by quantifying the apoptosis. The receiver operating characteristic analysis was performed with overall survival (OS) data, and low and high AI cut-offs were obtained. The relationship between AI and response and clinicopathological parameters was evaluated.

Results: A significant relationship was found between low AI in TC-BT and at least partial response (p=0.025), longer OS (p=0.01) and disease-free survival (p=0.01), and progesterone receptor-positive tumors (p=0.03). Her2-negative tumors were more prone to low AI. A significant decline in AI (p=0.001) and Ki67 proliferation index (p<0.001) was observed in resections AT.

Conclusions: These data suggested that the AI is a simple and cost-effective tool that may play an important role in determining response, and a low AI in TC-BT may have some value as a predictive marker in breast carcinomas.

Keywords: Apoptosis, apoptotic index, breast cancer, neoadjuvant chemotherapy, response

ÖZ

Amaç: Neoadjuvan kemoterapi (NAKT) lokal ileri meme karsinomu olan hastaların tedavisinde önemli rol oynamaktadır. Çoğu hasta NAKT'den fayda sağlasa da tedaviden sonra hala yüksek oranda tümör kalan bazı hastalar mevcuttur. NAKT mekanizması apopitozu indüklediğinden, tedavi sırasında veya sonrasında tru-cut biyopside (TKB) apopitozda bir artış görülmesi beklenir. TKB'den apoptotik indeksin (Aİ) değerlendirilmesinin tedavi öncesi (TÖ) yanıtı tahmin edip edemeyeceğini ve Aİ ile klinikopatolojik parametreler arasında herhangi bir korelasyon olup olmadığını araştırmayı amaçladık.

Yöntemler: Çalışmamıza 70 meme kanseri dahil edildi. Aİ, TÖ ve sonrası apopitoz sayılarak değerlendirildi. Alıcı işletim karakteristik analizi, genel sağkalım (GS) verileriyle yapıldı ve düşük ve yüksek Aİ eşik değerleri elde edildi. Aİ ile yanıt ve klinikopatolojik parametreler arasındaki ilişki değerlendirildi.

Bulgular: TÖ'de düşük Aİ ile kısmi yanıt (p=0,025), daha uzun GS (p=0,01) ve hastalıksız sağkalım (p=0,01), progesteron reseptör pozitif tümörler (p=0,03) arasında anlamlı ilişki görüldü. Her2-negatif tümörler, düşük Aİ'ye sahip olmaya daha yatkındı. Tedavi sonrası rezeksiyonlarda Aİ (p=0,001) ve Ki67 proliferasyon indeksinde (p<0,001) anlamlı düşüş gözlendi.

Sonuçlar: Bu verilerle, Aİ'nin tek başına yanıtın belirlenmesinde önemli bir rol oynayabilecek basit ve uygun maliyetli bir teknik olduğunu ve TÖ'deki düşük Aİ'nin meme karsinomlarında öngörücü bir belirteç olarak bir değeri olabileceğini düşündürmüştür.

Anahtar kelimeler: Apopitoz, apopitotik indeks, meme kanseri, neoadjuvan kemoterapi, yanıt

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INTRODUCTION

Neoadjuvant chemotherapy (NACT) is currently preferred in the treatment of patients who have large tumors or locally advanced breast cancer. Many agents used in the chemotherapy protocol substantially reduce the tumor size by inducing apoptosis, increasing the chance of breast-conserving surgery, and improving survival by preventing distant metastasis[1-4]. The success of NACT relies on clinical and pathologic responses. Pathologic response is determined by the percentage of residual tumor after resection. Although pathologic complete or partial response is commonly seen after treatment (AT), the percentage of residual tumor can still be high in some cases[5]. Patients with pathologic complete response (pCR) have longer survival than patients with partial or no response[3,6-10]. Some factors affect response such as patient age, tumor size, molecular subtype, tumor microenvironment, and type of tumor stroma[1,3,11-14]. However, no marker is routinely used to evaluate the response. Studies have shown that apoptosis is one of the signs to predict response and can be morphologically seen AT[1,3,15-19]. Moreover, tumor with a low apoptotic index (AI) during treatment or AT is known to be more resistant to therapy. Therefore, apoptosis plays a crucial role in response[1,4,6,11,18,20-24]. Identifying apoptosis morphologically can be challenging because resection is performed in 4-6 weeks AT. Furthermore, for pCR cases, no method of evaluating the AI is available owing to the lack of residual tumor. Thus, the evaluation of the AI from tru-cut biopsy before treatment (TC-BT) may pave the way for predicting response to NACT.

This study primarily aimed to examine whether the AI in TC-BT can predict the response and determine its applicability in routine practice. AI-BT and AI-AT were also compared. Moreover, the relationship between AI and other prognostic factors such as nuclear grade, status of hormone receptors, human epidermal growth factor receptor 2 (Her2) expression, and Ki67 proliferation index that affect the tumor response were studied.

MATERIALS and METHODS

Patient Selection

All patients who were diagnosed with invasive breast carcinoma and received NACT between 2012 and 2022 were retrieved from the hospital's electronic database. Informed consent was obtained from each patient before the surgical procedure. Hematoxylin and eosin-stained slides were retrieved from the pathology archive, and cases without tumor slides or clinical data were excluded.

Clinical Data

The age, details of the NACT protocol, status of recurrence or distant metastasis, and survival status were retrieved from the hospital and national electronic databases. The tumor size, hormone receptor status, Her2 expression, Ki67 proliferation index, and presence of lymphovascular and perineural invasion were obtained from pathology reports.

Outcomes

Overall survival (OS) and disease-free survival (DFS) were defined as the time from diagnosis to death and the time from diagnosis to the first recurrence, respectively, as well as the last evaluation dates of patients who did not relapse and were still alive at the time of evaluation.

Histopathologic Evaluation of the AI

The AI is defined as a percentage of apoptotic cells or bodies per all tumor cells. However, some authors use it to denote the number of apoptotic cells per 1000 tumor cells. Furthermore, in some investigations, apoptosis is measured as the number of apoptotic cells per 10 high-power fields (HPF). In our study, the best representative hematoxylin-eosin (H&E)-stained tumor slide was chosen, and the AI was evaluated by counting the cells with condensed chromatin, fragmented nuclei, and intense eosinophilic cytoplasm at 10 HPF under ×400 objective (Figure 1). The slides of both TC-BT and resection after treatment (R-AT) were evaluated. Counting was only conducted from tru-cut biopsy (TCB) for cases with pCR because no residual tumor was left after resection. The AI was statistically compared BT and AT. However, the number of cases with residual tumor was not statistically comparable. Moreover, to obtain an objective value, evaluation was performed without knowing the clinicopathologic parameters, and the area away from tumor necrosis was selected. According to the receiver operating characteristics (ROC), the cut-off value of the AI was 18.5 (area under curve), 0.742 [95% confidence interval (CI) 0.521-0.963; p=0.05] and 20.5 for BT and AT, respectively. AI values <18.5 for BT and 20.5 for AT were accepted as low, whereas >18.5 and 20.5 were considered high. For R-AT, the cut-off value was determined after excluding pCR cases.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). To determine an optimal AI cut-off for the prediction of recurrence or metastasis and survival status, ROC analysis was performed. According to the cut-off, cases were classified into low AI and high AI. The compliance

of numerical variables to a normal distribution was evaluated using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov or Shapiro-Wilk test). Continuous variables were compared between groups using the Wilcoxon test. To determine whether a correlation existed between the groups, categorical variables were evaluated using the chi-square (Pearson chi-square) and Fisher's Exact test. The Kaplan-Meier method was used for survival analysis and was evaluated with the log-rank test. P-values of <0.05 were considered statistically significant.

Ethical Approval

The ethical approval was provided by the Non-Interventional Clinical Research Ethics Committee Chairmanship of Recep Tayyip Erdogan University Faculty of Medicine (decision no: 2022/42, dated 02.17.2022).

RESULTS

Clinicopathologic Features

A total of 70 cases, including 63 (90%) cases of invasive ductal carcinoma and 7 (10%) cases of invasive lobular carcinoma, were studied. The clinicopathologic features of these cases are summarized in Table 1.

Table 1. Clinicopathologic features of the entire cohort.

	n (%)
Median age years (range)	58 (33-77)
Tumor type	
Invasive ductal carcinoma	63 (90)
Invasive lobular carcinoma	7 (10)
Molecular subtype	51 (73)
Luminal	
Her2	8 (11)
Triple negative	11 (16)
Lymph node status	
N0	33 (47)
N1	37 (53)
Lymphovascular invasion	
Present	25 (36)
Absent	45 (64)
Perineural invasion	
Present	9 (13)
Absent	61 (87)
Mean follow-up (months)	27
Status	
No evidence of disease	55 (79)
Alive with disease	9 (13)
Died of disease	6 (8)

The correlation between AI and clinicopathologic parameters is summarized in Table 2. The median AI-BT and AT were 10 (95% CI 3-46) and 5.5 (95% CI 0-85) and 11 (minimum-maximum 3-80) and 5 (minimum-maximum 0-85), respectively. The AI-BT was low in 54 (77%) cases. Low AI-BT and AI-AT were significantly related to a partial response (p=0.025 and p=0.04, respectively). Tumors with low nuclear grade were prone to have a low AI (Figure 1) compared with tumors with high nuclear grade (Figure 2). However, no significant relationship was found between the AI and the nuclear grade in TCB and resection (p=0.259 and p=0.37, respectively). The expression of progesterone receptor (PR) was significantly higher in the low AI group (p=0.035). Cases that did not express Her2 were more prone to have low AI in TC-BT than cases that were positive for Her2 (35 vs. 15 cases with low AI, p=0.24). The AI increased as the proliferation index increased in both TCB and resection. Cases classified in the luminal group had lower AI in TCBT than in other molecular subtypes. However, the molecular subtype and Ki67 proliferation index did not correlate with the AI (p=0.11 and p=0.48, respectively).

Interestingly, the frequency of metastatic axillary lymph nodes (ALNs) was low (6%) in the high AI group, and metastatic ALNs significantly correlated with low AI-BT and AI-AT (p<0.001 and p=0.03, respectively). The frequency of distant metastasis was low (16%) in the low AI group, and a significant relationship was observed between these two entities (p=0.03). Moreover, no significant relationship was observed between AI and lymphovascular and perineural invasion (p=0.10 and p=0.67, respectively).

AI in TCB and Survival

Six (8%) cases died of disease, and 4 (67%) of them had high AI. The outcome was significantly poor as the AI increased (p=0.02). A low AI significantly correlated with longer OS and DFS (p=0.01 and p=0.01, respectively) (Figure 3A and 3B). The follow-up period was short (8 months) in four cases because they were diagnosed recently. Therefore, these cases were excluded from the evaluation between AI and survival. Moreover, no patient had distant metastasis, and all were alive.

Comparison of AI-BT and AI-AT

When we compared the AI between TC-BT and R-AT, a significant decline was observed in the AI and Ki67 proliferation index in R-AT (p=0.001 and p<0.001, respectively).

Table 2. Correlation between apoptotic index and clinicopathologic parameters.

	AI <18.5 n (%)	AI >18.5 n (%)	p-value
Tumor type			
Invasive ductal carcinoma	48 (89)	16 (100)	0.316
Invasive lobular carcinoma	6 (11)	0	
Response to treatment			
No response	4 (7.4)	5 (31)	0.025
Partial and or complete response	50 (92.6)	11 (69)	
ER expression			
Positive	41 (76)	9 (56)	0.206
Negative	13 (24)	7 (44)	
PR expression			
Positive	39 (72)	7 (44)	0.035
Negative	15 (28)	9 (56)	
Her2 expression			
Positive	15 (30)	8 (53)	0.24
Negative	35 (70)	7 (47)	
Molecular subtype			
Luminal	42 (78)	9 (56)	0.11
Her2	5 (9)	3 (19)	
Triple negative	7 (13)	4 (25)	
Ki67 proliferative index			
<15%	14 (28)	2 (15)	0.48
>15%	36 (72)	11 (85)	
Lymphovascular invasion			
Present	22 (41)	3 (19)	0.10
Absent	32 (59)	13 (81)	
Perineural invasion			
Present	8 (15)	1 (6)	0.67
Absent	46 (85)	15 (94)	
Metastatic axillary lymph node			
Present	36 (67)	1 (6)	<0.001
Absent	18 (33)	15 (94)	
Distant metastasis			
Present	8 (16)	7 (44)	0.03
Absent	42 (84)	9 (56)	
Status			
Alive	48 (96)	12 (75)	0.02
Dead of disease	2 (4)	4 (25)	

ER: Estrogen receptor, PR: Progesterone receptor, Her2: Human epidermal growth factor 2, AI: Apoptotic index

DISCUSSION

NACT provides patients with a chance for conservative surgery and protects the patients from occult metastasis by reducing the tumor size. The mechanism is based on inducing apoptosis[1,3,18,19]. Therefore, we expect to see an increase in apoptosis AT. Moreover, the greater the tumor's ability to escape the apoptotic pathway, the greater the likelihood of resistance to therapy. Several studies have

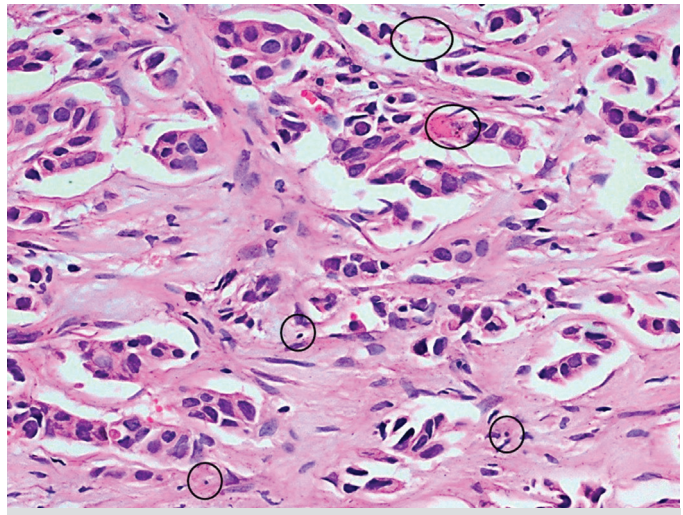

Figure 1. A case with a low nuclear grade and a low apoptotic index in tru-cut biopsy before treatment (×400, hematoxylin-eosin staining). The circles show apoptotic cells with condensed chromatin, fragmented nuclei, and intense eosinophilic cytoplasm. The slide belongs to a patient with a complete pathologic response after treatment.

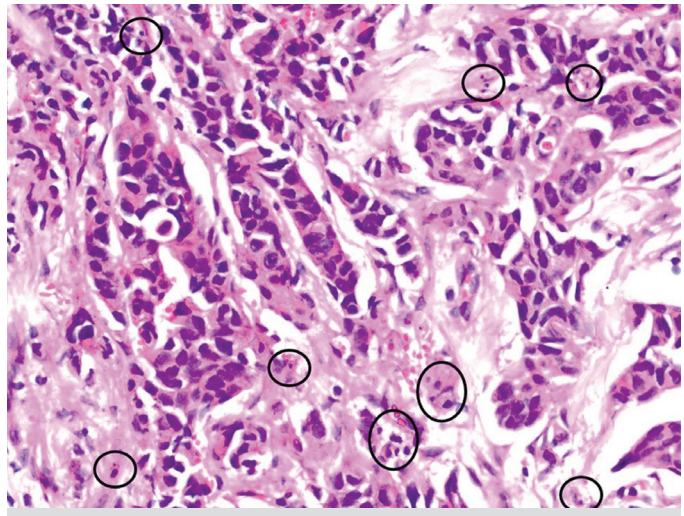

Figure 2. A case with high apoptotic index in tru-cut biopsy before treatment (×200, hematoxylin-eosin staining). The circles show apoptotic cells. The nuclear grade and proliferation index were high in this case.

examined the optimal time to examine apoptosis[1,4,20-22]. According to Burcombe et al.[21], a significant increase in the AI was observed on day 21. However, they also emphasized that 21 days after NACT can be too late for the evaluation of the AI and Ki67 and suggested earlier times to observe optimal response. For example, Tiezzi et al.[4] observed a significant increase in the AI following NACT and realized that 24 h was the optimal time to see apoptotic cells. However, according to Buchholz et al.[1], apoptosis was prominent 48 h AT. In these studies, patients had undergone several invasive procedures. Moreover, they did not compare the AI between TCB and resection. As a result, several studies have examined the AI; however, all focused either on determining the time interval when the AI is most prominent after NACT or the relationship between AI and prognostic parameters during treatment or AT. Some studied the relationship between the effect of the chemotherapeutic agent and the AI. The results suggest that chemotherapeutic drugs destroy the tumor by inducing apoptosis; therefore, tumors with high AI respond better to treatment, whereas those with low AI are more resistant to treatment[1,3,15-19]. By contrast, in our study, the survival rate was lower in the high AI group despite treatment, whereas the response and survival were higher in the low AI group.

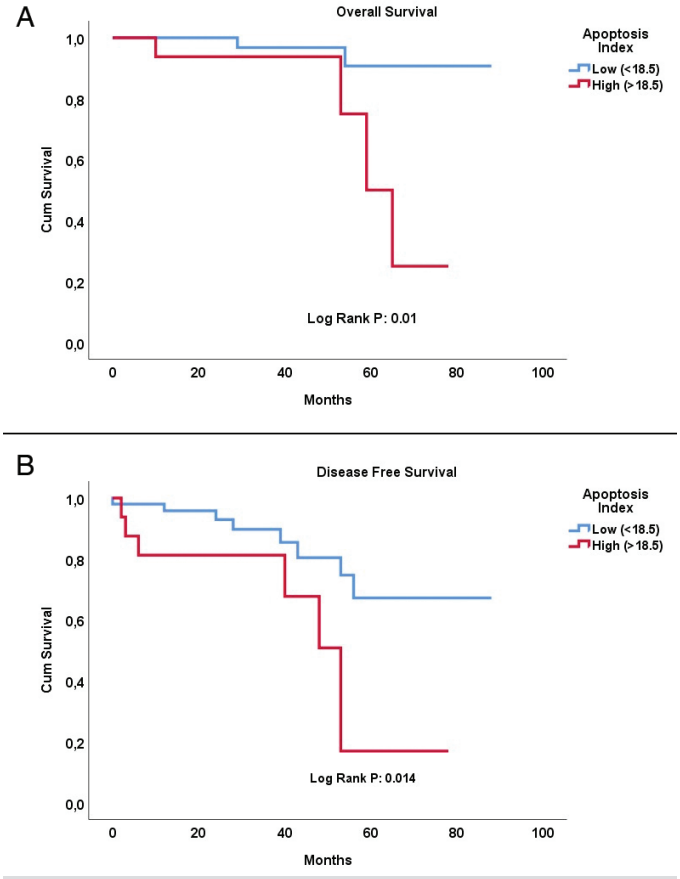

Figure 3. Receiver analysis characteristic curve for predicting survival according to the apoptotic index. Cases with low apoptotic index (<18.5) have longer overall survival (A, upper) and disease-free survival (B, lower).

However, in these studies, neither the pre-treatment assessment of the AI alone nor the comparison of the AI in biopsy and resection specimens BT and AT was made. In the present study, we aimed to determine a threshold value by evaluating the AI over TCBs taken BT, independent of post-treatment resection specimens, and understand whether this value can predict treatment response.

One of the advantages of our study was that neither additional biopsy nor additional staining method was needed for AI evaluation. Thus, we obtained a new morphological parameter in breast cancer, which has very limited data in predicting treatment response.

We also compared the AI in TC-BT and R-AT. We observed that 90% of the cases had declined AI-AT, and the majority of them had partial pathologic response and/or pCR (59% and 41%, respectively). Considering that, resection is done approximately 4-6 weeks AT; thus, it should not be surprising to see low AI in resections.

In addition, tumors with high Ki67 have a better response to NACT[17,25-27]. We observed that all cases with pCR and a decline in AI-AT also had high Ki67 in TC-BT. By contrast to previously published studies, we suggest that not only a decline in AI-AT but also high Ki67 could be a sign of at least partial response to NACT[28,29]. As a result, evaluating the AI only AT in our routine practice could not be a reliable method; thus, the comparison between BT and AT is needed to obtain an effective result.

We observed that tumors with low AI-BT have a better response and longer OS and DFS. This result can be explained by not only the apoptotic pathway but also the biology of the tumor. For example, tumors with low AI had a low Ki67 proliferation index and low nuclear grade, which means that they progress more slowly and had less potential to metastasize than tumors with high AI and high Ki67.

Ki67 is expressed only on proliferating cells, and tumors with high Ki67 and high AI progress rapidly and may metastasize. As mentioned above, chemotherapy is more effective on tumors with a high proliferation rate, and a decrease in Ki67 AT is a sign of response[4,17,18,20,27,30,31]. For example, Burcombe et al.[21] examined a significant decline in Ki67 on day 21 among patients with clinically

complete response. In the present study, the AI and Ki67 were compared in 63 cases because we could not obtain Ki67 stains from seven cases. Except for one case, tumors with high Ki67 and high AI had a partial response and/or pCR consistent with this theory. In addition, a statistically significant decline in Ki67, which was a sign of response, was also observed in the AI. The case with no pathologic response was a triple negative one, and Ki67 increased 1.5 times in R-AT. On the contrary, we observed more metastatic ALNs in patients with low AI tumors. However, in detail, the majority of them had a partial response and/or pCR (89% and 63%, respectively) and high Ki67 in TC-BT. In addition, 75% of the cases with no response had increased Ki67 in R-AT. Therefore, high Ki67 in TC-BT could determine local metastasis potential. However, despite having metastatic ALNs, tumors with low AI-BT could have a better response than tumors with high AI-BT.

Another important factor that affects the tumor response is the molecular subtype. Tumors that express estrogen receptor and PR or Her2 have better responses than the triple negative subtype regardless of the Ki67 proliferating index[8,32]. In the present study, PR expression significantly correlated with low AI. Moreover, the majority of them (89%) had a partial response and/or pCR consistent with this theory. On the contrary, low AI was more observed in Her2-negative tumors. However, improved survival was observed in Her2-positive cases, and a decline in Ki67 AT was also observed in this group. This explains the effectiveness of trastuzumab regardless of other clinicopathologic parameters such as lymph node or distant metastasis or treatment response. We suggest that apoptosis alone may not be reliable in predicting the response in TC-BT of Her2-positive tumors, whereas it predicts a better response in luminal subtypes.

Finally, the choice of agents in chemotherapy also alters the tumor response[33-36]. In this study, we did not observe any significant difference between the type of agent and AI-AT. In addition, no one was superior to others since no correlation was found between OS or DFS and the type of agent.

CONCLUSION

A low AI in TC-BT is related to a better response and longer OS and DFS. Despite revealing a better response, a high AI in TC-BT could be a sign of rapid tumor progression with local or distant metastasis. The evaluation of the AI in R-AT may not be reliable, especially in cases without residual tumors. Besides the AI in TC-BT, comparing the AI and Ki67 between TC-BT and R-AT could have more reliable results. As advantages, no additional invasive procedures and immunohistochemical stains were needed in this study. We preferred to use only H&E staining because it is easily accessible and cost-effective. As a result, we suggest that AI evaluation is a simple and cost-effective technique to help predict the response and prognosis of TC-BT in invasive breast carcinomas, particularly in luminal subtypes.

Ethics

Ethics Committee Approval: The ethical approval was provided by the Non-Interventional Clinical Research Ethics Committee Chairmanship of Recep Tayyip Erdogan University Faculty of Medicine (decision no: 2022/42, dated 02.17.2022).

Informed Consent: Informed consent was obtained from each patient before the surgical procedure.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Surgical and Medical Practices: G.A., Concept: G.A., C.O., S.D.O., B.S., R.B., Design: G.A., C.O., S.D.O., Data Collection and/or Processing: G.A., O.O., C.O., S.D.O., B.S., Analysis and/or Interpretation: G.A., O.O., C.O., S.D.O., B.S., R.B., Literature Search: G.A., O.O., R.B., Writing: G.A., R.B.

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