



Safety analysis of immediate breast reconstruction with a deep inferior epigastric perforator (DIEP) flap in the post-COVID-19 era: a comparison between pre- and post-pandemic cohorts

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Background: The demand for immediate breast reconstruction with a deep inferior epigastric perforator (DIEP) flap is recovering as coronavirus disease 2019 (COVID-19) transitions from a pandemic to an endemic. This study sought to evaluate the safety of resuming DIEP flap reconstruction in the post-COVID-19 era.

Methods: Consecutive breast cancer patients who underwent immediate breast reconstruction with a DIEP flap at the Comprehensive Breast Health Center, Ruijin Hospital were retrospectively included in the study. The patients were divided into a post-pandemic group (Group A) and a pre-pandemic group (Group B). The clinicopathological factors, surgical procedures, and rates of post-operative complications were compared between the two groups using the Mann-Whitney *U* test and Chi-squared test.

Results: A total of 167 patients were included in the study, of whom 119 (71.3%) were in Group A and 48 (28.7%) were in Group B. The two groups had similar clinicopathological features, including age ($P=0.988$), body mass index ($P=0.504$), and tumor, node, metastasis (TNM) stage ($P=0.932$). The Group A patients were more likely to receive single perforator DIEP flap transplantation than the Group B patients ($n=28$, 22.8% *vs.* $n=3$, 5.8%, $P=0.007$). There was a numerical decrease in the mean operating time of Group A patients compared to Group B patients (9.82 *vs.* 10.12 hours, $P=0.172$). The mean length of stay after the surgery was significantly shorter after the pandemic than before the pandemic (11.2 *vs.* 14.3 days, $P<0.001$). The complication rates between the two groups were similar.

Conclusions: This study provides evidence that resuming DIEP reconstruction is safe in the post-COVID-19 era.

Keywords: Breast cancer; breast reconstruction; deep inferior epigastric perforator (DIEP); coronavirus disease 2019 (COVID-19)

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Introduction

Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic in 2020, the healthcare system has faced significant challenges (1-3). As a result of the pandemic, the treatment patterns for early breast cancer changed significantly (4-6). During the pandemic, some patients experienced surgical delays or changes in their surgical plans, while others received neoadjuvant endocrine therapy as a bridge to their eventual surgery (4). Adjuvant therapy was also optimized to increase the safety of treatments during the pandemic (5).

Due to a rising need among breast cancer patients for the restoration of not only their physical but also their psychological health, breast reconstruction has become an important part of breast cancer treatment, and has been shown to have great positive effects on the personal image and quality of life of patients (7,8). Deep inferior epigastric perforator (DIEP) flap transplantation is a central component in the state-of-the-art practice of breast reconstruction (9). Under this procedure, adipocutaneous tissue of the anterior abdominal wall is harvested, while the continuity, width, and innervation of the rectus abdominis muscle is preserved. However, following the outbreak of COVID-19, due to the extensive surgical procedure and prolonged hospital stay, it was recommended that breast reconstruction using autologous flaps be postponed to conserve limited medical resources for critical circumstances

(10-13). The percentage of patients undergoing autologous breast reconstruction greatly decreased during the pandemic; several research studies in Europe, North America and east Asia reported that the decline ranged from 33.3% to 78.0% (14-16). According to a survey of Brazilian breast specialists, only 3.4% recommended autologous reconstruction for early breast cancer patients during the outbreak of COVID-19 (3).

However, as COVID-19 transitions from a pandemic to an endemic, and restrictions on resources gradually loosen, we are facing the challenge of how to resume autologous breast reconstruction. Due to the dynamic zero-COVID policy of the Shanghai government, the medical system worked continuously and steadily in Shanghai during the pandemic, which allowed our center to re-start autologous breast reconstruction surgery for breast cancer patients quickly. There have been several reports on the variation of implant-based breast reconstruction or microplastic surgery after the pandemic (17,18); however, there is still a lack of systemic research on the influence of COVID-19 on DIEP surgery.

This study sought to evaluate the safety of resuming immediate breast reconstruction with a DIEP flap in the post-COVID-19 era by comparing the surgical procedures and post-operative complication rates of patients who underwent breast reconstruction before and after the epidemic. We present this article in accordance with the STROBE reporting checklist (available at <https://gs.amegroups.com/article/view/10.21037/gc-23-143/rc>).

Highlight box

Key findings

- This study found no difference in the surgical complication rates of patients who underwent deep inferior epigastric perforator (DIEP) flap reconstruction before and after the coronavirus disease 2019 (COVID-19) pandemic.

What is known and what is new?

- The COVID-19 pandemic has caused delays in and cancellations of oncoplastic surgery for breast cancer patients.
- This study found that patients were more likely to undergo a single perforator DIEP flap transplantation after the pandemic than before the pandemic and that there was a numerical decrease in the mean time of performing the surgery.
- The mean length of stay after the surgery was significantly lower after the pandemic than before the pandemic.

What is the implication, and what should change now?

- DIEP reconstruction can be safely resumed in the post-COVID-19 era.

Methods

Patient eligibility

The patients were retrospectively recruited from the Comprehensive Breast Health Center, Ruijin Hospital. Consecutive patients with histologically proven breast cancer, who were older than 18 years at the time of diagnosis, and underwent immediate breast reconstruction between January 1, 2019, which was the first time we conducted immediate breast reconstruction with a DIEP flap, and December 31, 2021, were identified. The patients also had to meet the following inclusion criteria: (I) be female; (II) have undergone a total mastectomy (TM), nipple sparing mastectomy (NSM), or skin sparing mastectomy; and (III) have undergone immediate breast reconstruction with a DIEP flap.

Based on the time of surgery, the patients were divided into the following two groups: (I) Group A, which

comprised patients who underwent the surgery between March 1, 2020 and December 31, 2021, which was defined as the post-COVID-19 era; and (II) Group B: which comprised patients who underwent the surgery between January 1, 2019 and February 29, 2020, which was defined as the pre-COVID-19 era.

Patients' clinicopathological information, and details on the surgical procedures and the post-operative complications were collected from patients' medical records, including age, body mass index (BMI), smoking history, history of diabetes, pathology, pathologic tumor, node, metastasis (TNM) stage, intrinsic subtype, pre-operative systemic treatment, the number of the harvested perforator, surgery time, flap failure, emergent re-exploration, and fat necrosis. Follow-up information was collected by breast surgeons and specialist breast nurses at our center.

This study was approved by the independent Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (Approval code: 2020-309). The study was conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants gave informed consent before taking part in this study.

Interventions

At our center, all women undergoing TM for breast cancer were offered the option of breast reconstruction procedures. After assessing the suitability of each patient for reconstruction surgery, all forms of reconstruction and the associated additional possible exposure risk of COVID-19 were discussed with the patient. The final decision as to the type and timing of the reconstruction surgery was based on each patient's preference. Approximately 40% of the patients requiring breast reconstruction chose a DIEP flap transplantation. Before surgery, each patient routinely underwent a computed tomography angiography assessment of the perforators of the lower abdominal wall. All the DIEP flap reconstructions were performed by two senior surgeons using a standard technique. Usually, the first-choice recipient vessels were the internal thoracic vessels at the level of the third rib. In patients with large breasts and those with midline abdominal scars, unilateral reconstructions were performed using a bi-pedicled DIEP flap.

Statistical analyses

All the statistical analyses were carried out using Statistical

Package for the Social Sciences (SPSS) version 18.0 (SPSS, Inc., Chicago, IL, USA). Independent sample *t*-tests were used to compare the normally distributed continuous variables. Mann-Whitney *U* tests were used to compare the skewed distributed continuous variables. The results are presented as the median with the standard deviation. The categorical variables, such as the baseline characteristics between the groups, are presented as frequencies (with percentages), and were compared using the Chi-square or Fisher's exact test as appropriate. Patients with incomplete information were excluded from the study. All the statistical tests were two-tailed, and a *P* value <0.05 was considered statistically significant.

Results

Patient characteristics

Among the 2,654 breast cancer patients who underwent mastectomy during the 36-month study period, 175 DIEP flaps (eight bilateral and 159 unilateral) reconstructions were performed in 167 patients (mean age: 40.3 years; range, 26–66 years), who were retrospectively enrolled in the study. Notably, immediate breast reconstruction surgeries with a DIEP flap were postponed from February to March in 2020, due to the uncertainty of the epidemic and relevant policies of the hospital. Breast reconstruction surgery gradually recommenced in the late April 2020. In total, 119 patients underwent surgery after the pandemic, and the other 48 patients underwent surgery before the COVID-19 pandemic. The two cohorts had similar clinical characteristics (*Table 1*), including age (*P*=0.988), BMI (*P*=0.504), co-morbid conditions, such as smoking history (*P*=0.504) and diabetes mellitus (*P*=0.504), and pathological characteristics (*Table 1*), such as pathology (*P*=0.626) and TNM stage (*P*=0.932). In relation to the treatment features, no differences were observed between the two groups in terms of neoadjuvant chemotherapy (NAC) (*P*=0.356), breast surgery type (*P*=0.206) or axilla surgery type (*P*=0.778).

Surgical procedures

Group A comprised 105 TM and 18 NSM patients, while Group B comprised 48 TM and 4 NSM patients (*P*=0.267). In relation to the axilla surgery type, the proportion of patients who received only sentinel lymph node biopsies without axillary lymph node dissection in the pre-

Table 1 Patients' baseline clinicopathological characteristics

Characteristics	Entire cohort, n (%)	Group A, n (%)	Group B, n (%)	P value
Age (years)				0.988
<50	153 (91.6)	109 (91.6)	44 (91.7)	
≥50	14 (8.4)	10 (8.4)	4 (8.3)	
BMI (kg/m ²)				0.504
<25	145 (86.8)	102 (85.7)	43 (89.6)	
≥25	22 (13.2)	17 (14.3)	5 (10.4)	
Smoking history				0.504
No	165 (98.8)	118 (99.2)	47 (97.9)	
Yes	2 (1.2)	1 (0.8)	1 (2.1)	
Diabetes mellitus				0.504
No	165 (98.8)	118 (99.2)	47 (97.9)	
Yes	2 (1.2)	1 (0.8)	1 (2.1)	
NAC				0.356
No	124 (74.3)	86 (72.3)	38 (79.2)	
Yes	43 (25.7)	33 (27.7)	10 (20.8)	
Breast surgery				0.206
TM	153 (87.4)	105 (85.4)	48 (92.3)	
NSM	22 (12.6)	18 (14.6)	4 (7.7)	
Axilla surgery				0.778
No	3 (1.7)	2 (1.6)	1 (2.0)	
SLNB	101 (57.7)	69 (56.1)	32 (61.5)	
ALND +/- SLNB	71 (40.6)	52 (42.3)	19 (36.5)	
Pathology				0.626
DCIS	34 (19.4)	25 (20.3)	9 (17.3)	
IDC	126 (72.0)	89 (72.4)	37 (71.2)	
Others	15 (8.6)	9 (7.3)	6 (11.5)	
TNM stage				0.932
Stage 0	34 (19.4)	25 (20.3)	9 (17.3)	
Stage I	49 (28.0)	33 (26.8)	16 (30.8)	
Stage II	63 (36.0)	45 (36.6)	18 (34.6)	
Stage III	29 (16.6)	20 (16.3)	9 (17.3)	
Intrinsic subtype				0.533
Luminal A-like	53 (30.3)	36 (29.3)	17 (32.7)	
Luminal B-like	60 (34.3)	45 (36.6)	15 (28.8)	
TNBC	18 (10.3)	14 (11.4)	4 (7.7)	
Her-2 enriched	44 (25.1)	28 (22.8)	16 (30.8)	

BMI, body mass index; NAC, neoadjuvant chemotherapy; TM, total mastectomy; NSM, nipple-spared mastectomy; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; TNM, tumor, node, metastasis; TNBC, triple negative breast cancer.

Table 2 Surgical features

Features	Group A, n (%)	Group B, n (%)	P value
Side			0.281
Unilateral DIEP flap	114 (95.8)	44 (91.7)	
Bilateral	5 (4.2)	4 (8.3)	
Pedicle			0.294
Uni-pedicle flap	86 (69.9)	32 (61.5)	
Bi-pedicle flap	37 (30.1)	20 (38.5)	
Perforator			0.007*
Single perforator	28 (22.8)	3 (5.8)	
Two or more perforator	95 (77.2)	49 (94.2)	
Nipple reconstruction			0.045*
No	92 (74.8)	46 (88.5)	
Yes	31 (25.2)	6 (11.5)	

*, P<0.05. DIEP, deep inferior epigastric perforator.

Table 3 Operative time and hospitalization stay of breast reconstruction patients

Variables	Group A	Group B	P value
Post-operative hospital stay (days)	11.2±4.0	14.3±3.4	<0.001
Operative time (hours)	9.82±1.87	10.12±2.38	0.172

Data are presented as mean ± standard deviation.

COVID-19 period was comparable to that in the post-COVID-19 period (61.5% *vs.* 56.1%, P=0.778).

The proportion of patients who underwent bi-pedicle DIEP flap reconstruction was statistically similar between the two groups (Group A: 30.1% *vs.* Group B: 38.5%, P=0.294; *Table 2*). All eight bilateral patients underwent reconstruction with a bi-pedicle DIEP flap. The Group A patients had a numerically lower mean number of flap perforators than the Group B patients (mean: Group A =2.56 perforators *vs.* Group B =2.87 perforators, P=0.140). Additionally, the proportion of flaps with one perforator was significantly higher in Group A patients than in Group B patients (Group A: 22.8% *vs.* Group B 5.8%, P=0.007). Further, a significant higher rate of nipple reconstruction was observed after the pandemic than before the pandemic (25.2% *vs.* 11.5%, P=0.045).

There was a numerical decrease in the mean operating time for Group A patients compared to Group B patients (9.82 *vs.* 10.12 hours, P=0.172; *Table 3*). Moreover, we also found a significantly shorter post-operative hospital stay

(mean 11.2 *vs.* 14.3 days, P<0.001) for post-COVID-19 patients compared to pre-COVID-19 patients.

Post-operative complications

Post-operative follow-up data were collected for all the enrolled patients (*Table 4*). After a median follow-up period of 12.8 months (range, 9–22 months), the overall rate of post-operative complications was 10.2% (17/167), and the rates were comparable between the two groups (Group A: 10.1%, 12/119 *vs.* Group B: 10.4%, 5/48, P=0.949). In relation to the breast-related complications, the absolute rates of re-exploration (Group A: 2.5% *vs.* Group B: 4.2%, P=0.572), seroma (Group A: 1.7% *vs.* Group B: 4.2%, P=0.325), hematoma (Group A: 0.8% *vs.* Group B: 4.2%, P=0.199), and infection with the need for an intravenous injection (i.v.) of antibiotics (Group A: 0.0% *vs.* Group B: 2.1%, P=0.287) were similar between the pre-COVID-19 and post-COVID-19 patients, and the differences were not statistically significant. No abdominal-related complications

Table 4 Post-surgical complications

Characteristics	Whole group, n (%)	Group A, n (%)	Group B, n (%)	P value
Breast complication				
Re-exploration				0.572
No	162 (97.0)	116 (97.5)	46 (95.8)	
Yes	5 (3.0)	3 (2.5)	2 (4.2)	
Seroma				0.325
No	163 (97.6)	117 (98.3)	46 (95.8)	
Yes	4 (2.4)	2 (1.7)	2 (4.2)	
Hematoma				0.199
No	164 (98.2)	118 (99.2)	46 (95.8)	
Yes	3 (1.8)	1 (0.8)	2 (4.2)	
Infection requiring PO antibiotics				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Infection requiring IV antibiotics				0.287
No	166 (99.4)	119 (100.0)	47 (97.9)	
Yes	1 (0.6)	0 (0.0)	1 (2.1)	
Abdominal complication				
Abdominal flap necrosis				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Seroma				0.558
No	164 (98.2)	116 (97.5)	48 (100.0)	
Yes	3 (1.8)	3 (2.5)	0 (0.0)	
Hematoma				>0.99
No	165 (98.8)	117 (98.3)	48 (100.0)	
Yes	2 (1.2)	2 (1.7)	0 (0.0)	
Infection requiring PO antibiotics				>0.99
No	166 (99.4)	118 (99.2)	48 (100.0)	
Yes	1 (0.6)	1 (0.8)	0 (0.0)	
Infection requiring IV antibiotics				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Fat liquefaction				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	

Table 4 (continued)

Table 4 (continued)

Characteristics	Whole group, n (%)	Group A, n (%)	Group B, n (%)	P value
Umbilicus necrosis				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Hernia				NA
No	167 (100.0)	119 (100.0)	48 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Abdominal bulge				>0.99
No	165 (98.8)	117 (98.3)	48 (100.0)	
Yes	2 (1.2)	2 (1.7)	0 (0.0)	
VTE				>0.99
No	166 (99.4)	118 (99.2)	48 (100.0)	
Yes	1 (0.6)	1 (0.8)	0 (0.0)	

NA, not available; PO, peros; IV, infection of vein; VTE, venous thrombus embolism.

were observed in Group B (pre-COVID-19) patients. In the post-COVID-19 patients, the absolute rates of abdominal seroma, hematoma, infection with a need of peros (p.o.), antibiotics, abdominal bulge, and venous thrombus embolism were 2.5%, 1.7%, 0.8%, 1.7%, and 0.8%, respectively. No patients and none of the medical staff were affected by COVID-19 during the hospital stays of the patients in either the pre-operative or post-operative periods.

Discussion

Since the onset of the COVID-19 pandemic, surgical oncologists have strived to ensure that breast cancer patients receive optimal treatment, despite the huge impact that the COVID-19 prevalence has brought to our healthcare systems. During this period, it was recommended that as a non-essential surgery, immediate breast reconstruction, especially autologous breast reconstruction, be postponed (10-13). However, as COVID-19 transitions from a pandemic to an endemic, the recommencement of autologous breast reconstruction has become inevitable. By comparing the surgical procedures and post-operative complication rates of patients receiving immediate breast reconstruction with a DIEP flap before and after the onset of the COVID-19, our study sought to evaluate the safety of resuming autologous free-flap breast reconstruction in

the post-COVID-19 pandemic era.

The current study found no significant difference in the baseline clinicopathological and surgical procedures between the patients who underwent surgery in the two different periods. Notably, the proportion of patients who underwent NAC was numerically higher in the post-COVID-19 pandemic patients (Group A: 27.7% *vs.* Group B: 20.8%, $P=0.356$), as the treatment guidelines recommend neoadjuvant treatment as a bridge to the eventual surgery (10,19). Fewer patients received a DIEP flap with two or more perforators in the post-COVID-19 era than in the pre-COVID-19 era, and there was a slight and numerical decrease in the mean time of performing the surgery. Additionally, no significant differences in the post-operative complications were observed between the two groups, which supports the recommencement of immediate breast reconstruction using a DIEP flap during and after the COVID-19 pandemic. The findings of this study have important implications for surgical care as we begin to recover from the COVID-19 pandemic, which has caused widespread and numerous delays in medical services.

In the adapted recommendations of the European Society for Medical Oncology (ESMO) and American College of Surgeons (20,21) for the COVID-19 era, immediate reconstruction is only recommended when hospital resources permit, while autologous reconstruction should be deferred. The American Society of Plastic Surgeons also

recommend that implant-based breast reconstruction be evaluated on a case-by-case basis, depending on the risk of complications, the utilization of medical resources and each patients' risk, and that autologous reconstruction be delayed (22). Consequently, a huge decrease in elective breast reconstruction surgery was observed as a result of the initial phase of the COVID-19 response. As a result, a great decrease in the rate of immediate autologous breast reconstruction was observed in North America (23) and European countries (24) following the outbreak of the pandemic. Boyd *et al.* reported a 43% decrease in immediate autologous reconstruction during the pandemic (25). A British study of 34 breast plastic surgery units reported the almost complete cessation of breast reconstruction (in 30 out of 34 units) (26).

The availability of a reliable screening test(s) for COVID-19 is a prerequisite for the return to elective surgery. Screening for temperature, upper respiratory tract symptoms, or a history of recent travel will reduce the risk of COVID-19 infection. In our study, we ensured that the COVID-19 pandemic was stable and that there were adequate medical resources before we resumed breast reconstruction surgery with the DIEP flap. Additionally, we introduced a number of strategies to optimize our standard operating procedure of hospitalization and operation preparation, including a rapid pre-operative assessment, a shortened pre-operative waiting period, a shortened operative period, and an accelerated post-operative rehabilitation plan. Other studies have suggested that patients with minimal co-morbidities be selected (27) and peri-operative management be optimized to ensure that the breast reconstruction operation could be conducted safely and effectively during and after the COVID-19 period (28).

Adopting the aforementioned procedure, we performed immediate pedicle-flap breast reconstruction safely at our center after the pandemic. None of our patients or staff members was infected with COVID-19, and we also maintained low rates of complications. To our knowledge, this is the largest-scale report on the safety and outcomes of autologous reconstruction in the post-COVID-19 period. Our findings are in line with several previous studies with small sample sizes. In a small retrospective study by Ho *et al.*, 29 patients underwent breast reconstruction with DIEP flaps in the "post-COVID" period (27). Less cases were performed than in the same period before the pandemic; however, there was no significant difference in the post-operative complications and secondary surgery pre- and post-COVID, which provides evidence of the

safety of autologous breast reconstruction throughout the COVID-19 pandemic. Similar results were also reported in a retrospective analysis by Vj *et al.* (29). In their study, 25 patients received immediate autologous free-flap breast reconstruction in the COVID-19 era with service reconfiguration. Such studies provide further support that the feasibility of immediate breast reconstruction in selected women should not be neglected due to the COVID-19 pandemic.

Autologous reconstruction is routinely performed at our center, depending on the experience of the multi-disciplinary treatment team. Besides the relatively more natural shape achieved by DIEP flap transplantation, DIEP flap transplantation is also preferred to implant reconstruction due to the effect of COVID-19 vaccines on plastic surgery. Mahrhofer *et al.* reported immune reactions, including seroma, swelling, and sterile abscesses, after vaccinations in breast-implant patients (30). Moreover, in a retrospective analysis of 4,073 patients, Taghioff *et al.* revealed the potential protective effect of the COVID-19 vaccine among patients receiving microsurgery who not only had a lower risk of post-operative adverse outcomes, such as surgical site infections, intensive care unit admissions, and generalized infections, but who also had a lower risk of flap failure (31). These findings support breast reconstruction with DIEP flaps in the post-COVID-19 era.

The major limitation of the current study relates to its retrospective nature. In addition, it is inevitable that the improvement of the skills and experience of breast surgeons in the reconstruction operation might have affected the surgery time and the rate of complications, which might have led to a bias.

After battling the spread of the virus for 3 years, the world has lifted most of its pandemic-era restrictions and adopted a living-with-COVID strategy. As we witness a decline in new cases and the gradual loosening of restrictions on resources, the need to resume and ramp-up surgical services has become critical. The "Full Recovery" phase will occur with the establishment of a "new" normal in which regular surgical activities, including immediate autologous breast reconstruction, gradually resume.

Conclusions

We found that DIEP flap breast reconstruction following mastectomy for breast cancer was a safe procedure during and after the COVID pandemic, and observed a low incidence of flap loss or major complications in both

unilateral and bilateral cases. As COVID-19 continues to transition from a pandemic to an endemic, the recommencement of DIEP flap breast reconstruction is an inevitability that we will all have to embrace.

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Footnote

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Ethical Statement: The authors are 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. This study was approved by the independent Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (Approval code: 2020-309). The study was conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants gave informed consent before taking part in this study.

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