Using carbon nanoparticles to localize nonpalpable breast masses in breast conservative surgery: a single-arm pilot study

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Received: 19 March 2022
Revised: 17 April 2022
Accepted: 26 April 2022
Published: 04 January 2023

The Egyptian Journal of Surgery 2023, 41:690–696

Introduction
The development of highly sensitive screening methods for early detection of breast cancer (BC) resulted in an epidemic of newly identified nonpalpable BC masses. In the era of breast conservative surgery where minimizing resections and maximizing aesthetics is the goal, exploring new techniques to fulfill these targets is mandatory. Carbon nanoparticles (CNs) display a good profile and may be a significant tool in localizing these lesions.
Aims and objectives
To examine the feasibility of CNs in localizing nonpalpable lesions, to explore its effects on cancer-free resection margins, and to evaluate the aesthetic outcomes of surgery guided by these techniques.
Patients and methods
This is a prospective, single-arm, open-label pilot study of 20 patients (*N*=20) with nonpalpable breast masses undergoing breast conservative surgery with CN localization. All patients had preoperative staging procedures and metastasis workup. Mean age was 50.05±9.8 years. Initial staging revealed that 18 (90%) patients were T1N0M0 and two (10%) patients were T2N1M0, those two patients were downstaged with six cycles of neoadjuvant chemotherapy.
Results
Successful localization occurred in all patients (100%) with no complications or allergic adverse outcomes. Mean diameter of tissues removed was 4.35±1.1 cm. Aesthetic outcomes postoperatively (based on Rose classification) were as follows: 14 (70%) excellent, three (15%) good, two (10%) fair, and one (5%) poor. No postoperative complications (dye persistence, incision infection, significant seroma, or mortality) occurred in any patient.
Conclusions
CN injection for early localization of nonpalpable BC lesions is effective, with a high safety profile and largely acceptable aesthetic outcomes.

Keywords:
carbon nanoparticles, early breast cancer, nonpalpable masses, oncoplasty

Egyptian J Surgery 2023, 41:690–696

1110-1121

Introduction

Breast cancer (BC) is the most prevalent cancer among women, GLOBOCAN statistics shows that it is the fifth cause in cancer-related deaths with an incidence of more than 2.3 million cases annually [1]. In the United States, 5-year incidence has increased by 0.3% per year, which is attributed mainly to rises in local stage and hormone-positive disease [2]. Data derived from the Global Burden of Disease study showed that disability-adjusted life years for BC worldwide was around 17.42 million years, and the rates for disability-adjusted life years was highest among Africans and sub-Saharan Africans [3]. Furthermore, BC affects individual health-related quality of life, both aesthetically and with a complex of symptoms that is directly attributed to the disease [4]. In recent years, a lot of efforts were paid to develop highly sensitive and cost-effective diagnostic methods to diagnose the disease early, with the added benefits of better overall survival, disease-free survival, distant disease-free survival, and limited surgical interventions [5].

Breast conservative surgery (BCS) with radiotherapy, chemotherapy, or systemic endocrine therapy is the standard of care for early, low-stage BC. The publication of the NSABP B-06 trial showed equivalent disease-free survival, distant disease-free survival, and overall survival among women who had undergone partial mastectomy with radiation compared with radical mastectomy [6]. This was confirmed by the 20-year follow-up of the study population and validated the initial results [7]. Furthermore, NSABP B-17 confirmed

the role of radiotherapy which reduced recurrence rates of both invasive and noninvasive disease [8]. After establishing BCS, a constant controversy existed on the ideal tradeoff between width of excision that achieves least recurrence rates with cancer-free margins on pathological examination, and aesthetic outcomes that is reached by reducing the diameter of the healthy tissues removed.

Many of early BC lesions are nonpalpable and appear surprisingly on mammography [9]. The gold standard for nonpalpable masses localization is wire-guided needle localization, as described by Dodd *et al*. [10]. The procedure has high versatility; however, certain serious limitations incur: first, wire insertion is done within the radiology department and coupling the schedules of both the surgeon and the radiologist may be difficult. Second, radiologist chooses the point of entry, which may not be the best entry point for wound placement, and this may complicate the aesthetic outcomes of oncoplastic surgery. Third, wire is left hanged from the patient's skin, which may be liable for displacement, migration, and patient distress. Fourth, it requires high and complicated technical expertise [11].

Many new techniques were proposed to combat these limitations, of which, carbon nanoparticles (CNs) seem to offer high durability with better complication profile [11]. Evidence showed that CNs did not diffuse into the surrounding tissues, and this may be attributed to their size that may enter lymph vessels but not blood vessels. CNs have a diameter of 150–200 nm, this theoretically makes them able to enter the gap between lymphatics which have a diameter of 120–500 nm; however, they cannot enter blood vessels, which have a diameter of 20–50 nm [12]. Still, the use of CNs in localizing nonpalpable breast masses needs further evaluation.

Aims and objectives

This study aims to examine the feasibility of CNs visualizing mass lesions. It also aims to examine the value of this technique on negative resection margins as well as the amount of tissues removed and its aesthetic outcomes.

Patients and methods

This is a prospective, single-arm pilot study to examine the feasibility and adverse outcomes of CNs in localizing nonpalpable, early-stage BC masses. The study took place between October 2020 and October 2021, and all the surgical procedures were performed within El-Demerdash Surgical Hospital. A total of 20 female patients with nonpalpable masses with diagnosis of early BC were enrolled. Staging procedures were performed routinely and as a part of the study for patients. All the procedures were performed by an experienced surgeon in the field of breast surgery. This study was conducted in full accordance with all applicable Ain Shams University Hospital and its scientific research committee principles and all applicable state laws. Full information sheet together with anticipated benefits and risks were provided to patients. This research was performed at the Department of General Surgery, Ain Shams University Hospitals. Ethical Committee approval and written, informed consent were obtained from all participants.

Study population

Inclusion criteria

(1) Patients with nonpalpable masses that are suggestive of early BC and those who have small masses and monocentric tumors.
(2) TNM Staging T1mi N0M0, TisN0M0, T1N0M0, and T2N0M0 (in dense breast and not palpable clinically).
(3) Patients who fulfilled their neoadjuvant treatment with downsizing of breast masses.
(4) Cases with vanishing masses with clips.

Exclusion criteria

(1) Patients who refused to participate in the study.
(2) Patients with larger masses who responded poorly to neoadjuvant chemotherapy.
(3) Patients with multicentric diseases.
(4) Patients who are pregnant in their first and second trimester.
(5) Patients with locally advanced disease.

Study outcomes

Primary outcome

(1) Margin-free samples as identified by the gold standard histopathological frozen section.

Secondary outcomes

(1) Success rates of preoperative localization.
(2) Maximum diameter of removed healthy tissues.
(3) Adverse outcomes such as nonvisualization, tissue staining, and wound infection.

Study interventions

Preoperatively

All patients had proper staging and risk stratification. This started first with full detailed history and physical examination together with all the relevant data that may impair surgical treatment or hinder BCS.

Patients had radiological assessment using either breast ultrasound, sonomammography, or breast MRI, according to their age. A detailed metastatic workup with axillary staging was also done. Tru-cut biopsies from breast masses were taken. If axillary disease was clinically evident, Tru-cut biopsies were also taken. All cases were discussed among the multidisciplinary team to determine the best management plan.

Procedure

(1) Carbon injection took place 1 day before the scheduled surgery or on the day of operation.
(2) Patient adequately exposed, area of interest was sterilized with betadine.
(3) Patient lay supine, ultrasound-guided local anesthesia (lidocaine) around 5–7 ml was injected subcutaneously at the site of lesion reported
(4) CNs of 120–150 nm was prepared on set mixed with distilled water at a ratio of 1: 1, average amount of 7–10 ml solution using a 16-G needle that was injected perilesional (5 mm away from the lesion) in four points anterior and posterior on both medial and lateral sides of the lesion with marking of the skin at sites of entries as shown in Figs 1 and 2.

Intraoperatively

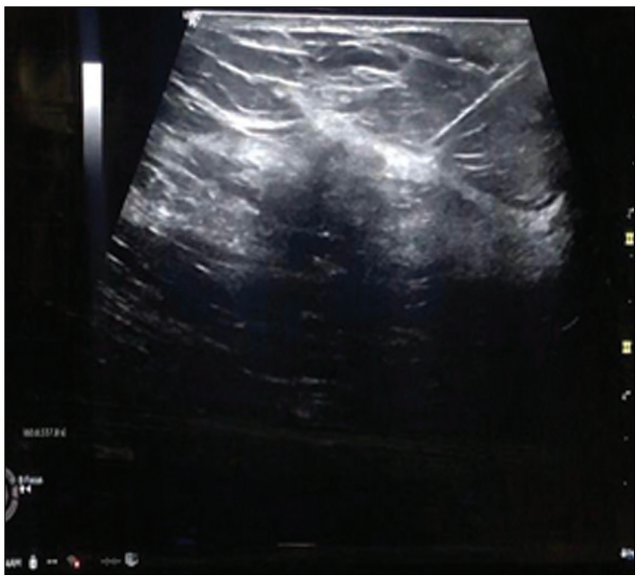
BCS not only aims to preserve the breast, but also it considers the aesthetics. A case-by-case approach was designated to plan surgical resection. This considered the size of the mass, its location within the breast, and placement of the incision. We followed the oncoplastic concepts developed by the French surgeon Krishna Clough. As most of our patients had tumors in the upper or lower outer quadrant, the most commonly used techniques were either round block or superior pedicle reduction. The lesion was removed en bloc along the outer edge guided by CN staining. Extracted masses were routinely fixed and sent for routine pathological examination as shown in Fig. 3. Axillary dissection was done according to the multidisciplinary team decisions individually for each case.

Postoperatively: all 20 patients received postoperative chemotherapy consisting of six cycles. Eighteen patients (Luminal A and Luminal B) received endocrine therapy. All of them received local radiotherapy 4 weeks after completion of adjuvant chemotherapy course. Patients were examined 2 weeks postoperatively to document any complications or adverse outcomes and to follow their progression. Follow-up continued up to 6 months to 1 year, during which patients were subjected to routine physical examination, standard blood tests, and colored ultrasound examination of the breasts. Patients had ultrasound examination of the abdomen, mammography, and chest radiograph done every 6 months. Aesthetic appearance of the breast was evaluated through the Rose method, where a separate examining physician evaluates the breast as excellent, good, fair, or poor.

Statistical analysis

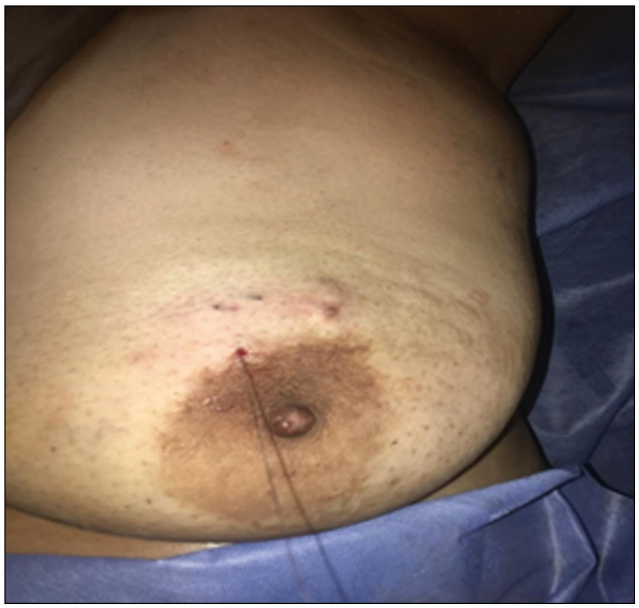
Data entry was done through Microsoft Excel spreadsheet. Continuous variables were expressed as mean±SD as a measure of variability in the data. Frequency data were summarized as percentages. Tabulation and statistical analysis were done using

Figure 1



Ultrasound-guided localization of mass with carbon nanoparticles.

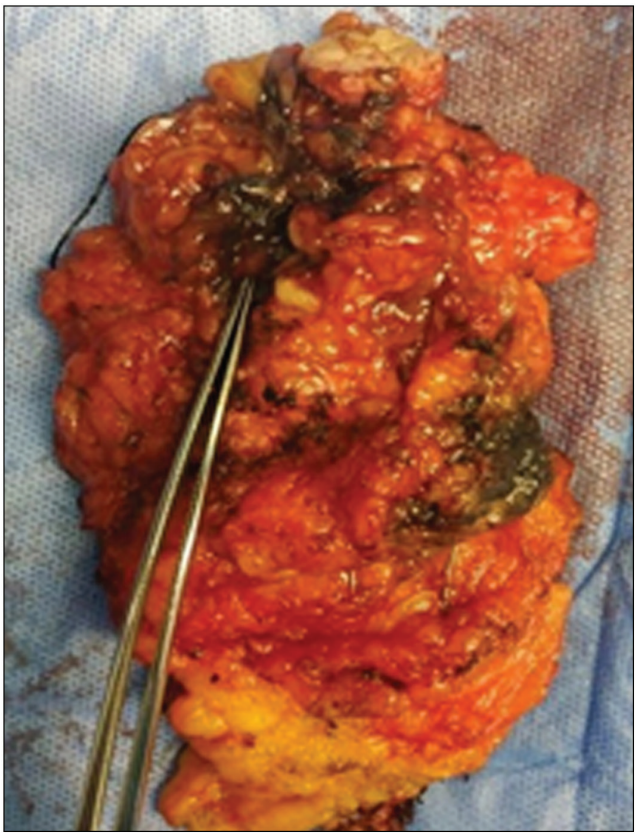
Figure 2



Marking of the skin at sites of entries.

IBM SPSS statistics for windows, Version 23.0 (Armonk, NY, USA: IBM Corp.).

Figure 3



Postoperative after excision of the mass.

Results

Baseline and preoperative characteristics

This study enrolled 20 female patients (*N*=20) who had undergone mass localization with CNs. Mean age was 50.05 ± 9.8 (range, 30–66 years). Eleven (55%) patients had tumors in the upper outer quadrant, six (30%) patients had tumors in the lower outer quadrant, two (10%) in the upper inner quadrant, and one (5%) in the lower inner quadrant. Median distance from the nipple was 5 cm and the mean distance was 4.75 ± 1.65 cm. All 20 (100%) patients had invasive ductal carcinoma on pathological examination. Ten (50%) patients had Luminal A, eight (40%) patients had Luminal B, and two (10%) patients were triple negative. On initial TNM staging, 18 (90%) patients were T1N0M0, two (10%) patients were T2N1M0, those two patients received six cycles of neoadjuvant chemotherapy and were downstaged to T1N0M0. Seven (35%) patients were injected with CNs the day before the surgery and 13 (65%) patients were injected on the same day of surgery. Table 1 summarizes these findings.

Resection margins and adverse outcomes

Preoperative localization of the mass was successful in all the patients (100%) and no complications or allergic adverse events occurred during the procedure. Only one lesion was identified in all patients. The diameter of the removed tissues ranged between 3 and 6 cm, with a mean diameter of 4.35 ± 1.1 cm. Frozen section examination of the resected specimens was negative for tumor cells in all resection margins (0%). A separate

Table 1 Baseline and preoperative characteristics of patients

	Value (percent/SD)	Notes
Mean age (in years)	50.05 (±9.8)	Range 30–66 years
Tumor location by quadrant		
Upper outer	11 (55)	
Lower outer	6 (30)	
Upper inner	2 (10)	
Lower inner	1 (5)	
Distance from the nipple by (cm)		
Median	5	Distance from nipple ranged between 2 and 7 cm
Mean	4.75 (±1.65)	
Histology		
Invasive ductal carcinoma	20 (100)	–
Luminal A	10 (50)	
Luminal B	8 (40)	
Triple negative	2 (10)	
Initial TNM staging		
T1N0M0	18 (90)	Those 2 patients who were T2N1M0 had 6 cycles neoadjuvant therapy and were downstaged to T1N0M0
T2N1M0	2 (10)	
Timing of injection		
Day before	7 (35)	–
Same day	13 (65)	

investigator evaluated the aesthetic outcomes of the breast and identified 14 (70%) patients as excellent outcome, three (15%) as good aesthetic outcome, two (10%) as fair, and one (5%) as poor outcome.

No postoperative complications (dye persistence, incision infection, serious seroma, or mortality) occurred (0%) during the follow-up period (6–12 months). Table 2 gives a summary of these findings. Figure 4 shows a scatter plot for the diameter of tissues removed from the breast.

Discussion

In this prospective, single-arm, open-label pilot study with 20 patients enrolled to examine the feasibility and safety profile of CNs, all the patients had negative resection margins identified by frozen section examination. Mean diameter of the removed tissues was 4.35 ± 1.1 cm. None of the patients had any serious adverse outcomes, most of the patients had acceptable aesthetic outcomes.

With the growing improvements in imaging techniques for detecting BC, namely mammography, breast ultrasound, MRI and core biopsy, together with increased patients' awareness, many nonpalpable breast masses are being identified [13]. These masses represent a clinical dilemma, and many controversies exist regarding their optimal management. Localization of the mass preoperatively helps to reduce false-negative results on histopathological examination and minimizes the volume of healthy tissue removed, and this may augment aesthetical outcomes [14]. Traditional criteria for adequacy of BCS focused mainly on durability, easiness, and patient and surgeon convenience [11]. These criteria are still relevant in today's world; however, with the increasing adoption of 'no tumor on ink' criteria for invasive BCS, this was subjected to newer discussions [15].

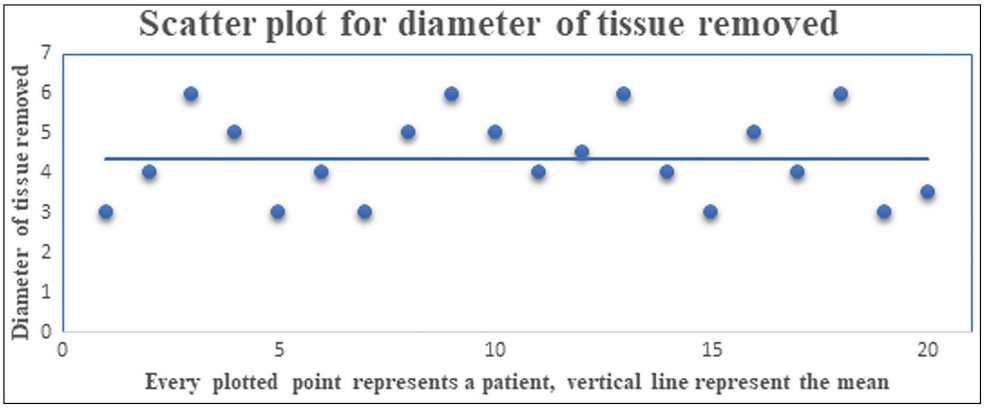
BCT became the standard of care for more than 25 years now; despite this, there was no clear consensus on which microscopic margin width is considered sufficient. Only the NSABP B-06 trial required clear microscopical margins, identified as 'no tumor on ink' [6]. This consequently resulted in one in four women who had a BCS undergoing re-excision, and approximately half of the women had no tumor on ink [16]. This resulted in further discomfort for the patients, increased resources expenditure, increased surgical complications, and poor cosmetic outcomes [17]. In response to these clinical question, the Society of Surgical Oncology (SSO) and American Society of Radiation Oncology (ASTRO) conducted a multidisciplinary expert panel to examine the relationship between resection margins and ipsilateral breast tumor recurrence. Moran *et al.* [15] examined 33 studies involving more than 28 162 patients and concluded that positive margins on ink was associated a twofold increase in ipsilateral breast tumor recurrence, this effect persisted after adjustment for tumor biology, endocrine therapy, or radiation. Tamirisa *et al.* [18] examined the effect of implementing these guidelines on reducing re-excision rates. They found that re-excision rates decreased after guideline implementation (23.5 vs 19.3%, $P<0.001$), and many of these re-excision operations were due to lobular-type carcinoma.

After Dodd and colleagues described their pioneering needle localization tool, many new techniques were described to improve patients' outcomes. Newer techniques can be classified as noninvasive, for example, intraoperative ultrasound guidance and radar reflectors' and injectables (toluidine/methylene blue dye, cryo-assisted localization, and radioguided occult lesion localization). Many of these techniques are promising; however, many of them have multiple limitations. Methylene blue may diffuse into the surrounding breast tissues, so timing between injection and operation should be carefully chosen, and if too much delay occurred between injection and operation this may result in inaccurate localization. Cryo-assisted localization may distort histopathological examination of the sample due to the effect of freezing; it also leads to considerably longer operative times. The radioguided occult lesion localization technique can be complicated with a phenomenon known as 'shine-through artifacts'

Table 2 Operative and postoperative outcomes

Outcome	*n* (%)
Successful preoperative localization	20 (100)
Complication during injection	0
Mean diameter of tissue removed (±SD)	4.35 ± 1.1 cm
Aesthetic outcomes (Rose criteria)	
Excellent	14 (70)
Good	3 (15)
Fair	2 (10)
Poor	1 (5)
Postoperative complications (dye persistence, incision infection, significant seroma, or mortality)	0%

Figure 4



A scatter plot for diameter of tissues removed from the breast.

where effects of high-energy positrons result in faulty mapping in sentinel lymph node biopsy [11].

CNs are being used increasingly in many fields of cancer therapy. Huang *et al.* [19] used it to protect parathyroid functions during thyroid surgery; these results was suggested by decreasing symptomatic postoperative hypocalcemia. However, a study conducted by Liu *et al.* [20] suggested a marginal benefit in thyroid surgery but not reaching statistical significance. This study also highlighted the importance of further evaluation of this new technique. Yan *et al.* [21] used CNs track lymph node metastases in T1-2 colorectal cancer. They reported that overall sensitivity, specificity, and accuracy of identifying LNs were 91.67, 100, and 98.63%, respectively.

Similar to our scope, Zhou and colleagues conducted a comparative study between CNs and methylene blue for the localization of nonpalpable breast masses. They concluded that both techniques are safe and feasible in localizing these masses with a more favorable profile in CN arm. They found a significant correlation in the duration between injection time and dyeing area in the methylene blue arm but not in the CN arm, suggesting that methylene blue is likely to diffuse into the surrounding tissue by time, unlike CNs. Operative time in CN arm was significantly lower than the methylene blue arm (P<0.001). Total resection volume was significantly lower in the CNs arm (P=0.016). No dye persisted in the methylene blue arm; however, this occurred in 19.4% of CNs (P=0.001) [22].

Jiang *et al.* [23] conducted another small study (N=16) with the aim of evaluating feasibility of CNs in localizing both nonpalpable breast masses and axillary lymph nodes. The resection margins were free in all 16 patients. The aesthetic appearance was 'good' in 13 patients, 'fair' in two, and 'acceptable' in the remaining patient. The findings of the last two studies mirror our findings which showed that negative resection margins were obtained in all samples, alleviating the need for further re-excisions. Furthermore, the aesthetic outcomes were reported to be comparable with the study conducted by Jiang and colleagues. Compared with Zhou's study, dye persistence occurred only in 10% of the patients (compared with 19.4%).

The advantages of these technique seem to be its high durability and allowing good visualization of the lesions, minimal surrounding tissue penetration, and lower relative cost compared with the other techniques mentioned above. This, however, needs to be confirmed in larger randomized studies to clearly examine the cause–effect relationship.

Conclusions

Use of CNs for preoperative localization of nonpalpable, early BC masses is efficacious, safe, and with acceptable aesthetic outcomes.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest.

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