

## Original article

## Does breast composition influence late adverse effects in breast radiotherapy?



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## ABSTRACT

**Background:** Large breast size is associated with increased risk of late adverse effects after surgery and radiotherapy for early breast cancer. It is hypothesised that effects of radiotherapy on adipose tissue are responsible for some of the effects seen. In this study, the association of breast composition with late effects was investigated along with other breast features such as fibroglandular tissue distribution, seroma and scar.

**Methods:** The patient dataset comprised of 18 cases with changes in breast appearance at 2 years follow-up post-radiotherapy and 36 controls with no changes, from patients entered into the FAST-Pilot and UK FAST trials at The Royal Marsden. Breast composition, fibroglandular tissue distribution, seroma and scar were assessed on planning CT scan images and compared using univariate analysis. The association of all features with late-adverse effect was tested using logistic regression (adjusting for confounding factors) and matched analysis was performed using conditional logistic regression.

**Results:** In univariate analyses, no statistically significant differences were found between cases and controls in terms of breast features studied. A statistically significant association ( $p < 0.05$ ) between amount of seroma and change in photographic breast appearance was found in unmatched and matched logistic regression analyses with odds ratio (95% CI) of 3.44 (1.28–9.21) and 2.57 (1.05–6.25), respectively.

**Conclusions:** A significant association was found between seroma and late-adverse effects after radiotherapy although no significant associations were noted with breast composition in this study. Therefore, the cause for large breast size as a risk factor for late effects after surgery and optimally planned radiotherapy remains unresolved.

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## Background

There is good evidence that large breast size is associated with increased risk of late adverse effects after breast conserving surgery and radiotherapy for early stage breast cancer [1–6]. Suboptimal dosimetry in large-breasted women explains at least part of this

association [4,5,7]. A randomised trial ( $N = 306$ ) comparing 3D and 2D radiation dosimetry showed that minimisation of unwanted radiation dose inhomogeneity in the breast significantly reduces late adverse effects [8]. However, a study by Goldsmith et al. [3] suggests that residual dose inhomogeneity in patients treated with optimal dosimetry was insufficient to explain the increased risks of late adverse effects associated with large breast size.

Assessment of late adverse effects is made by clinicians, using patient-reported outcomes and from patient photographs as recorded in several breast radiotherapy trials [9–11]. The effects of radiotherapy most easily noticed on photographs are changes in

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size and shape, with breast shrinkage being the commonest effect. There are suggestions that the shrinkage may result from atrophy of adipose cells in the breast. Preclinical studies suggest that radiation induces a significant reduction in both number and mean size of adipocytes with consequent reduction in fat pad weight in mice [12]. In large breasted women, the major component of the breast by volume is adipose tissue [13,14]. This raises the possibility that a higher proportion of adipose tissue in large breasted women may, in some way, be responsible for some of the effects after radiotherapy, particularly breast shrinkage.

The purpose of this study was to test the association of breast composition and fibroglandular tissue distribution with late adverse effects after breast conservation surgery and radiotherapy. This association, if found, could then be used for prediction of late adverse effects of breast radiotherapy.

## Methods

### Patients

All patients from The Royal Marsden who had participated in The Royal Marsden FAST Pilot and UK FAST Trial of radiotherapy hypofractionation for treatment of early breast cancer (ISRCTN62488883; CRUKE/04/015) [9,10] were identified. The FAST Pilot study recruited 30 patients aged  $\geq 50$  years with early invasive breast cancer (tumour size  $< 3$  cm, clear resection margins, negative axillary node status and no requirement for cytotoxic therapy). They were prescribed 30 Gy in five fractions over 15 days to the whole breast using tangential 6–10 MV X-ray beams and three-dimensional dose compensation. The UK FAST Trial recruited 915 women with similar characteristics as above from 18 centres, 75 of which were from The Royal Marsden. Patients were randomly assigned to 50 Gy in 25 fractions versus 28.5 or 30 Gy in 5 once-weekly fractions of 5.7 or 6.0 Gy, respectively, to the whole breast. Both trials collected standardised pre-radiotherapy baseline photographs of the breasts followed by repeat photographs at 2 years post-radiotherapy, along with annual clinical assessments. Change in breast appearance (size and shape) were scored by three observers on a 3-point scale (none, mild or marked change) from the serial photographs at 2 years post-radiotherapy compared with pre-radiotherapy baseline photographs [11].

Inclusion criteria for this study were, treatment at The Royal Marsden, availability of CT planning scan images and a baseline and 2-year photographic assessment of change in breast appearance. All eligible cases with mild or marked change in photographic breast appearance were matched on fractionation schedule, breast size and surgical deficit (both scored as small/medium/large from baseline photographs) to controls defined as having no change in photographic breast appearance at 2 years (Table 1). Where there was more than one possible matched control per case, all of these controls were selected for inclusion in the study. Other known confounding factors such as chemotherapy, lymphatic radiotherapy and radiotherapy boost were exclusion criteria for the FAST Pilot and FAST trials.

### Breast outlining

The CT data consisted of 3 mm axial slices (GE HiSpeed XQ/I (GE Healthcare Ltd, Buckinghamshire, UK)). On each slice whole breast was delineated by a single clinician (MB). The Hounsfield units of scar and seroma are similar to fibroglandular tissue and therefore these tissues are not easily differentiated using segmentation methods [15]. The contralateral breast was used for the analysis of breast composition and tissue distribution, in order to avoid the effects of post-surgical seroma and scar on breast tissue

**Table 1**

Summary of patient and treatment characteristics of cases and controls.

	Cases N = 18	Controls N = 36
Age in years		
Median (range)	66 (50–76)	65 (51–77)
Breast size, <sup>a</sup> n (%)		
Small	6 (33%)	13 (36%)
Medium	8 (44%)	19 (53%)
Large	4 (22%)	4 (11%)
Surgical deficit, <sup>a</sup> n (%)		
Small	7 (39%)	23 (64%)
Medium	7 (39%)	7 (19%)
Large	4 (22%)	6 (17%)
Fractionation schedule, n (%)		
Fast pilot trial – 30 Gy in 5 fractions over 3 weeks	5 (28%)	14 (39%)
Fast trial – 50 Gy in 25 fractions over 5 weeks	3 (17%)	8 (22%)
Fast trial – 30 Gy in 5 fractions over 5 weeks	6 (33%)	8 (22%)
Fast trial – 28.5 Gy in 5 fractions over 5 weeks	4 (22%)	6 (17%)

<sup>a</sup> Assessed from baseline photographs.

segmentation. The contralateral whole breast was outlined using anatomical landmarks on planning CT scans for the treated breast as described by Kirby et al. [16].

In some cases a portion of the breast was not contained in the field of view of the CT scanner (see Fig. 1b). These patients were excluded, with some exceptions:

1. In 5 patients for whom only the skin was marginally not contained in the field of view, the anterior/surface margin was brought right up to the visible edge.
2. In 2 patients for which a significant proportion of the contralateral breast was missing but the treated breast had a well-defined seroma, the treated breast and seroma was outlined with the seroma excluded for the breast analysis (Fig. 1b).

Seroma was delineated using previously published guidelines [17]. Examples of whole breast contours marked on a single CT slice are shown in Fig. 1.

### Breast tissue automatic segmentation

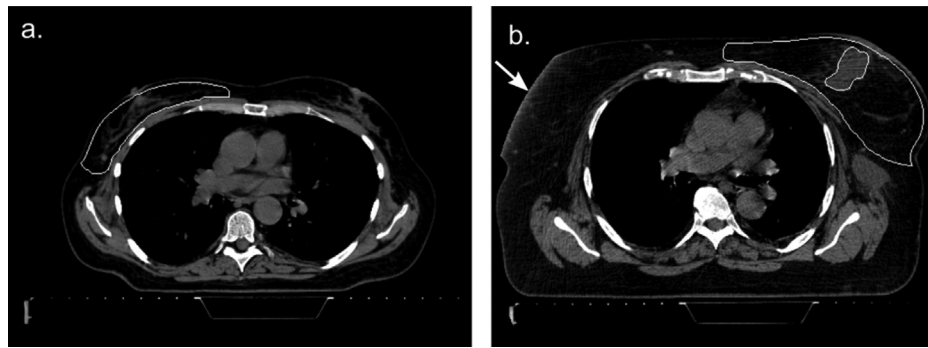
The whole breast as defined by the clinician on CT scans (see Fig. 1) was segmented into adipose and fibroglandular tissue using an automated segmentation method. Our previous study [15], showed that the fuzzy c-means clustering method with three classes (adipose, fibroglandular, and background) (FCM3) gave the most accurate breast tissue segmentation when validated against expert segmentation. FCM3 was used to find the volume of adipose tissue and percentage breast composition (BC) was calculated for all patient datasets using the following equation:

$$BC = 100 * V_{adi} / V_{tot}$$

where,  $V_{adi}$  and  $V_{tot}$  are the volume of adipose tissue and whole breast respectively.

### Clinician assessment of breast composition, fibroglandular tissue distribution, seroma and scar

Planning CT scan images were assessed by clinicians blind to patients' case/control status. Breast composition and fibroglandular tissue distribution were also ranked by 3 clinicians (AK, MB and NS), and seroma and scar were scored by a single clinician (MB) using the following methods:



**Fig. 1.** Sample mid-breast CT images with whole breast contour marked: a. Contralateral breast; b. Treated breast with seroma outlined for exclusion in breast analysis, as the contralateral breast profile was not fully contained within the field of view (shown by arrow).

**Breast composition:** For each patient the percentage of adipose tissue was assessed for the whole breast and given one of the following ranks: 1) <25%, 2) 25–50%, 3) 50–75% and 4) >75% of adipose tissue.

**Fibroglandular tissue distribution:** Our previous study [15] showed that fibroglandular tissue distribution affects the accuracy of breast tissue segmentation. This was studied further [18] and texture features were evaluated to identify the tissue distribution. Four representative ranks (on scale of 1–4, where 1 is the most sparse, i.e. very thin strands of fibroglandular tissue) were chosen based on 24 planning CT images from our previous studies [15], see Fig. 2. The distribution of fibroglandular tissue was ranked by the clinicians using the contralateral breast, where possible, in order to avoid the effects of seroma and scar tissue which have Hounsfield units similar to fibroglandular tissue. Where the contralateral breast was not fully contained within the field of view, the ipsilateral breast distribution was also considered for ranking. The whole breast was assessed and ranked. The images were viewed using the abdomen CT window setting (grey-level value range 294–2650).

**Assessment of seroma and scar tissue:** The amount of seroma and scar tissue in the treated breast at the time of radiotherapy planning were scored by the clinicians according to the following criteria. Seroma score: 1 = no seroma, 2 = some seroma, 3 = large seroma and scar score: 1 = no scar, 2 = some scar, 3 = large scar.

#### Statistical methods

Since the number of available matching cases and controls was small, the data were initially analysed using univariate analyses to compare the cases and controls in terms of clinician-ranked and algorithm-derived scores of breast density, amount of scar/seroma fluid, and distribution of fibroglandular tissue. BC as measured by auto-segmentation was compared between cases and controls using the t-test. Expert ranked breast composition and fibroglandular tissue distribution, and expert scored seroma and scar tissue were compared between cases and controls using Fisher's exact test. In some categories (rank/score) there were very small numbers (<3) of patients and therefore for statistical testing categories were combined. For breast composition, ranks 1 and 2, and ranks 3 and 4 were combined to form two categories: breast with  $\leq 50\%$  adipose tissue and breast with  $>50\%$  adipose tissue. For fibroglandular tissue distribution, ranks 1 and 2, and ranks 3 and 4 were combined to form sparse and non-sparse breast groups. For seroma and scar, scores 2 and 3 (some and large seroma/scar) were combined and compared with score 1 (no seroma/scar). Following univariate analysis, all the features were tested using logistic regression to compare cases and controls, adjusting for fractionation schedule,

breast size and surgical deficit. Finally a matched analysis was performed using conditional logistic regression. The primary analysis was unmatched in order to maximise the sample size, as some of the matched sets included more than one case and control. The Wald test was used to assess statistical significance in the regression models [19].

#### Results

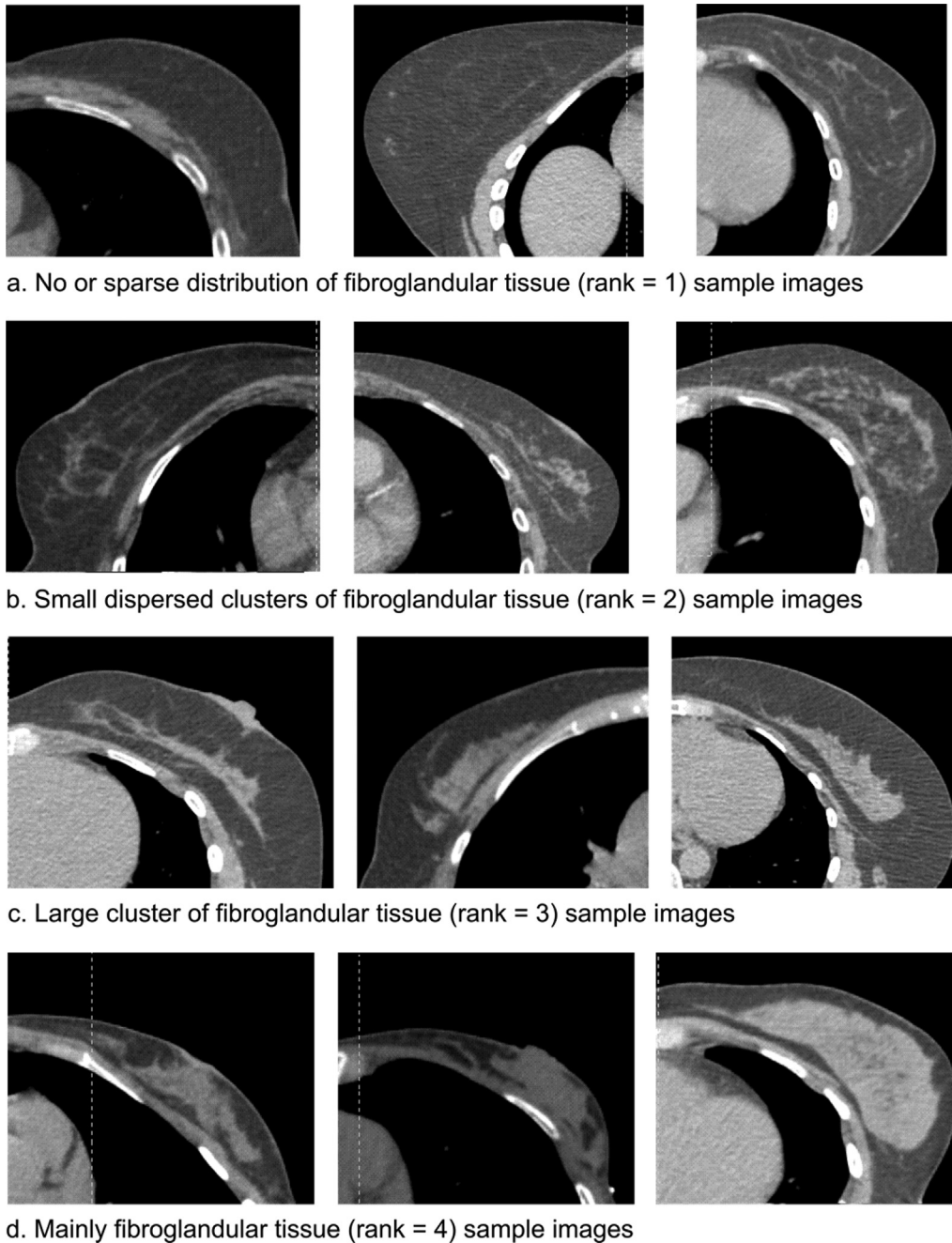
In total there were 18 cases with mild ( $n = 16$ ) or marked change ( $n = 2$ ) in photographic breast appearance at 2 years eligible for inclusion into the study. Thirty-three matched controls were selected, with at least one matched control per case (median number of controls per case = 1); there were 14 matched sets altogether, ranging in size from 1 case with 1 control to 3 cases with 4 controls. In addition, three unmatched controls were included in the dataset in order to increase the sample size. The analysis was based on these 54 patients who had evaluable planning CT images. Table 1 summarises the patient and treatment characteristics of the study sample.

#### Univariate analysis

Table 2 summarises the results from univariate analysis for the association of breast composition, fibroglandular tissue distribution, seroma, and scar with change in photographic breast appearance at 2 years. No significant differences were found between cases and controls in terms of breast composition, tissue distribution and scar. There was some evidence of higher seroma scores in the cases (78% with some or large seroma compared with 47% of controls, although this was only of borderline statistical significance ( $p = 0.07$ )).

#### Logistic regression

Results from the logistic regression analysis and matched analysis using conditional logistic regression are given in Table 3. Logistic regression analysis suggested that after adjusting for fractionation schedule, breast size and surgical deficit, there was a statistically significant association between amount of seroma and change in photographic breast appearance (odds ratio 3.44, 95% CI 1.28–9.21,  $p = 0.01$ ). Breast composition, breast tissue distribution and scar were not significantly associated with the measure of late effects. The significant association between amount of seroma and change in photographic breast appearance was also evident in the matched analysis (odds ratio 2.57, 95% CI 1.05–6.25,  $p = 0.04$ ).



**Fig. 2.** Sample mid-breast CT images for various distribution of fibroglandular tissue in the breast based on expert ranking: a. No or sparse fibroglandular distribution (rank = 1); b. Small clusters of fibroglandular tissue (rank = 2); c. Large cluster of fibroglandular tissue (rank = 3); d. Mainly fibroglandular tissue (rank = 4).

## Discussion

A number of studies have shown that large breast size is associated with an increased risk of late adverse effects [1–7]. This raises the possibility that the increased proportion of adipose tissue in large breasted women is, in some way, more sensitive to the effects of radiotherapy. We investigated the association of patient-related (breast composition, and tissue distribution), and treatment-related (seroma and scar) breast features with late adverse effects after breast conservation surgery and radiotherapy. Breast composition was evaluated by auto-segmentation method and by clinicians ranking of planning CT scan images. Against our expectation, the present analysis failed to confirm that breast

composition may explain the association between large breast size and late adverse effects. Breast tissue distribution and the presence of scar tissue were also not significantly associated with late adverse effects.

Accurate estimation of breast composition using tissue segmentation algorithms is difficult, even in case of 3D data such as MRI (magnetic resonance imaging) or CT. Nie et al. [20] noted in their study of breast MRI, a 3–6% variation in measurements of fibroglandular composition with body positioning. In our previous study [15], unexpectedly large variations (>10%) between prone and supine fibroglandular composition measurements were found for 7 of 24 patients. Furthermore clinician ranking of breast composition is widely variable [21]. Another limitation of the



**Table 2**

Results from univariate analysis, testing the association of breast composition measured by the auto-segmentation method and clinicians rank, fibroglandular tissue distribution, seroma, and scar with the late adverse effects.

Feature		Cases	Controls	p-Value
Breast composition	Auto-segmentation mean BC (SD)	83.2 (15.3)	81.1 (21.8)	0.72 <sup>a</sup>
	Ranks 1 & 2, n (%)	3 (16.7%)	9 (25.0%)	0.73 <sup>b</sup>
	Ranks 3 & 4, n (%)	15 (83.3%)	27 (75.0%)	
Fibroglandular tissue distribution	Sparse (ranks 1 & 2), n (%)	9 (50.0%)	14 (38.9%)	0.63 <sup>b</sup>
	Non-sparse (ranks 3 & 4), n (%)	9 (50.0%)	22 (61.1%)	
Seroma	None (score 1), n (%)	4 (22.2%)	19 (52.8%)	0.07 <sup>b</sup>
	Some/large (scores 2 & 3), n (%)	14 (77.8%)	17 (47.2%)	
	Scar	None (score 1), n (%)	6 (33.3%)	
Some/large (scores 2 & 3), n (%)	12 (66.7%)	22 (61.1%)		

<sup>a</sup> t-Test.

<sup>b</sup> Fisher's exact test.

**Table 3**

Results of unmatched and matched logistic regression analyses testing associations between breast features and late adverse effects.

	OR <sup>a</sup> (95% CI) p-value	OR <sup>b</sup> (95% CI) p-value
Automated segmentation: breast composition	0.98 (0.94–1.03) p = 0.46	0.99 (0.93–1.05) p = 0.70
Clinician ranked: breast composition	1.50 (0.53–4.24) p = 0.45	2.03 (0.71–5.79) p = 0.19
Breast tissue distribution	0.90 (0.32–2.58) p = 0.85	0.57 (0.20–1.62) p = 0.29
Seroma	3.44 (1.28–9.21) p = 0.01	2.57 (1.05–6.25) p = 0.04
Scar	1.19 (0.33–4.24) p = 0.79	1.07 (0.35–3.22) p = 0.91

<sup>a</sup> Odds ratio from logistic regression adjusted for fractionation schedule, breast size, and surgical deficit.

<sup>b</sup> Odds ratio from matched analysis using conditional logistic regression.

current work is that the analysis was based on the assumption that the breast composition and tissue distribution is the same when measured in contralateral and ipsilateral breast; this might not always be true. Alonzo-Proulx et al. [22] in their study, using digital mammograms of 15,351 individual women, found that the average relative difference in right and left breast density (percentage of fibroglandular tissue in the breast) was 21%.

However, our results showed that a larger seroma is significantly associated with late effects, measured using change in photographic breast appearance. The odds ratio of 2.57 obtained from the matched analysis suggests that for increase in the category of seroma (none to some or very large), the risk of being a case (i.e. of developing late effects) increases by an estimated factor of around 2.5, although the 95% confidence interval was wide (1.05–6.25). Previously Mukesh et al. have shown that the presence of seroma is an independent risk factor for late normal tissue toxicity (whole breast and tumour bed induration) at 2 and 5 years [23]. Their results suggested that seroma is associated with overall cosmesis, taking into account both surgical and radiotherapy changes using serial photographs. In the current study, cases and controls were matched for breast size to remove the influence of breast size on the outcomes.

Both seroma and scar could be defined more objectively through quantification of their respective sizes rather than visual assessment as performed in this study. However, these volumes are small and likely to have substantial variability in outlines with intra and inter-expert variations. Seroma and scar are often used as a surrogate for tumour bed in breast radiotherapy, where applicable. For tumour bed, substantial outline variability has been noted in a number of studies, including van Mourik et al. [24] who found mean conformity index to be 0.44 (range: 0.10–0.83). It is likely that seroma and scar will have at least similar uncertainty in outlining as tumour bed. Therefore, in the absence of accurate

measurement of seroma and scar sizes, qualitative visual assessments for these were used.

One limitation of this study is the small sample size, and in particular the number of eligible cases. In order to detect an odds ratio of 2 for a risk factor with 50% prevalence in the controls, we would need 200 cases and 200 controls, assuming a 2-sided significance of 0.05 and 80% power. It would therefore be interesting to investigate a patient cohort with a larger number of cases with the analysis approach developed here. It should be noted that the analysis presented in this study is based on photographic scores at 2 years follow-up and it is certain that not all patients have expressed their final level of toxicity. Therefore, investigation of late adverse effects at 5 years after the completion of radiotherapy is warranted. Furthermore, most of the cases (16 out of 18) had mild change in photographic breast appearance which may also have contributed to the lack of association with breast composition. The current Intensity Modulated Partial Organ Radiotherapy (IMPORT) High trial [25] is aimed at delivering an escalated dose to the tumour bed in the test groups for women at a higher than average risk of recurrence. This randomised trial of around 2500 patients might provide a good dataset for studying the association of breast composition with late adverse effects. Investigation of these large numbers of patients will need to be automated as much as possible through the validated methods in literature [15,18].

## Conclusions

In conclusion, this study found that seroma is significantly associated with late adverse effects of radiation as recorded by change in breast appearance on photographs at 2 years. Our preliminary results suggest that breast composition and tissue distribution, and a larger scar are not associated with late adverse effects.

However, this needs to be interpreted with caution given the small data set.

### Authors' contributions

All authors were involved in the design of the study, analysis and interpretation of the data and contributed to the writing of the report.

### Conflict of interest statement

None.

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