Research paper

Reproductive hormone analyses and effects of adjuvant zoledronic acid in early breast cancer – An AZURE (BIG 01/04) sub-study

Caroline Wilsona,⁎, Samantha Hinsleyb, Helen Marshallb, David Camerond, Richard Belld, David Dodwelle, Robert E. Colemana

a Academic Unit of Clinical Oncology, Weston Park Hospital, University of Sheffield, Sheffield, UK
b Leeds Cancer Clinical Trials Unit, University of Leeds, Leeds, UK
c Edinburgh Cancer Research Center, ECRC and Western General Hospital, University of Edinburgh, UK
d Deakin University, Geelong, Victoria 3220, Australia
e St James University Hospital, University of Leeds, UK

ARTICLE INFO

Keywords:
Adjuvant Bisphosphonate Breast Reproductive hormones Recurrence

ABSTRACT

Purpose: Adjuvant bisphosphonates have been shown to improve disease outcomes in early breast cancer in women who are postmenopausal at the start of treatment. We explored the influence of pretreatment serum levels of reproductive hormones in the hypothalamic-pituitary-gonadal (HPG) axis from a subset of patients included in the AZURE trial to investigate their impact on disease recurrence and whether reproductive hormone measurements are of value in selecting patients for treatment with adjuvant zoledronic acid.

Patients and methods; The AZURE trial is an academic, multi-centre, international phase III trial that randomised patients to standard adjuvant therapy (chemotherapy and/or endocrine therapy) ± intravenous zoledronic acid, 4 mg for 5 years. Serum from 865 patients taken at randomisation was stored at −80 °C prior to central batch analysis for inhibin A, oestradiol and follicle stimulating hormone (FSH). We assessed the clinical value of pretreatment hormone levels for predicting invasive disease free survival (IDFS), skeletal recurrence and distant recurrence and response to treatment with zoledronic acid.

Results: Oestradiol in the postmenopausal range ( < 50 pmol/l) was associated with a significantly shorter IDFS (HR 1.36 95%CI: 1.05–1.78 p=0.022), predominantly due to distant recurrence (HR 1.33 95%CI: 0.98–1.81 p=0.065), compared to oestradiol ≥50pmol/l. In contrast, FSH in the postmenopausal range ( > 26 IU/l) was associated with a longer time to bone first recurrence (HR 0.66 95%CI: 0.41–1.04 p=0.072) compared to an FSH ≤26 IU/l. When all 3 hormone levels were within the assay specified postmenopausal range, a trend to improved IDFS was seen with addition of zoledronic acid in biochemically postmenopausal women only (postmenopausal HR=0.81; 95%CI: 0.54–1.22, non-postmenopausal HR=0.99; 95%CI: 0.69–1.39) with risk reductions that mirrored the results of the main AZURE study, although the interaction between menopausal status and treatment effect was not statistically significant (p=0.47).

Conclusion: Oestradiol and FSH may influence the pattern of disease recurrence with postmenopausal levels possibly creating a less conducive environment for the formation of bone metastases, therefore disseminated tumour cells could seek alternative niches outside of bone. Biochemical evaluation of a panel of reproductive hormones may be helpful to assist selection of patients for adjuvant zoledronic acid when menopausal status is unknown.

1. Introduction

Bone is a common site for breast cancer metastases [1] and spread of tumour cells to bone may be an early event in the natural history of the disease, occurring before the primary tumour is clinically detected. These disseminated tumour cells (DTCs) have stem cell like properties [2] with the capacity to form new tumour colonies in bone. The presence of DTCs at diagnosis is an independent poor prognostic factor and 50% of patients with detectable DTCs will relapse within 10 years [3]. This relapse may be within the bone or at extra skeletal sites and can occur at any time over at least 20 years after the primary tumour diagnosis. The propensity of breast cancer for late relapse suggests that these DTCs may be held in a state of dormancy within the bone microenvironment with increasing evidence suggesting that dormant...
cells can reside in hematopoietic stem cell (HSC) and osteoblastic niches within the bone [4]. Within these niches the tumour cells are under the same local bone environmental factors that influence HSCs and may revert to a non-dividing phenotype showing cell cycle arrest [5] that confers resistance to adjuvant therapy [6].

The bone marrow microenvironment is therefore key in determining the fate of these DTCs, and can be modified by both bone targeted therapy such as bisphosphonates [7–9] and also host factors including the levels of reproductive hormones within the HPG axis such as oestradiol, FSH and inhibin A (an ovarian secreted hormone that inhibits FSH) [10–12].

Menopausal status has been shown to affect breast cancer recurrence (higher incidence and prevalence in premenopausal women) [3,13] indicating that hormones within the HPG axis may influence both the homing of breast cancer cells to bone and subsequent progression to established bone metastases.

Clinical trials of the bone targeting agents, bisphosphonates, have been conducted over the past 20 years with the aim of preventing the formation of bone metastases. Bisphosphonates are analogues of pyrophosphate that bind avidly to bone and are taken up by osteoclasts in which they induce apoptosis [14,15]. When combined with chemotherapy they appear to have direct anti-tumour effects in vivo [16]. Bisphosphonates such as zoledronic acid have been shown to affect cells within the bone microenvironment and influence the ability of tumour cells to both home to bone niches and establish as metastases. For example, zoledronic acid can reduce the proliferation and migration of HSC's, and thereby decrease their ability to attract tumour cells [17]. In clinical studies, zoledronic acid decreased the number of DTCs in bone marrow aspirates from breast cancer patients [18–20] suggesting either the DTCs had been killed, moved to another site in the body or had entered into a state of dormancy with altered surface protein expression that could not be detected by the tumour cell extraction techniques utilized in these studies.

The interplay between bisphosphonates, menopausal status and breast cancer recurrence was demonstrated in large prospective adjuvant phase III trials of zoledronic acid with improvements in disease outcomes with zoledronic acid demonstrated only in women who were either naturally in established menopause [7,8] or had undergone a chemically induced menopause [9]. A meta-analysis of individual patient data from 18,766 women treated with adjuvant bisphosphonates confirmed that women who were postmenopausal (defined clinically) at the initiation of adjuvant bisphosphonates had reduced recurrence rates at all distant sites (RR 0.82, 0.74–0.92; 2p=0.0003), in bone specifically (0.72, 0.60–0.86; 2p=0.0002) and

### Table 1
Baseline characteristics for the 806 patients included in the serum population analysis (patients receiving HRT, tibolone or endocrine therapy at baseline are excluded) in addition to the overall AZURE population.

<table>
<thead>
<tr>
<th>Overall study</th>
<th>Serum population</th>
<th>Standard treatment alone</th>
<th>Standard treatment+Zoledronic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>0</td>
<td>62</td>
<td>1.8</td>
<td>7</td>
</tr>
<tr>
<td>One–three nodes involved</td>
<td>2075</td>
<td>61.8</td>
<td>246</td>
</tr>
<tr>
<td>&gt; four nodes involved</td>
<td>1211</td>
<td>36</td>
<td>147</td>
</tr>
<tr>
<td>Unknown involvement</td>
<td>11</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1065</td>
<td>31.7</td>
<td>116</td>
</tr>
<tr>
<td>T2</td>
<td>1717</td>
<td>51.1</td>
<td>212</td>
</tr>
<tr>
<td>T3</td>
<td>456</td>
<td>13.6</td>
<td>56</td>
</tr>
<tr>
<td>T4</td>
<td>117</td>
<td>3.5</td>
<td>16</td>
</tr>
<tr>
<td>ER status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER positive</td>
<td>2634</td>
<td>78.4</td>
<td>304</td>
</tr>
<tr>
<td>ER negative</td>
<td>705</td>
<td>21.1</td>
<td>95</td>
</tr>
<tr>
<td>ER unknown</td>
<td>20</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Clinical menopausal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>1503</td>
<td>44.7</td>
<td>195</td>
</tr>
<tr>
<td>Less than/equal to 5 years post</td>
<td>490</td>
<td>14.6</td>
<td>57</td>
</tr>
<tr>
<td>More than 5 years post</td>
<td>1041</td>
<td>31</td>
<td>119</td>
</tr>
<tr>
<td>Menstrual status unknown</td>
<td>324</td>
<td>9.6</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>3359</td>
<td>100.0</td>
<td>400</td>
</tr>
</tbody>
</table>

### Table 2
Clinical and biochemical menopausal categorisation of patients in the serum population (patients receiving HRT, tibolone or endocrine therapy at baseline are excluded).

<table>
<thead>
<tr>
<th>Menopausal status (clinical categorisation)</th>
<th>Pre-menopausal</th>
<th>Less than or equal to 5 years since menopause</th>
<th>More than 5 years since menopause</th>
<th>Menstrual status unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Non post-menopausal</td>
<td>357</td>
<td>92.0</td>
<td>66</td>
<td>57.4</td>
<td>40</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>31</td>
<td>8.0</td>
<td>49</td>
<td>42.6</td>
<td>197</td>
</tr>
<tr>
<td>Total</td>
<td>388</td>
<td>100.0</td>
<td>115</td>
<td>100.0</td>
<td>237</td>
</tr>
</tbody>
</table>
after a median follow up of approximately 84 (IQR 71
first recurrence and distant recurrence. 966 DFS events were observed
improved overall breast cancer mortality (0.82, 0.73
pausal levels of hormones.
IDFS outcomes, skeletal and distant recurrence for non-postmenopausal vs postmeno-
surgically excised.
4 mg for 5 years, initially q3-4 wk and then with increasing intervals
and statin use. Patients received standard adjuvant therapy (che-
motherapy and/or endocrine therapy) ± intravenous zoledronic acid,
study centre status (premenopausal, unknown), type and timing of systemic therapy, study centre
randomized study treatment (zoledronic acid or control), and assess
the following minimization criteria; number of involved lymph nodes,
recruited 1:1 using a computer generated system which included
phase III trial that recruited 3360 women with node positive Stage
monitoring. Thirty-one (8%) clinically premenopausal women were biochemi-
treatment rather than postoperative adjuvant therapy as per the
in the AZURE trial at the time of the last data-lock on April 30th, 2013
5 years postmenopausal, > 5 years postme-
total 72.7 months.
Inhibin A analysis was performed on an automated ACCESS
Chemiluminescence immunoassay system from Beckman Coulter Ref
AZURE (BIG 01/04) trial taken prior to the commencement of
concentrations of follicle stimulating hormone (FSH), oestradiol and
< 3.6
Distant recurrence 0.99 (0.63–1.57) 0.972
Skeletal recurrence 1.1 (0.81–1.49) 0.534
Distant recurrence 1.15 (0.9–1.53) 0.239
Skeletal recurrence 0.99 (0.63–1.57) 0.972
Skeletal recurrence 0.96 (0.74–1.26) 0.779
Skeletal recurrence 0.66 (0.41–1.04) 0.072
Distant recurrence 0.86 (0.63–1.16) 0.331
The primary endpoint of AZURE was disease free survival (DFS)
assay (Roche 602 Elecsys electrochemiluminescence immunoassay). All assays
levels of reproductive hormones.
Baseline serum samples from 865 patients were collected following
informed consent and prior to randomized treatment allocation with
either standard adjuvant therapy alone (control) or standard adjuvant
therapy plus zoledronic acid. Serum was stored at −20 °C or below at
recruiting sites prior to shipment frozen for central storage at −80 °C in
Sheffield prior to central batch analysis. 59 patients were excluded due to
recent or current medication (hormone replacement therapy, tibolone and tamoxifen) at the time of serum collection that may alter
levels of reproductive hormones.
Assay specific reference ranges were used to compare postmeno-
pausal and non-postmenopausal levels of hormones. The following
hormone levels fulfilled the definition of postmenopausal; FSH >
26 IU/l, oestradiol < 50 pmol/l. Inhibin A < 3.6 pg/ml. If any one of
these criteria were not fulfilled the patient was classified as non-
postmenopausal. IDFS was selected as the primary endpoint for
analysis. Additional endpoints assessed were skeletal recurrence and
distant recurrence. Kaplan-Meier survival curves were used to assess
rates of DFS and cumulative incidence function curves were used to
assess rates of bone as first recurrence and distant recurrence. Differences in outcomes between groups were assessed using the log-
rank test and Cox’s proportional hazards model and analyses of
different hormone levels were adjusted for the factors found to be
significant for the relevant endpoint in the main AZURE analyses (i.e.
alyses were adjusted for randomised treatment only if this was
significant in the relevant main AZURE analysis). IDFS was adjusted
for tumour stage, ER status, lymph node involvement and use of neo-
adjuvant therapy rather than postoperative adjuvant therapy as per the
main AZURE analyses. Time to bone as first recurrence was adjusted
for randomised treatment allocation, tumour stage and lymph node
involvement (not adjusted for ER as this was not significant in the main
AZURE analysis). Time to first distant recurrence analyses were adjusted
for tumour stage, ER status and lymph node status. When
assessing the interaction of individual hormone levels with treatment,
alyses were adjusted for randomised treatment allocation. P-values
were considered significant at α < 0.05 with no adjustments made for
multiple testing as all findings are considered exploratory and hypothes-
is generating. All analyses were performed with the use of SAS
software version 9.2 or 9.4.

2. Results

3.1. Patient characteristics

860 patients were eligible for inclusion in the reproductive hor-
mone analysis, and the disease outcomes used for this analysis were
IDFS (230 events), bone as first recurrence (74 events) and distant
recurrence (174 events). Patient disease characteristics and menopau-
sal status of these 860 patients and the overall AZURE study popula-
tion are presented in Table 1. The characteristics of the subgroup
available for analysis were very similar to the overall AZURE popula-
tion. Thirty-one (8%) clinically premenopausal women were biochemi-

<table>
<thead>
<tr>
<th>Analysis</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol pmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 50pg vs ≥50)</td>
<td>1.36 (1.05–1.78)</td>
<td>0.022</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>1.15 (0.71–1.83)</td>
<td>0.575</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>1.33 (0.98–1.81)</td>
<td>0.065</td>
</tr>
<tr>
<td>Inhibin pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.6pg vs ≥3.6)</td>
<td>1.17 (0.9–1.53)</td>
<td>0.239</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>0.99 (0.63–1.57)</td>
<td>0.972</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>1.1 (0.81–1.49)</td>
<td>0.534</td>
</tr>
<tr>
<td>FSH IU/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt; 26pg vs ≥26)</td>
<td>0.96 (0.74–1.26)</td>
<td>0.779</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>0.66 (0.41–1.04)</td>
<td>0.072</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>0.86 (0.63–1.16)</td>
<td>0.331</td>
</tr>
</tbody>
</table>

If HR > 1 then the risk of experiencing an event is greater in the reference group (Φ).

2. Patients and methods

2.1. Study population

The AZURE trial was an academic, multi-centre, international
phase III trial that recruited 3360 women with node positive Stage
II/III breast cancer between 1.9.03 and 16.2.06. Patients were randomized 1:1 using a computer generated system which included
the following minimization criteria; number of involved lymph nodes,
clinical tumour stage, oestrogen receptor status, clinical menopausal
status (premenopausal, ≤5 years postmenopausal, > 5 years postme-
opausal, unknown), type and timing of systemic therapy, study centre
and statin use. Patients received standard adjuvant therapy (che-
motherapy and/or endocrine therapy) ± intravenous zoledronic acid,
4 mg for 5 years, initially q3-4 wk and then with increasing intervals
between doses [8]. Eligibility criteria for the AZURE trial have been
reported previously [8] and included patients aged ≥18 years with
histologically confirmed breast cancer with N1 disease or a T3-4
primary tumour. Patients were excluded if there was clinical or imaging
evidence of metastases or if the primary tumour could not be fully
surgically excised.

2.2. Patient evaluation

The primary endpoint of AZURE was disease free survival (DFS)
with pre-specified subgroup analyses planned for the minimization
criteria. Secondary endpoints included invasive disease free survival
(IDFS, excluding in situ recurrence)<sup>α</sup>, overall survival (OS), bone as
first recurrence and distant recurrence. 966 DFS events were observed
in the AZURE trial at the time of the last data-lock on April 30th, 2013
after a median follow up of approximately 84 (IQR 71–92) months.

*Table 3*

IDFS outcomes, skeletal and distant recurrence for non-postmenopausal vs postmeno-
pausal levels of hormones.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol pmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 50pg vs ≥50)</td>
<td>1.36 (1.05–1.78)</td>
<td>0.022</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>1.15 (0.71–1.83)</td>
<td>0.575</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>1.33 (0.98–1.81)</td>
<td>0.065</td>
</tr>
<tr>
<td>Inhibin pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.6pg vs ≥3.6)</td>
<td>1.17 (0.9–1.53)</td>
<td>0.239</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>0.99 (0.63–1.57)</td>
<td>0.972</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>1.1 (0.81–1.49)</td>
<td>0.534</td>
</tr>
<tr>
<td>FSH IU/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt; 26pg vs ≥26)</td>
<td>0.96 (0.74–1.26)</td>
<td>0.779</td>
</tr>
<tr>
<td>Skeletal recurrence</td>
<td>0.66 (0.41–1.04)</td>
<td>0.072</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>0.86 (0.63–1.16)</td>
<td>0.331</td>
</tr>
</tbody>
</table>
cally classified as postmenopausal, of these 1 woman was aged 29 and the remainder were aged ≥44 years. Forty (16.9%) clinically > 5 years postmenopausal women were biochemically classified as non-postmenopausal (see Table 2). Of these 43 women, the biochemical classification of postmenopausal status was not attained in the majority due to an oestradiol level still within the premenopausal range (70%).

3.2. Baseline oestradiol and FSH may influence disease recurrence patterns

Non-postmenopausal vs postmenopausal levels of FSH, oestradiol and inhibin A were compared for IDFS, bone as first recurrence and distant recurrence (see Table 3). An oestradiol of < 50 pmol/l (postmenopausal range) was associated with a significantly shorter IDFS compared to an oestradiol of ≥50 pmol/l (HR=1.36; 95% CI 1.05–1.78 p=0.022) (see Fig. 1A). Time to bone as first recurrence was not significantly different between postmenopausal and non-postmenopausal levels of oestradiol (HR=1.15; 95% CI 0.71–1.83, p=0.575), however, an oestradiol of < 50 pmol/l was associated with a shorter time to distant recurrence compared to an oestradiol ≥50 pmol/l (HR=1.33; 95% CI 0.98–1.81 p=0.065).

IDFS did not significantly differ between postmenopausal and non-postmenopausal levels of FSH (HR=0.96; 95% CI 0.74–1.26, p=0.7794); however, an FSH of > 26 IU/l (postmenopausal range) was associated with a longer time to bone as first recurrence compared to an FSH ≤26 IU/l (HR=0.66 95% CI: 0.41–1.04 p=0.072) although this was not statistically significant at the 5% level (Fig. 1B).

No significant differences in disease outcomes were seen between postmenopausal and non-postmenopausal levels of inhibin A.

The effect of age on recurrence, independent of treatment, was evaluated in the control group of patients (no zoledronic acid) in the overall AZURE population (n=1678). Comparison of recurrence rates by age categories showed age to be a significant prognostic factor with a
Fig. 2. Overall invasive disease free survival according to age. Data represents adjusted hazard ratios (HR) and 95% confidence interval (CI).

Table 4
IDFS outcomes, skeletal and distant recurrence according to age.

<table>
<thead>
<tr>
<th>Analysis according to age category</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IDFS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–49 vs &lt; 40</td>
<td>0.87 (0.66–1.15)</td>
<td>0.0025</td>
</tr>
<tr>
<td>50–59 vs &lt; 40</td>
<td>0.81 (0.61–1.07)</td>
<td></td>
</tr>
<tr>
<td>60–69 vs &lt; 40</td>
<td>1.22 (0.91–1.64)</td>
<td></td>
</tr>
<tr>
<td>≥70 vs &lt; 40</td>
<td>1.44 (0.92–2.26)</td>
<td></td>
</tr>
<tr>
<td><strong>Skeletal recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–49 vs &lt; 40</td>
<td>0.81 (0.52–1.25)</td>
<td>0.12</td>
</tr>
<tr>
<td>50–59 vs &lt; 40</td>
<td>0.75 (0.48–1.17)</td>
<td></td>
</tr>
<tr>
<td>60–69 vs &lt; 40</td>
<td>0.82 (0.5–1.35)</td>
<td></td>
</tr>
<tr>
<td>≥70 vs &lt; 40</td>
<td>1.82 (0.92–3.58)</td>
<td></td>
</tr>
<tr>
<td><strong>Distant recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–49 vs &lt; 40</td>
<td>0.86 (0.58–1.29)</td>
<td>0.068</td>
</tr>
<tr>
<td>50–59 vs &lt; 40</td>
<td>0.79 (0.53–1.19)</td>
<td></td>
</tr>
<tr>
<td>60–69 vs &lt; 40</td>
<td>1.25 (0.82–1.91)</td>
<td></td>
</tr>
<tr>
<td>≥70 vs &lt; 40</td>
<td>1.32 (0.71–2.47)</td>
<td></td>
</tr>
</tbody>
</table>

If HR < 1 then the risk of experiencing an event is greater in the reference group (< 40).

Fig. 3. Invasive disease free survival (IDFS) outcomes in the main AZURE population and the serum AZURE population. Data represents adjusted hazard ratios (HR) and 95% confidence interval (CI). Postmenopausal (PM) represents patients clinically > 5 years PM in the main AZURE population or biochemically postmenopausal in the serum population, and non-postmenopausal (non-PM) represents patients < 5 years PM, premenopausal and unknown menopausal status in the main AZURE population or biochemically non-postmenopausal in the serum population. The heterogeneity of treatment effect according to menopausal status was statistically significant in the main AZURE population (p=0.0300) but not in the serum AZURE population (p=0.4699).
shorter IDFS in patients < 40 years and ≥60 years (p=0.0025) (see Fig. 2). The recurrence sites in women < 40 years suggest they experienced more skeletal recurrences as their first recurrence than any other age group (excluding women ≥70 years in whom there were fewer events and thus wide confidence intervals around the recurrence risk estimates). Women ≥60 years experienced more distant recurrence compared to women < 40 years which appears to be driven by recurrence outside the bone (see Table 4).

3.3. Biochemically postmenopausal patients continue to experience improved IDFS outcomes with zoledronic acid

The effect of zoledronic acid on IDFS was compared in biochemically postmenopausal women and non-postmenopausal women using a combined assessment of oestradiol, FSH and inhibin A (see Fig. 3). The risk reduction for IDFS with the addition of zoledronic acid to standard therapy in biochemically postmenopausal women (HR=0.81; 95% CI 0.54–1.22) was similar to that seen in the overall study (HR for women > 5 years postmenopausal 0.77; 95% CI 0.63–0.96) although, probably due to relatively small number of events, the upper 95% confidence interval overlaps unity. In biochemically non-postmenopausal women, IDFS was unaffected by treatment allocation (HR=0.98; 95% CI 0.69–1.39), mirroring the findings of the overall study (HR for women not > 5 years postmenopausal=1.03 95% CI 0.89–1.2). However, unlike in the study as a whole and probably due to the limited number of events, the interaction between menopausal status and treatment was not statistically significant (p=0.47). There was a trend towards a reduced skeletal recurrence in biochemically pre- and postmenopausal patients (postmenopausal HR 0.81; 95% CI 0.37–1.8, premenopausal HR 0.82; 95% CI 0.46–1.46) with additional benefit in distant non skeletal disease recurrence suggested in postmenopausal women only (HR 0.91; 95% CI 0.5–1.7). These findings were not statistically significant again perhaps due to a limited number of events. Further follow-up with the addition of more events should help clarify these findings.

4. Discussion

These results suggest that women with postmenopausal levels of oestradiol at diagnosis have a significantly shorter IDFS, which may be driven by recurrence outside the bones. This may be due to the effect of the hormones on bone cell function creating a microenvironment that is either less attractive to DTCs or less conducive to their autonomous growth into metastasis, and potentially influencing them to seek alternative ‘metastatic niches’ outside of bone. The increased distant recurrence rate in women with low oestradiol was probably linked to selection of older women with more aggressive disease for the trial rather than poorer disease outcomes per se in older women. The suggested increase in skeletal recurrence in the under 40 s may infer that FSH can influence bone recurrence as this age group would be expected to have low FSH levels due to negative feedback on the pituitary from high levels of cycling ovarian hormones. However, the relatively small number of events limits our study and further data are required to address this definitively.

Our findings are supported by clinical studies evaluating the presence of DTCs from breast cancer in the bone marrow at diagnosis. A large meta-analysis (n=4700) of the prevalence of bone marrow DTCs demonstrated that premenopausal patients had a significantly higher prevalence compared to postmenopausal women (premenopausal 32.7%, post menopause 29.5% p=0.02) [3] and a retrospective evaluation of recurrence patterns in 7064 women diagnosed with breast cancer showed younger women were significantly more likely to develop bone metastases [13], suggesting premenopausal bone may be a more attractive microenvironment for survival of tumour cells. In addition, data on recurrence patterns of 6792 breast cancer patients entered into trials conducted by the International Breast Cancer Study Group showed that younger patients (< 35 years) had significantly higher incidences of bone metastases occurring during the course of their disease [22].

Improving disease outcomes by addition of zoledronic acid to standard therapy was evaluated in the AZURE study [8] and the ABCSG 12 study [23]. In these studies sub-group analyses showed that the beneficial effects of zoledronic acid on disease outcomes were driven by women in whom very low levels of HPG activity would be expected > 5 years since menopause in AZURE (overall survival; HR=0.75; 95%CI 0.59–0.96 p=0.02) and > 40 years and receiving goserelin in ABCSG-12 (disease free survival; HR=0.58, 95% CI 0.40–0.84). The beneficial effects of adjuvant bisphosphonates have been confirmed in a large meta-analysis of pre- and postmenopausal patients treated in adjuvant bisphosphonates trials. For the meta-analysis, patients were defined clinically as pre- or postmenopausal. In postmenopausal women there was a significant reduction in breast cancer recurrence, particularly in bone, and a clinically important reduction in breast cancer mortality [21]. Biochemical analysis of hormones was not performed in all of these trials and therefore its utility in selecting patients for adjuvant bisphosphonates has not been evaluated in a large patient cohort. Our results suggest that selection of patients for adjuvant bisphosphonates where menopausal status is unknown might be done using a biochemical analysis of inhibin A, FSH and oestradiol to confirm levels are within the postmenopausal range for the assay used.

Pre-menopausal/younger patient is associated with relatively low levels of osteoclast activity and thus alternative mechanisms may be driving the homing to and establishment of DTCs in bone. This may explain why bisphosphonates, as osteoclast targeting drugs, are not affecting disease outcomes in premenopausal women and there remains a need to identify bone-targeting drugs with efficacy for tumour prevention in this group of younger patients. The molecular mechanisms underlying the failure of non-postmenopausal women to derive benefit from zoledronic acid are not yet identified. Our data suggest that oestradiol and FSH may be able to influence the homing to and survival of DTCs within specific tumour ‘niches’ either in bone or in other distant sites. The efficacy of zoledronic acid in affecting survival of tumour cells may then be different according to whether the burden of DTCs are within bone or not. This remains hypothesis generating and requires further evidence from both pre-clinical and clinical research.

Funding

Funding support for this study was received for research time from Weston Park Cancer Charity (Grant no. CA101A, CA140) and the Sunita Murali Charitable fund.

Acknowledgements

Thanks to Sheffield Teaching Hospitals NHS Foundation Trust laboratories for performing the hormone analyses. Thanks to H Shulver (supported by ECMC) and J Horsman for their support in the laboratory and with data management. Many thanks to the AZURE patients, who consented to collection of their blood for future research.

References


7