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Research paper

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

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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 as 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

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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.

	Overall study		Serum population			
			Standard treatment alone		Standard treatment+Zoledronic acid	
	Number	Percent	Number	Percent	Number	Percent
Lymph nodes						
0	62	1.8	7	1.8	9	2.2
One–three nodes involved	2075	61.8	246	61.5	240	59.1
= > four nodes involved	1211	36	147	36.8	155	38.2
Unknown involvement	11	0.3	0	0.0	2	0.5
T stage						
T1	1065	31.7	116	29.0	136	33.5
T2	1717	51.1	212	53.0	188	46.3
T3	456	13.6	56	14.0	69	17.0
T4	117	3.5	16	4.0	13	3.2
ER status						
ER positive	2634	78.4	304	76.0	316	77.8
ER negative	705	21	95	23.8	88	21.7
ER unknown	20	0.6	1	0.3	2	0.5
Clinical menopausal status						
Pre-menopausal	1503	44.7	195	48.8	193	47.5
Less than/equal to 5 years post	490	14.6	57	14.3	58	14.3
More than 5 years post	1041	31	119	29.8	118	29.1
Menstrual status unknown	324	9.6	29	7.3	37	9.1
Total	3359	100.0	400	100.0	406	100.0

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

	Menopausal status (clinical categorisation)								Total	
	Pre-menopausal		Less than or equal to 5 years since menopause		More than 5 years since menopause		Menstrual status unknown			
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Menopausal status (biochemical categorisation)										
Non post-menopausal	357	92.0	66	57.4	40	16.9	42	63.6	505	62.7
Post-menopausal	31	8.0	49	42.6	197	83.1	24	36.4	301	37.3
Total	388	100.0	115	100.0	237	100.0	66	100.0	806	100.0

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 bone 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