A comparison of the third-generation aromatase inhibitors and their effects on postmenopausal women with early hormone sensitive breast cancer

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For Mum, Dad, Maggie, Jamie, Andrew and Morven who have loved, supported and encouraged me through many years of study.

'A dream doesn't become reality through magic; it takes sweat, determination and hard work'.

Colin Powell

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ABSTRACT

Breast cancer is the most common malignancy and the second highest cause of cancer death in women. Many of these tumours are oestrogen dependent and can be treated using compounds which are themselves antioestrogenic or reduce the production of oestrogen. Until recently, the selective oestrogen receptor modulator (SERM) tamoxifen was prescribed as standard adjuvant therapy for postmenopausal women with hormone sensitive tumours. Several studies have shown that in postmenopausal women, the newer aromatase inhibitors (AIs) are effective at suppressing plasma oestrogen levels and are more efficacious than tamoxifen. Although their use is increasing there remain concerns about their side-effects. There are two classes of AIs: type 1 which are irreversible, steroidal inhibitors and include the drug exemestane; type II that are reversible, non-steroidal inhibitors and include the drugs letrozole and anastrozole. Each has similar but different levels of oestrogen suppression and it remains to be determined whether these differences in suppression of aromatase translate into differences in clinical benefits and side-effects.

The purpose of the following studies was to assess the pharmacodynamic differences between the AIs anastrozole, letrozole and exemestane in postmenopausal women with early hormone sensitive breast cancer. The oestrogen-depriving effects of AIs have been reported to be associated with an increased risk of osteoporosis and cardiovascular disease alongside a potential reduction in the risk of thromboembolic events. These studies therefore investigated the differential effects of the more commonly used AIs, anastrozole, letrozole and exemestane on bone turnover markers, lipid profiles, coagulation parameters and quality of life (QOL).

ALIQUOT (Anastrozole vs Letrozole, an Investigation of Quality of Life and Tolerability) was an open randomised pharmacodynamic study in which 185 patients

were randomised to receive either 3 months s of letrozole followed by 3 months of anastrozole or 3 months of anastrozole followed by 3 months of letrozole. 39 patients had received prior tamoxifen therapy. Blood and urine samples were collected at baseline and after 3 months of each drug. Hormone naïve (i.e. those who did not receive prior tamoxifen) patients were switched to tamoxifen after 6 months and further samples obtained after an additional 3 months. Validated QOL questionnaires were collected during treatment.

ALEX (a randomised study of the effects of Anastrozole, Letrozole and Exemestane on bone turnover, lipid metabolism and coagulation) was an open randomised pharmacodynamic study in which 120 patients were randomised to receive 4 months of either drug and then switched to tamoxifen. Similar blood and urine samples were collected at baseline, after 3, 4 and 12 months.

Results demonstrated that each AI increased bone turnover markers. The non-steroidal AI exemestane showed a greater increase in markers of bone turnover compared with the non-steroidal AIs. Significant changes in bone turnover were observed when tamoxifen was withdrawn and a non-steroidal AI commenced. The data from these studies suggest that any benefit from tamoxifen in increasing bone density is likely to be lost in the months and years after treatment is stopped. Patients who take anastrozole or letrozole following tamoxifen need the same bone monitoring as any patient taking anastrozole or letrozole alone.

The non-steroidal AIs had different effects on lipid profiles compared to the steroidal group. Exemestane caused an increase in atherogenic ratios and a decrease in the cardioprotective high density lipoprotein (HDL) compared to the non-steroidal AIs. This supports studies suggesting that exemestane may have a negative impact on lipid levels and may increase the risk cardiovascular disease (CVD).

There were no significant differences between coagulation parameters in the patients treated with non-steroidal AIs. However exemestane caused a significant reduction in several anticoagulants predisposing to a potential increased risk of thromboembolic disease.

Data presented in this thesis indicate that steroidal and non-steroidal AIs have different metabolic effects on bone, lipids and coagulation and suggest each will have different side effect and morbidity profiles. These observations have important implications when considering which AI to use in the clinical setting and how patients on different drugs should be monitored.

Declaration

I declare that this thesis is entirely my own work. I was responsible for project management of the trials including patient recruitment and obtaining specimens. The bone marker measurements were performed with Dr Rosemary Hannon's team at the Metabolic Bone Centre, Northern General Hospital, Sheffield. Lipid analysis were performed with Professor RA Riemersma and Dr AF Howie in the Centre for Cardiovascular Science, Queen's Medical Research Institute, Royal Infirmary of Edinburgh. Coagulation analysis were performed by Professor Chris Ludlam and Mrs Pamela Dawson in the Department of Clinical Haematology, Royal Infirmary of Edinburgh. Statistical analysis were performed by Dr Linda Williams at the Centre for Population Health Sciences, University of Edinburgh.

This thesis has not been submitted in candidature for any other degree, postgraduate diploma or professional qualification.

22/05/12

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Abbreviations

*	p<0.05
**	p<0.01
***	p<0.001
ABC	Advanced breast cancer
ACPR	Activated protein C resistance
AE	Adverse events
AH	Atypical hyperplasia
AI	Aromatase inhibitor
ALEX	A randomised study of the effects of Anastrozole, Letrozole and Exemestane on bone turnover, lipid metabolism and coagulation
ALIQUOT	Anastrozole vs Letrozole, an Investigation of Quality of Life and Tolerability
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
Apo	Apolipoprotein
ASCO	Americal society of clinical oncology
ATAC	Anastrozole vs tamoxifen vs combined
BCE	Bone collagen equivalents
BMD	Bone mineral density
BRM	Joint pain score
Cr	Creatinine
CRF	Clinical research folder
CV	coefficient of variation
CVD	Cardiovascular disease
DCIS	Ductal carcinoma in situ
DEXA	Dual energy X-ray absorptiometry
DFS	Disease free survival
ER	Oestrogen receptor
ER+ve	Oestrogen receptor positive
FACT-B+ES	Functional assessment of cancer therapy - breast + endocrine subscale
FNAC	Fine needle aspiration cytology
FSH	Follicle stimulating hormone
GnRH	Gonaotrophin-releasing hormone
HDL	High-density lipoprotein
HER2	Human erb receptor 2
HRT	Hormone replacement therapy
LCIS	Lobular carcinoma in situ
LDL	Low-density lipoprotein
LEAP	Letrozole, exemestane and anastrozole in healthy postmenopausal women
LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
MRI	Magnetic resonance imaging
NPI	Nottingham prognostic index
NSABP	National Surgical Adjuvant Breast & Bowel Project
NST	No specific type
OS	Overall survival
OPG	Osteoprotegerin
PAI	Plasminogen activating inhibitor antigen
PGE	Prostaglandin E
PgR	Progesterone receptor
PINP	Procollagen type 1 N-terminal propeptide
PTH	Parathyroid hormone
QOL	Quality of life

RANKL	Nuclear factor-kappa B ligand
sCTX	Serum C-terminal cross-linked telopeptide
SD	Standard deviation
SERM	Selective oestrogen regulating modulator
SNP	Single nucleotide polymorphism
TDLU	Terminal duct lobular unit
UICC	Internationial Union Against Cancer
uNTX	Urinary N-terminal cross-linked telopeptide
VLDL	Very low density lipoproteins
VTE	Thromboembolic events
vWF	von Willebrand factor
WHO	World health organisation

SECTION 1: INTRODUCTION

1.1 Aims and objectives

AIs reduce circulating oestrogen and therefore affect not only hormone dependent breast tumours but also have consequences on other non-tumour related systems affected by oestrogen. Although extensive research on AIs has been undertaken, there is very little research directly comparing the effects of the different drug types on morbidity, tolerability and quality of life (QOL).

The aim of the ALIQUOT study was to evaluate the effects of the non-steroidal AIs anastrozole and letrozole on bone, lipid and quality of life profiles in postmenopausal women with oestrogen receptor positive (ER+ve) breast cancer.

The aim of the ALEX study was to further evaluate the effects of non-steroidal AIs and also the effects of the steroidal AI, exemestane on bone, lipid, coagulation and quality of life profiles in postmenopausal women with ER+ve breast cancer.

1.2 Breast cancer epidemiology

1.2.1 Incidence and prevalence

Since 1950 the incidence of breast cancer has been rising. Breast cancer remains a significant public health problem which impacts on the wellbeing of women worldwide with considerable social and economic cost. By 2010 the annual global burden of new breast cancer cases was expected to reach 1.5 million with an ever-increasing number from developing countries[1]. The increasing incidence may be explained by a number of factors including greater longevity, delays in starting a family, the use of hormone replacement treatment (HRT) and an increased breast cancer detection rate. There was a marked increase in the UK incidence of breast cancer following the implementation of the National Breast Cancer Screening Programme which was introduced in 1988. Breast cancer affects 1 in 10 women by the age of 80 and is the most frequent cancer affecting elderly women[2].

Despite the increase in incidence, there has been an improvement in overall survival which is thought to be due to a combination of earlier diagnosis resulting from improved public health initiatives including screening programmes; better treatments including the use of tamoxifen; an increase in care specialisation; and a recent fall in the use of HRT. Notwithstanding this, breast cancer remains the leading cause of UK cancer death in women and resulted in 12,116 deaths in 2008[3]. The UK incidence and mortality rates are shown in figure 1.1.

The prevalence of breast cancer is also increasing due to the increased incidence and improvement in survival. Approximately 172,000 women are currently living with breast cancer in the UK and over 75% of women diagnosed with breast cancer are alive at least after five years after diagnosis[3].

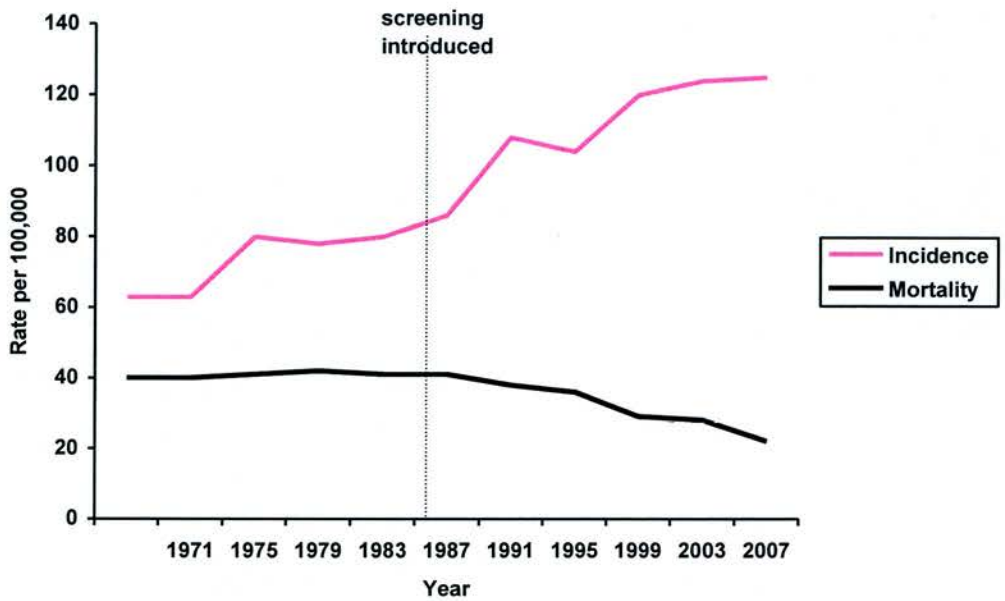


Figure 1.1 Incidence and mortality rates from breast cancer in UK
(Adapted from the Office for National Statistics www.statistics.gov.uk 2008[3])

1.2.2 Geography

In the UK breast cancer is the leading cause of death in women under 54 years of age and is surpassed only by lung cancer mortality in those over this age. In 2007 45,972 women were diagnosed with breast cancer and 12,116 died from their disease in 2008[3]. Age standardised incidence and mortality in the UK is among the highest in the world. Of interest, some particular areas are associated with significantly lower rates of breast cancer e.g. Wales and Yorkshire compared with the South West of England. To a degree, these variations reflect the positive relationship between breast cancer and socio-economic class with higher socio-economic status being associated with increased risk. This disparity is largely explained by reproductive risk factors e.g. women in lower socio-economic strata have more children at a younger age. It may also be related to more affluent women attending screening more readily and they therefore have an increased detection rate[3].

There exists a marked variation in the rates of breast cancer among populations worldwide with the highest incidence among women in the USA and Western and Northern Europe. The lowest incidence is found in Asia, India and Africa however this gap is closing with the transition towards Western lifestyles including a change in diet and reproductive trends. Migrants from low to high risk countries acquire the risk of their host country within two generations as observed in women from Japan who migrated to USA[4].

Despite the fact that breast cancer is predominantly viewed as a disease of developed countries, the majority of breast cancer deaths each year occur in the developing world[1]. The international incidence of female breast cancer is shown in figure 1.2.

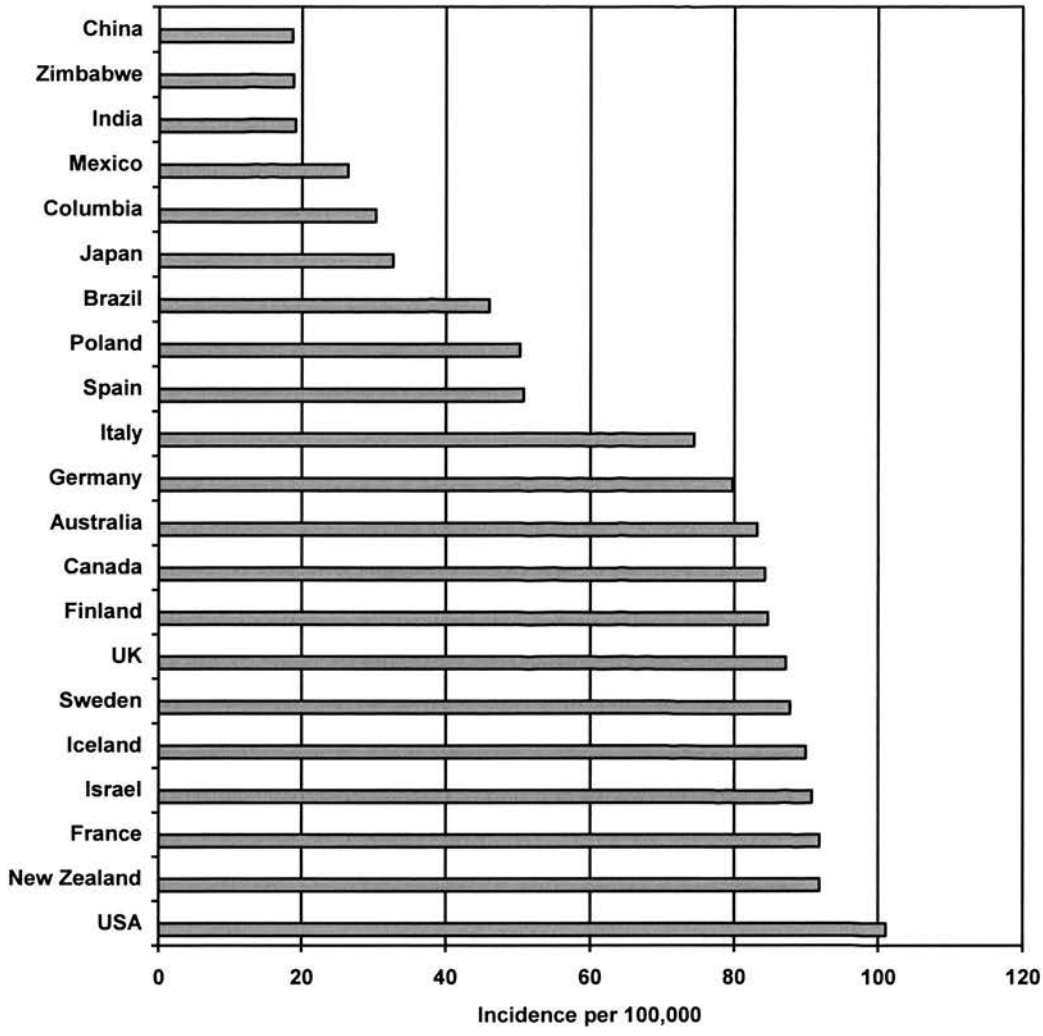


Figure 1.2 International variation in breast cancer incidence among women from 2000-2005, per 100,000 women-years
(Data adapted from GLOBOCAN[5] and Cancer Research UK 2002[3])

1.2.3 Age

The incidence of breast cancer increases sharply with increasing age as shown in figure 1.3. It is extremely rare in those under the age of 20 years and uncommon in those under the age of 30 years. The incidence of breast cancer continues to rise through life but slows down between the ages 45-50 years. Non-hormone dependent cancers do not exhibit this change of incidence and this is clear evidence suggesting that breast cancer is caused by reproductive hormones[6]. Eighty per cent of cases occur in those over the age of 50 years.

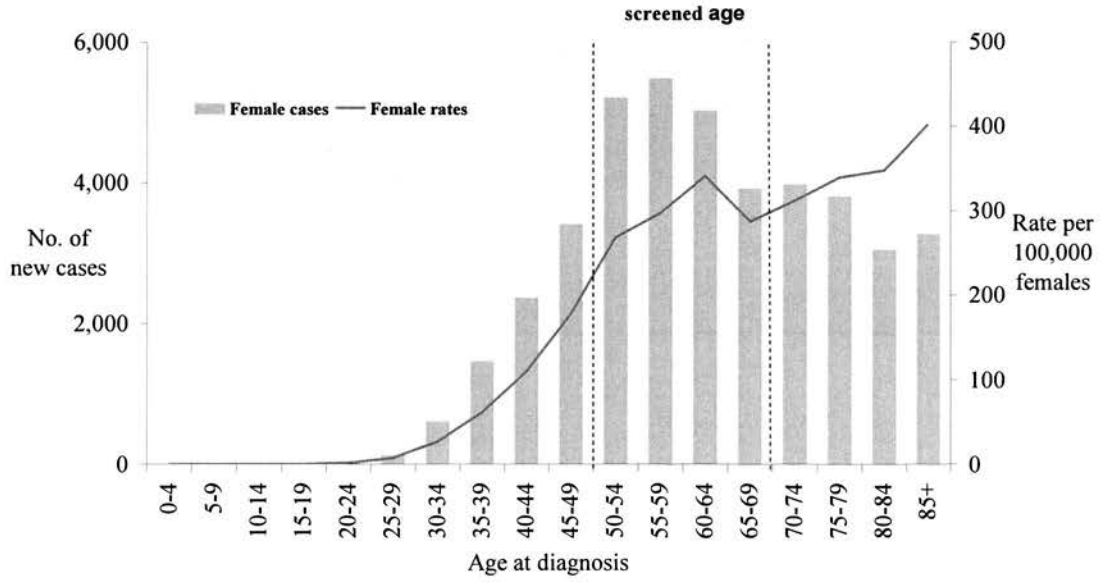


Figure 1.3 Incidence of UK female breast cancers and rates by age
(Adapted from cancer statistics, Cancer Research UK 2001[3])

1.3 History of breast cancer and hormonal treatments

Breast cancer is an ancient disease which transcends race, class and time. It was first documented by the Egyptians as far back as 3000 years before the birth of Christ[7]. During ancient times, breast cancer *was* cancer. People died of other malignancies but few of these cancers were visible to the naked eye. Breast cancer however could be easily and logically correlated with the external manifestation of the disease and ultimately with the death that followed, it was therefore greatly feared[8].

Breast cancer was later described by the godfather of Western Medicine, Hippocrates born around 460BC. He taught medicine in the temple dedicated to the Asclepios, the Greek god of medicine and healing. Hippocrates supplied the earliest and by far the most enduring, description of cancer. Its appearance provoked Hippocrates to name it 'karkinos', a Greek work for crab. He found on close examination that tumours were seen to have tentacles, like the legs of a crab, reaching out and grasping normal tissue. From karkinos evolved the modern term 'carcinoma'. During these times it was viewed as a systemic illness. Clarissimus Galen, the second century Greek physician succeeded Hippocrates[8] and he attributed breast cancer to a coagulum of black bile within the breast and therapy was aimed at getting rid of this excess bile by diets, purgation, venesection, cupping and leeching[9]. His humoral theories disappeared into the dust of history until approximately 1000 years later when monastic scribes translated Arab texts back into Latin in the late Middle Ages.

The professor of medicine at Padua University, Bernardino Ramazzia ruminated on the puzzling epidemiology of breast cancer. In 1713 he documented that these tumours were found more often in nuns than in any other women and blamed the nuns' celibacy. He failed to make the true association with nulliparity[8].

The infamous American surgeon William Stewart Halsted graduated in 1877 and became the professor of surgery at John Hopkins Hospital in Baltimore, USA. He performed the first 'radical mastectomy' in 1882 which involved an 'en bloc resection' removing the entire breast, skin and subcutaneous tissue alongside the pectoralis major and minor muscles and axillary lymph nodes. The procedure represented the most successful technique for treating cancer of its time and was carried out worldwide throughout the first half of the twentieth century. Although his surgery boosted survival, women were left feeling handicapped[7,8]. Prior to this era, tumours were treated by local excision however the results were disappointing and Sir James Paget concluded in 1863 that this operation was futile in terms of curative potential. It soon became evident that radical mastectomy reduced local recurrence rates but achieved little in terms of overall survival.

The first oophorectomy was carried out by a German surgeon, Alfred Hegar in 1872 for benign disease. Within one month, the American Robert Battey performed the first bilateral oophorectomy on a young woman with multisystem malady and the absence of regular menses. It was not until 1882 when Thomas William Nunn reported the relationship of ovarian function to breast cancer. He described a perimenopausal woman with breast cancer who went into remission after menstruation ceased. In 1889 the German Albert Schinzinger proposed surgical oophorectomy as a treatment for breast cancer. He suggested the possibility of removing a patient's ovaries before mastectomy as a way of getting at more tumour tissue. He believed oophorectomy would render the mammary glands atrophic, giving rise to the possibility that cancer nodes might become demarcated in the shrinking tissue. Despite this he never carried out the surgery himself[8].

It is now over 100 years since the Glaswegian surgeon George Thomas Beatson reported the effects of ovarian ablation on breast cancer in his paper titled 'On The

Treatment Of Inoperable Cases of Carcinoma Of The Mamma: Suggestions for a New Method Of Treatment, With Illustrative Cases' published in 1896[10]. Beatson's interest in the connection between the breast and ovary started when he embarked upon his MD thesis. He observed lactation in sheep, cattle and rabbits and described 'the close intimacy between the ovary and the mamma, as seen in the absence, as a rule, of the menstrual function during lactation'. He questioned the possible effects of oophorectomy on the progress of cancerous growths of the breast. On June 15th 1882 he performed his first bilateral salpingo-oophorectomy on a young woman with recurrent breast tumour. The patient was also given ovine thyroid extract, pre and post-operatively, as it was thought that this was a powerful lymphatic stimulant which might help cure her disease. Her tumour regression was remarkable and she survived for a further four years. The case was published along with several others. Hence, Beatson demonstrated that many breast cancers were dependent on the ovaries for growth and oophorectomy could result in tumour regression.

In 1886 Stanley Boyd carried out his first bilateral oophorectomy on a woman with metastatic breast cancer and eleven years later performed the first oophorectomy as adjuvant therapy for the treatment of metastatic breast cancer[11].

Radical mastectomy remained the surgical treatment of choice until a revolutionary change of view occurred during in the 1970s regarding the mechanism by which breast cancers grow and spread. The earlier belief that these tumours remained confined to the breast and lymphatics before metastasising at a later date was challenged. Studies showed that only 12% of Halsted's patients undergoing radical mastectomies survived beyond one decade. The concept that tumours spread early in the disease and that metastases were not clinically apparent led to the development and subsequent introduction of adjuvant treatment. Both Hippocrates and his

successor Galen believed that breast cancer was undoubtedly a systemic disease, and that extirpation of the primary tumour made matters worse[11].

Ethamoxytriphetol (MER25) was described by Lerner and co-workers in 1954 as the first non-steroidal anti-oestrogen and was used as a treatment for a wide range of gynaecological conditions including breast cancer. Its main side-effect was neurotoxicity including hallucinations and it was therefore withdrawn from further development[12]. Research in the triphenylethylene-based compounds continued and this culminated in the introduction of clomiphene which was used to induce ovulation and then in the late 1960s, tamoxifen which was first investigated as a contraceptive and then used for the treatment of breast cancer[11]. Tamoxifen is a SERM which has anti-oestrogenic effects on breast tissue and oestrogenic effects on endometrium and bone. It was first used to treat advanced breast cancer in the UK in 1973 (ICI Pharmaceuticals, now known as Astra Zeneca). This pioneering advance was not without concerns. In the mid-1980s it was predicted that tamoxifen would increase the risk of endometrial cancer in post-menopausal women and later studies confirmed that the incidence increased four-fold. However its overall toxicity was low and it remains an extremely successful endocrine treatment and has been the mainstay hormonal agent for breast cancer for the last 30 years. It has also been used for breast cancer prevention however studies have demonstrated that the risk of serious adverse events (AEs) is significant and a lack of survival benefit has led to its selective use as a preventative medicament. To date, tamoxifen continues to be associated with gynaecological problems including an increased risk of endometrial bleeding, endometrial thickening and endometrial carcinoma together with an increased risk of thromboembolism. These risks have reduced its use in prevention and have resulted in the development of newer endocrine treatments including AIs.

AIs have not only been shown to be more efficacious compared to tamoxifen but they appear to have a more favourable side-effects profile, increasing the likelihood of drug uptake and compliance.

Aromatase inhibition was first shown to be an effective clinical treatment for postmenopausal breast cancer using the first generation AI, aminoglutethimide. The development of aminoglutethimide as an endocrine therapy for breast cancer resulted from the observation that the drug inhibited adrenal steroidogenesis during its earlier investigation as an antiepileptic[13]. Its use was based on the principal that adrenal androgens form the basic substrate for the synthesis of plasma oestrogens by aromatase in the peripheral tissues of postmenopausal women. Removal of androgens were therefore expected to lessen the oestrogenic stimulus to breast cancer cells by a process of medical adrenalectomy. When administered in sufficient doses, the drug inhibits the production of adrenal steroids by inhibiting cholesterol side chain cleavage in the early steps of steroidogenesis and causing partial blockade of 11β-, 18- and 21-hydroxylases in the adrenal. Corticoid replacement is therefore routinely required to avoid adrenal insufficiency[14]. Studies demonstrated that the aminoglutethimide-corticoid regimen blocked the peripheral conversion of androgens to oestrogen and suppressed circulating oestrogens in postmenopausal women with breast cancer[15]. Amininoglutethimide's lack of specificity results in the need for routine glucocorticoid replacement and largely results from its actions on other cytochrome P450 systems[16]. Although early studies established aminoglutethimide as a feasible treatment for advanced breast cancer in postmenopausal women, it was poorly tolerated, required high doses to achieve oestrogen deficiency and had several marked side effects including marked nausea, lethargy, and headache which affected 60%, 52% and 43% of patients respectively[17]. Other side effects include skin rashes and serious bone marrow suppression[14]. Direct comparisons between

aminoglutethimide and tamoxifen in relation to their effects on breast cancer demonstrated equal clinical efficacy but fewer side effects and toxicity with tamoxifen in the 1970s[18]. Second generation AIs were then developed in an attempt to reduce associated toxicity and to improve specificity and efficacy[19,20].

These second generation AIs fadrozole and formestane were better tolerated but continued to lack selectivity. Formestane was introduced in 1993 and was administered by intramuscular injection twice monthly rendering it less acceptable to patients. The third-generation AIs letrozole, exemestane and anastrozole were respectively introduced in 1993[21], 1995[22] and 1996[23]. They have greater specificity with fewer side-effects[24].

Despite the extensive progress that has been achieved over the last century, there are still many questions that remain unanswered about the treatment of this complex disease.

1.4 Breast development, anatomy and physiology

The development of breast tissue begins during the fifth week of gestation and commences independent of hormonal influences. It evolves in utero from a primitive milk streak which develops from the axilla to the groin. This then regresses to form a mammary ridge in the thorax which gradually develops under the influence of placental sex hormones to form mammary ducts, lobulo-alveolar structures and the nipple-areolar complex.

During puberty there is a gradual enlargement in female breast tissue which occurs under the control of the ovarian hormones, follicle stimulating hormone (FSH) and luteinising hormone (LH). FSH initiates the development of ovarian follicles into Graafian follicles which secrete oestrogen, primarily oestradiol. Prior to menarche, ovarian oestrogen synthesis predominates over luteal progesterone synthesis and these hormones together produce complete ductular-lobular-alveolar development. The relative role of each remains unclear however studies have demonstrated that oestrogen appears to have a much greater role in breast development[6].

The mammary gland lies on the anterior chest wall between the second and sixth ribs and extends into the axilla. It consists of glandular tissue, supporting dense fibrous stroma and interlobar adipose tissue. The glandular tissue consists of approximately 8 to 20 lobules which produce milk and are arranged in a 'spoke-like' pattern. Multiple ducts carry milk from the milk-secreting lobular units to the lactiferous sinus at the base of the nipple. These lobules and ducts constitute the functional unit of the breast which is otherwise known as the terminal duct lobular unit (TDLU). Most breast cancers form in the TDLU and spread along the ductal system in the radial axis of the lobe. At least half of all tumours are found in the upper outer breast

quadrant where there is a greater abundance of glandular tissue. It has been shown that women with invasive breast cancer and those with a familial pattern of cancer have a different architectural pattern from those with normal tissue. Women carrying the BRCA1 gene or related genes may have a different branching pattern during lobular development[25].

There are three phases of breast activity: puberty, ovulatory cycles and pregnancy. Breast development requires the influence of various hormones, the most important being oestrogen. After puberty, mammary tissue undergoes profound cyclical changes during each ovulatory cycle under the control of oestrogen and progesterone. During the follicular phase, FSH and LH increase the levels of oestrogen secreted by the ovarian follicles which in turn stimulates breast epithelium proliferation as shown in figure 1.4. During this proliferation the epithelium undergoes increased cellular mitoses. Ovulation occurs at the time of maximum oestrogen synthesis (mid-cycle). A second peak of oestrogen occurs in the mid-luteal phase when luteal progesterone is at its maximum. Premenstrual breast fullness can be explained by the increased ducto-lobular proliferation and interlobular oedema which occurs under the influence of these hormones. At the end of the menstrual cycle there is a marked fall in hormone levels and a resulting loss of breast cells due to apoptosis. These normal monthly changes in breast proliferation and apoptosis which occur in relation to the ovulatory cycle have been shown to differ from the effects found in endometrium where the maximum mitotic activity occurs during the follicular phase (days 6-14). The estimated peak for breast mitosis was shown to be at day 25 while that for apoptosis was at day 28[26]. Breast apoptosis occurs in response to decreasing levels of oestrogen and progesterone which occur toward the end of the menstrual cycle. In summary, breast lobules of 'resting' human breast

show a response, in terms of mitosis and apoptosis, which is a biorhythm in phase with the ovulatory cycle[26].

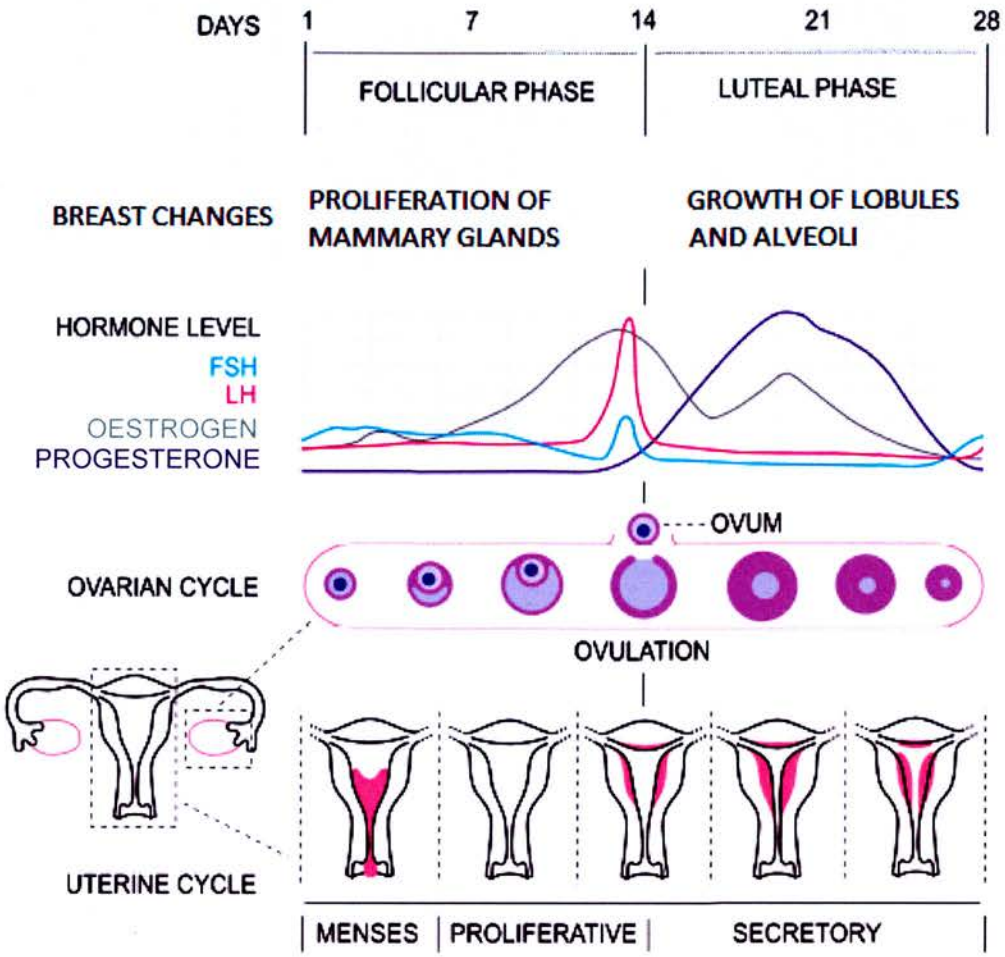


Figure 1.4 Cyclical changes affecting breast tissue

Pregnancy is associated with very high serum levels of oestrogens and progesterone which stimulate growth of breast epithelium and promote differentiation of epithelial tissue, reducing the number of epithelial structures most vulnerable to malignant transformation. The long term effect of pregnancy therefore reduces the risk of breast cancer[27]. Steroid hormone levels fall immediately after parturition and lactation is

initiated by prolactin. After weaning there is rapid involution of the differentiated lobular unit.

Further involutional changes occur within the breast with increasing age as a result of decreasing oestrogen and progesterone levels which occurs with defaulting ovarian function. The menopause results from follicular atresia of more than 400,000 follicles initially present in the human female foetus[6]. Breast involution leads to the replacement of stroma by adipose tissue and shrinkage of lobules. This results in softer and more ptotic breasts which are less dense and in turn result in mammograms which reveal tumours more readily.

1.4.1 Oestrogen

The endogenous oestrogens of greatest significance are oestradiol (E2, 17B-oestradiol) and the structurally similar, but less potent, oestrone (E1). Estriol (E3) does not play a significant oestrogenic role outside of pregnancy and is the main oestrogen secreted by the placenta. It is a thousand-fold less potent than oestradiol[28]. Oestrogens are synthesised from cholesterol via the pathway shown in figure 1.5. All oestrogens are synthesised through the action of the aromatase enzyme, which is a product of the CYP19 gene. This enzyme converts the androgens testosterone and androstenedione to the oestrogens oestradiol and oestrone, respectively. The conversion from androgens involves removal of the methyl group at C-19. E1 and E2 are freely interconvertible by the action of oestrogen dehydrogenases[19].

Oestradiol is the predominant form of circulating oestrogen in premenopausal women and is the most potent activator of ERs. Ovarian granulosa cells have a rich supply of aromatase and are the source of >90% of plasma oestrogens secreted in a cyclical pattern in women before the menopause. More than 95% of circulating oestrogens are bound to carrier proteins, oestradiol and oestrone are bound weakly to albumin and with high affinity to sex hormone-binding globulin (SHBG). Only approximately 2% of oestradiol is in the free form and is generally thought to influence tissue uptake and biological activity, although some debate still exists on this point. As ovarian function abates with the onset of the menopause, oestrogens are synthesised in increasing amounts by extragonadal sites. Mean plasma oestradiol levels fall to less than 10% of the premenopausal levels. Oestrone is the predominant postmenopausal oestrogen and is produced mainly from androstenedione and also

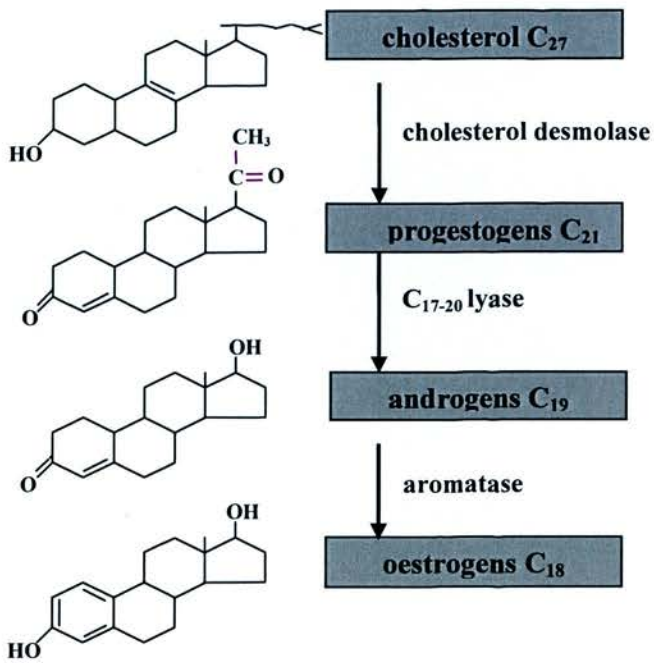


Figure 1.5 Pathway of oestrogen synthesis from cholesterol

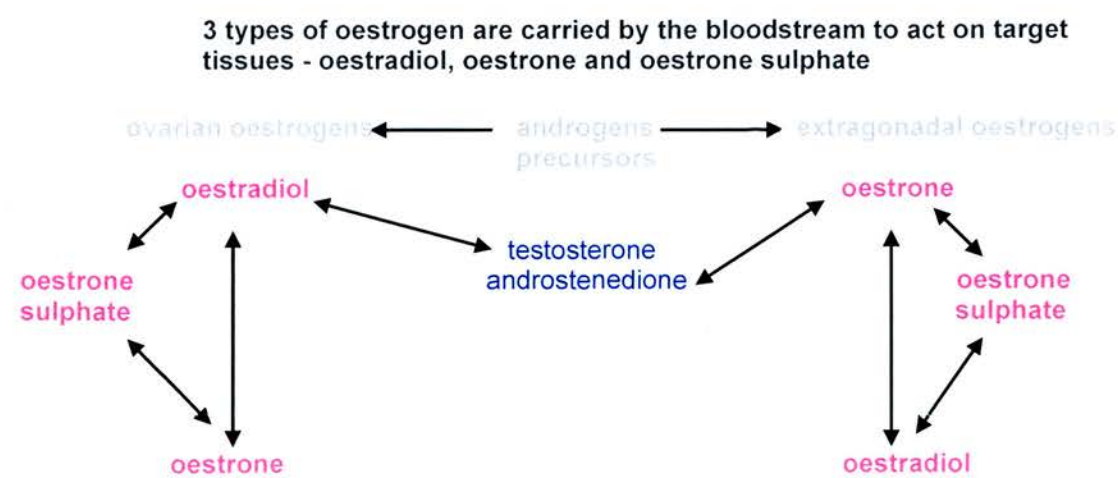


Figure 1.6 Types of circulating oestrogen in postmenopausal women

testosterone[29]. Figure 1.6 demonstrates the types of circulating oestrogens in the postmenopausal/non-pregnant female.

A significant proportion of oestrogen circulates as oestrone sulfate (E1S) which serves as a circulating reserve for the more active primary oestrogens. Both oestradiol and oestrone can be converted into this water-soluble conjugate. Although it circulates at much higher concentrations, it is only biologically active after conversion back to oestrone via estrone sulphatase[19].

In premenopausal women hormone production in the ovary is cyclical and the typical reference ranges for oestrogens depend upon the time point measured. Both premenopausal and postmenopausal normal oestrogen reference ranges are shown in table 1.1.

Premenopausal reference range Oestradiol levels		**Postmenopausal reference range Oestradiol levels**
Follicular phase	184 – 532 pmol/L	<80 pmol/L
Luteal phase	184 – 885 pmol/L	
Pre-ovulation	411 – 1626 pmol/L	
Post-ovulation	37 – 130 pmol/L	
Oestrone levels		**Oestrone levels**
Follicular phase	111 – 370 pmol/L	<80 pmol/L
Luteal phase	333 – 592 pmol/L	
Pre-ovulation	>550 pmol/L	
Oestrone sulphate levels		**Oestrone sulphate levels**
Follicular phase	184 – 532 pmol/L	<217 pmol/L
Luteal phase	184 – 885 pmol/L	
Pre-ovulation	411 – 1626 pmol/L	
Post-ovulation	37 – 130 pmol/L	

Table 1.1 Typical adult female oestrogen reference ranges[30]

Oestradiol is the most potent activator of oestrogen receptors and predominantly circulates in plasma bound to proteins. Approximately only 2% is free (not bound to protein) and is able to enter cells. Residual oestradiol is bound weakly to albumin or to sex hormone binding globulin with high affinity. As ovarian function abates with the onset of the menopause, oestrogens are synthesised in increasing amounts by extragonadal sites (including adipose tissue, liver, muscle, skin, brain and the stromal and epithelial cells of many breast cancers. Mean plasma oestradiol levels fall to less than 10% of the premenopausal levels. Oestrone is the predominant postmenopausal oestrogen and is produced mainly from androstenedione and also testosterone[29]. Figure 1.6 demonstrates the types of circulating oestrogens.

Conversion of oestradiol and oestrone into the water-soluble conjugate oestrone sulphate also occurs and circulates at much higher concentrations than the primary oestrogens. However it is only biologically active after conversion back to oestrone via estrone sulphatase.

Oestrogens have a marked proliferative effect on breast epithelial tissue with both endogenous and exogenous oestrogens stimulating breast epithelial cell mitosis, increasing the number of cell divisions and thus the opportunity for random genetic errors. They not only exert effects on breast and reproductive tissues but also mediate changes upon other systems including those affecting bone, lipids and coagulation.

Exposure to exogenous oestrogen is common with the use of hormonal preparations for the use of contraception and to a lesser extent to combat menopausal symptoms. HRT has been associated with a 2.3% excess risk of developing breast cancer for each year of use. The risk is higher with preparations combining oestrogen and progesterone compared with the use of oestrogen or progesterone alone[31]. Its use has diminished in recent years following the publication of the Women's Health

Initiative study which showed that long-term HRT not only increased the risk of breast cancer but also increased the risk of stroke, thromboembolic and cardiovascular disease[32]. HRT has a positive effect on bone density and is related to a reduced incidence of osteoporosis and bone fractures.

Tibolone, an analogue of the progestin norethynodrel, is a synthetic selective tissue oestrogenic activity regulator (STEAR) which relieves menopausal symptoms. It is a steroid with weak oestrogenic, progestrogenic and androgenic properties which affects multiple tissues in different ways. Tibolone is metabolised in the gastrointestinal tract to the 3α and 3β metabolites, which circulate in their inactive sulphated form and become oestrogenically active when desulphated[33,34]. Its global effect is predominantly oestrogenic in target organs. In breast tissue, the 3β metabolites strongly inhibit sulphatase, blocking the conversion of oestrone sulphate to oestrone which causes a reduction of bioactive oestrogen. This induces inhibition of proliferation and stimulates apoptosis and differentiation of breast cancer cells and supports a potential lower risk of breast cancer with tibolone compared with other forms of HRT[35]. However it has been associated with an increased risk of postmenopausal breast cancer in some studies but results are not univocal[35]. Tibolone is almost as effective as combined HRT at relieving menopausal symptoms and is as effective as oestrogen-progestin therapy at preventing bone loss.

The Million Women UK study (appendix F) was set up to investigate the effects of specific types of HRT on breast cancer incidence and fatal breast cancer. Results demonstrated that current HRT users were more likely to develop breast cancer and die from it (adjusted relative risk 1.66 and 1.22 respectively) compared with those that had never used HRT. Incidence was significantly increased for current users of oestrogen only HRT, oestrogen-progestagen HRT and tibolone (adjusted relative risks 1.30, 2.00 and 1.45 respectively)[32]. The findings of this study were criticised as

they were at odds with most of other studies comparing breast cancer risk in HRT users. Data from the Danish Nurse Cohort study (appendix F) also demonstrated a significant increased risk of breast cancer with the current use of tibolone RR 4.27 [95% CI 1.74-10.51[36], The use of combined HRT in the Womens' Health Initiative study demonstrated an estimated hazard ratio of 1.26 [95% CI 1.00-1.59] for breast cancer[37]. Subsequent studies have demonstrated no increase in breast cancer risk associated with tibolone use as demonstrated in the UK General Practice Research Database RR0.86 [CI 0.65-1.13][38] and LIFT study relative hazard 0.32 [95% CI 0.13-0.80][39](appendix F). The higher risk demonstrated in the Million Women study may be attributed to the preferential prescribing of tibolone to higher risk women, patients being given combined HRT prior to entry into the study which may increase their risk of breast cancer and the inadequacy of breast cancer screening prior to entry into the study[35].

A further study, LIBERATE (appendix F) was undertaken to demonstrate non-inferiority in breast cancer recurrence of tibolone versus placebo in women with menopausal symptoms who had undergone surgery for primary breast cancer within five years. After a median follow-up of 3.1 years 15.2% of women on tibolone had a cancer recurrence, compared with only 10.7% taking placebo (p=0.001). The study was stopped prematurely because of the 1.40 relative hazard risk of developing breast cancer. Although tibolone relieved vasomotor symptoms and prevented bone loss, it increased the risk of recurrence in breast cancer patients[40]. A Cochrane database systemic review was recently published detailing the randomised controlled trials comparing tibolone versus placebo, oestrogens or combined HRT by assessing menopausal symptoms and the occurrence of safety outcomes in postmenopausal women. Data on the long term safety of tibolone is concerning given the increase in the risk of breast cancer in women who had already suffered from breast cancer in

the past and in a separate trial the increase in the risk of stroke in women whose mean age was over 60 years. The overall risk-benefit profiles for combined preparations of HRT are better known[41].

1.5.1 Osteoporosis, bone metabolism and the effects of oestrogen

Osteoporosis is a common bone disease amongst postmenopausal women which is characterised by reduced BMD, micro-architectural deterioration in bone tissue and an increased fracture risk[42]. It occurs due to excess bone being removed during bone resorption compared with the amount remodelled during bone formation. Its pathogenesis is complicated with many factors involved, although oestrogen deficiency in postmenopausal women appears to be the most important. Predictors of future fractures related to osteoporosis include low BMD, prior fragility fractures, advancing age (bone mass peaks between the ages of 20 – 40 years in normal healthy females), smoking, low body weight, a family history of osteoporotic fractures and excess alcohol.

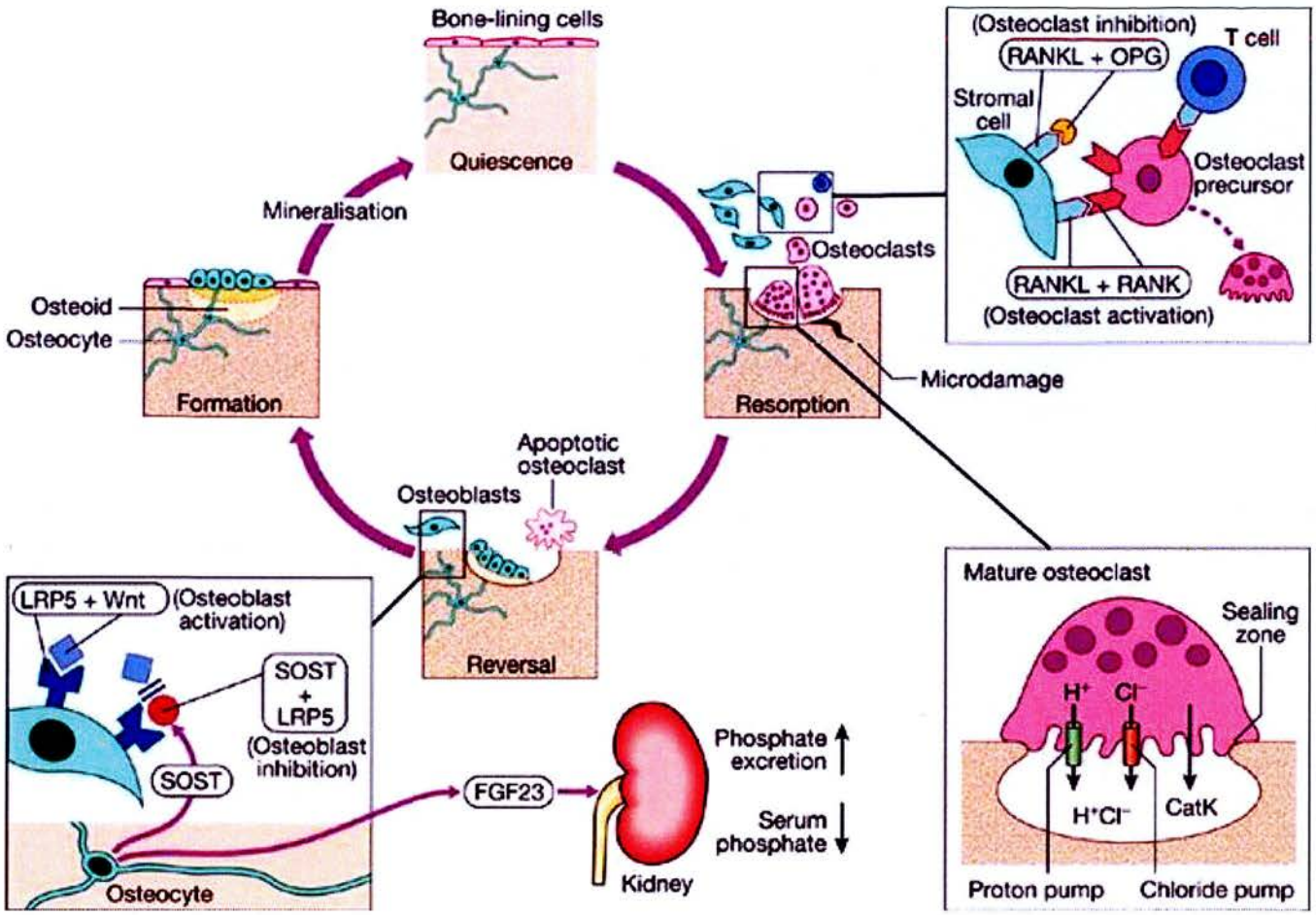
Bone has several functions which include maintaining blood calcium levels, providing mechanical support to soft tissues including muscle, supporting haematopoiesis and also providing protection to vital structures including the brain and spinal cord[43]. It is a dynamic tissue which is constantly changing through a process of remodelling which occurs through the resorption of old bone by osteoclasts and the formation of new bone by osteoblasts. The three most important effects on bone include the homeostatic demands for calcium, mechanical adaptation to changes in loading and finally the influence of sex hormones. Genetic factors also play an important role in regulating bone mineral density (BMD), skeletal geometry and bone turnover[44] as demonstrated in twin studies[43]. Their effects on bone metabolism result from a combination of genetic and environmental influences. Genome-wide linkage studies have identified several loci that show linkage with BMD and studies involving mice have supported the involvement of multiple genes[44].

Oestrogens and androgens play important roles in bone metabolism and homeostasis. Sex steroids influence the remodelling of bone in adults and alongside oestrogen are vital for maintaining bone mass in women. The closely coupled process of bone resorption followed by bone formation is a delicate balance between osteoclasts and osteoblasts and is responsible for renewing the skeleton while maintaining its anatomical and structural integrity[45]. The purpose of remodelling is believed to not only prevent bone aging and facilitate repair, but also to ensure appropriate blood calcium levels necessary for correct cell function. Approximately 25% of trabecular bone is replaced annually in adults whereas only 3% of cortical bone is replaced. This may be explained by the high surface-to-volume ratio of trabecular bone to bone marrow with approximately 70-85% of the bone surface being in contact with marrow[39].

Osteoclasts are multinucleated cells of haematopoietic origin which arise from granulocyte-macrophage colony-forming units whereas osteoblasts arise from the mononuclear cells of mesenchymal stem cells of the marrow stroma, fibroblast colony-forming units, and are responsible for the production of osteoid and subsequent formation of bone. Haemopoietic precursors of osteoclasts are unable to develop without the presence of stromal-osteoblastic cells which produce factors which mediate osteoclastic development[45]. Another important cell type involved in bone homeostasis includes osteocytes which are cells of myeloid origin. They are the most abundant cells in mature bone and are formed from osteoblasts. They have long cytoplasmic processes that make contact with their neighbouring osteoblasts by tight junctions. Osteocytes sense mechanical forces imposed on the bone, specifically mechanical distortion, which initiates bone remodelling. Osteocytes are not only responsible for the sensing and responding to mechanical loading of the skeleton but

they are also involved in phosphate metabolism and the exchange of calcium between extracellular fluid and bone[28,42,46].

Under normal circumstances bone resorption and formation are closely linked within spatiotemporal anatomic structures called the basic multicellular unit (BMU). Bone remodelling is demonstrated in figure 1.7 and is accomplished by cycles which involve the resorption of old bone by osteoclasts and the subsequent formation of new bone by osteoblasts. These cells are replenished from their haematopoietic progenitors within the bone marrow[45]. Osteoclast precursors are attracted from peripheral blood by chemotactic factors. These precursors then differentiate into mature osteoclasts in response to receptor activator of nuclear factor-κ β ligand (RANKL) which activates RANK receptors which are expressed on osteoclasts and their precursors. Osteoprotegerin inhibits osteoclast formation and blocks RANK receptors. Mature osteoclasts adhere to bone and break it down via acidification and proteolytic digestion. They secrete hydrochloric acid and enzymes including cathepsin K which dissolves matrix and breaks down collagen respectively. Once resorption is complete they undergo apoptosis and osteoblasts subsequently invade the area and start the process of bone formation by secreting osteoid which is a matrix of collagen and protein which finally becomes mineralised. Some osteoblasts become trapped within the bone matrix and differentiate into osteocytes. Once bone formation is complete, the bone surface becomes covered by terminally differentiated osteoblasts[45,46].



CatK - cathesin K
FGF23 - fibroblast growth factor -23, regulator of serum phosphate and calcitriol levels
LRP - lipoprotein receptor protein 5
OPG - osteoprotegerin
RANK - receptor activator of nuclear factor κ-β
RANKL - RANK ligand
SOST - sclerostin
Wnt - proteins which bind to and activate LRP5

Figure 1.7 The bone remodelling cycle[46]

Increased mechanical loads stimulate bone formation and suppress resorption, whereas unloading has the opposite effect[47]. The rate of remodelling is coupled locally so that when the rate of one increases or decreases then the other usually follows. The rate of resorption is however faster than the rate of formation and it usually takes at least three months to rebuild bone which is resorbed in only 2-3

weeks. Increased bone resorption therefore causes bone loss even though it is coupled with increased formation[43].

The regulation of bone remodelling is very complex and many cytokines and growth factors are involved, it is principally under autocrine-paracrine control. Systemic hormones control the production of local mediators in the bone microenvironment[48] and include circulating hormones such as parathyroid hormone (PTH), calcitriol, cortisol and sex steroids: vitamin D_3 is also involved. Molecules that govern bone remodelling include proteins which belong to the tumour necrosis factor (TNF) superfamily: osteoprotegerin, RANKL and their receptor RANK. Other molecules include TNF-α, IL-1 and IL-6 which are important mediators involved with the effects of oestrogen deficiency[48]. TNF-α is a multifunctional cytokine produced by activated monocytes-macrophages and is one of the most potent osteoclastogenic cytokines produced in inflammation, it also induces IL-1 synthesis. Several cytokines and colony-stimulating factors are specifically involved in the development of osteoclasts including IL-1, IL-3, IL-6, IL-11, TNF, granulocyte-macrohage colony-stimulating factor, macrophage colony-stimulating factor, leukaemia inhibiting factor and stem-cell factor. Cytokines which specifically stimulate bone resorption include IL4, IL-10, IL-18 and IFN-γ[48].

There are several important factors regulated by oestradiol which are involved in regulating the differentiation and function of osteoclasts and osteoblasts. Suppression of osteoclastic bone resorption and stimulation of osteoblastic bone formation form the basis for the bone-preserving effects of oestogen[49]. Oestrogen inhibits bone resorption by reducing osteoclast numbers and is thought to involve lineage allocation of monocyte/macrophage/osteoclast precursors through the effects on regulatory cytokines including IL-1, IL-6, TNF-α and prostaglandin E (PGE). In

osteoblasts, oestrogen stimulates the synthesis and secretion of the anabolic growth factor IGF-1 and inhibits that of the cytokines, IL-1, TNF and IL-6 which are involved in bone resorption[50]. Oestrogen deficiency results in the increased production of IL-6 in bone marrow cells[45]. Interestingly IL-6 only appears to be important for osteoclastogenesis in the pathological state and has been shown to have no effect on the development of osteoclast precursors in marrow with sufficient sex steroid levels i.e. in the oestrogen replete state. Oestradiol also stimulates the synthesis and secretion of osteoprotegerin (OPG), a protein with a critical role in inhibition of the function of osteoclasts. OPG, osteoclast differentiation factor (ODF) and RANK regulate osteoclastic differentiation and function[49]. After the menopause approximately 10% of bone is lost annually due to increased bone turnover. Oestrogen deficiency increases the activation of BMUs which induces this imbalance in remodelling. Bone loss related to oestrogen deficiency occurs mainly at the expense of trabecular bone as a result of excessive osteoclast activity. This is in contrast to the process associated with ageing, which affects primarily cortical bone and is most likely due to a reduction in the supply of osteoblasts compared to demand[19,45].

BMD is measured using dual energy X-ray absorptiometry (DEXA). Table 1.2 demonstrates the simple diagnostic criteria for low BMD used by the World Health Organisation (WHO)[19]. A decrease in 1 standard deviation (SD) in BMD leads to a doubling of the risk of fractures[51]. WHO have estimated that approximately 30% of postmenopausal women have osteoporosis and 54% have osteopenia. Osteoporosis has been labelled a 'silent epidemic' because patients are often asymptomatic up until the point of bone fracture[19].

WHO diagnostic criteria for osteopososis	
Normal	BMD not more than 1 SD below the mean value of peak bone mass in a normal adult T-score > -1
Osteopenia	BMD within -1 SD and -2.5 SD of the mean value of peak bone mass in a normal adult T-score -2.5 - -1
Osteoporosis	BMD more than 2.5 SD below the mean value of peak bone mass in a normal adult T-score < -2.5
Severe osteoporosis	BMD more than 2.5 SD below the mean value of peak bone mass in a normal adult T-score < -2.5 with the presence of fractures

SD – standard deviation

Table 1.2 WHO criteria of BMD and osteoporosis

There is clear evidence that bone turnover markers are strong predictors of future fractures[52,53] and can be used independently of BMD measurements to assess future risk. Increased bone turnover has a detrimental effect on bone microarchitecture and fragility[54]. Biochemical bone turnover markers include enzymes and proteins released during bone formation and degradation products produced during bone resorption. They can be easily measured, often with autoanalysers, using a variety of biochemical markers in either serum or urine. They can be divided into two categories as shown in table 1.3: markers of bone resorption, which reflect osteoclast activity and are for the most part degradation products of type I collagen; markers of bone formation, which reflect osteoblast activity and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes. The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine have recommended that one bone resorption marker (sCTX) and one bone formation marker (sPINP) should be used as reference markers and measured by standardised assays in future observational and intervention studies[55].

Bone formation	Bone resorption
Byproducts of collagen synthesis	Collagen degradation products
Procollagen type I C-terminal propeptide[s]	Hydroxyproline[u]
Procollagen type I N-terminal propeptide[s]	Pyridinoline[u,s]
	Deoxypyridinoline[u,s]
Matrix protein	Cross-linked telopeptides of type I collagen
Osteocalcin[s]	N-terminal cross-linked telopeptide[u,s]
Osteoblast enzyme	C-terminal cross-linked telopeptide[u,s]
Total alkaline phosphatase[s]	C-terminal cross-linked telopeptide generated by matrix metalloproteinases[s]
Bone alkaline phosphatase[s]	Osteoclast enzymes
	Tartrate-resistant acid phosphatase[s]
	Cathepsin K[s]

Table 1.3 Commonly used biochemical markers of bone turnover

[s]measured in serum
[u]measured in urine

Recent studies have demonstrated that markers of bone turnover appear even more strongly associated with fracture risk reduction than BMD. They support the use of markers of bone turnover as surrogates for fracture risk reduction, perhaps even more so than BMD. They have the advantage over BMD in that they provide information about mechanism of effect and changes are often observed more rapidly[56].

Oestrogens slow the rate of bone remodelling and protect against bone loss. Postmenopausal bone loss is due to oestrogen withdrawal which results in increased bone turnover due to an increase in bone resorption and a decrease in bone formation (osteoblast apoptosis increases). These changes are mediated via an increase in the production of cytokines with osteoclastogenic and osteoblastogenic properties.

Ageing also alters the cellular activity of bone marrow resulting in the likely reduction in the formation of osteoblast precursors[45]. Epidemiological and experimental studies have identified oestrogen deficiency as one of the most important risk factor for osteoporosis. Other factors include dietary calcium, parathyroid function and genetic background[43].

1.5.2 Cardiovascular disease, lipid metabolism and the effects of oestrogen

Cardiovascular disease (CVD) is a major health problem. It is the single most important cause of premature death in Europe and the leading cause of death in Scotland[57]. In the UK (population 59 million), it is estimated that 1 in 4 women die from coronary heart disease; these statistics are amongst the highest in Western Europe. Lipoprotein disorders are also common and are a major risk factor for all types of CVD. Cholesterol is a major component of lipid-containing atherosclerotic plaques and raised levels of circulating lipids correspond with increased risk of CVD.

Lipids have multiple functions and are localised primarily within three compartments: plasma, adipose tissue and biological membranes. There are several different types of lipid, the simplest form are fatty acids which are found primarily within plasma. Others include triglycerides (comprises 90% of dietary lipids and are stored primarily within adipose tissue), phospholipids (the major class of cell membrane lipids), cholesterol and sterols[58]. These are assimilated from the diet and transported and metabolised throughout the body. The majority are insoluble and are transported in plasma as particulate complexes with proteins, known as lipoprotein. The structure of lipoprotein is demonstrated in figure 1.8 and there are several types including chylomicrons, VLDL(very low-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein) and HDL (high-density lipoprotein). The protein components of the lipoproteins are called apolipoproteins (Apo) and are a complex family of polypeptides that promote and control lipid transport and uptake. There are four main groups, ApoA, B, C and E, some of which are subdivided[42].

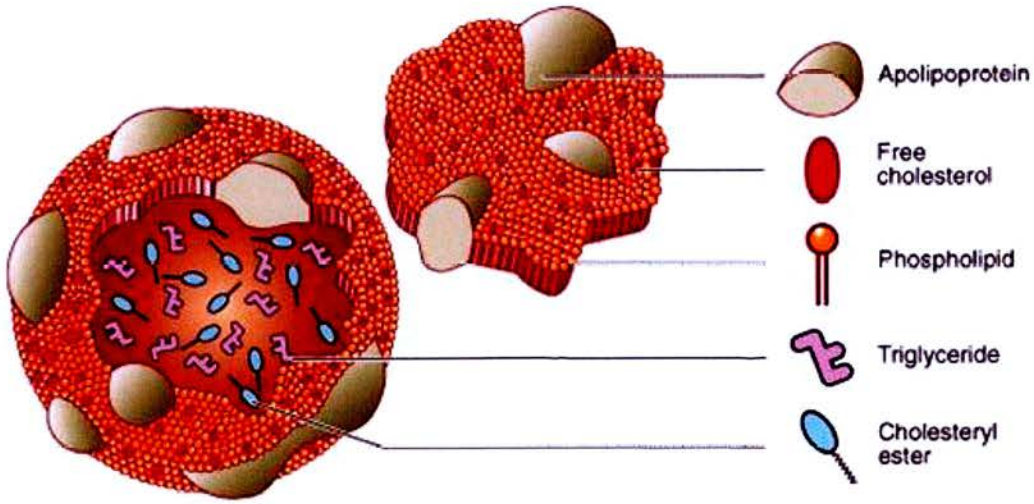


Figure 1.8 Structure of lipoproteins{[42]}

Apolipoproteins combine with lipids to form spherical lipoproteins which possess a hydrophobic core and a less hydrophobic coat.

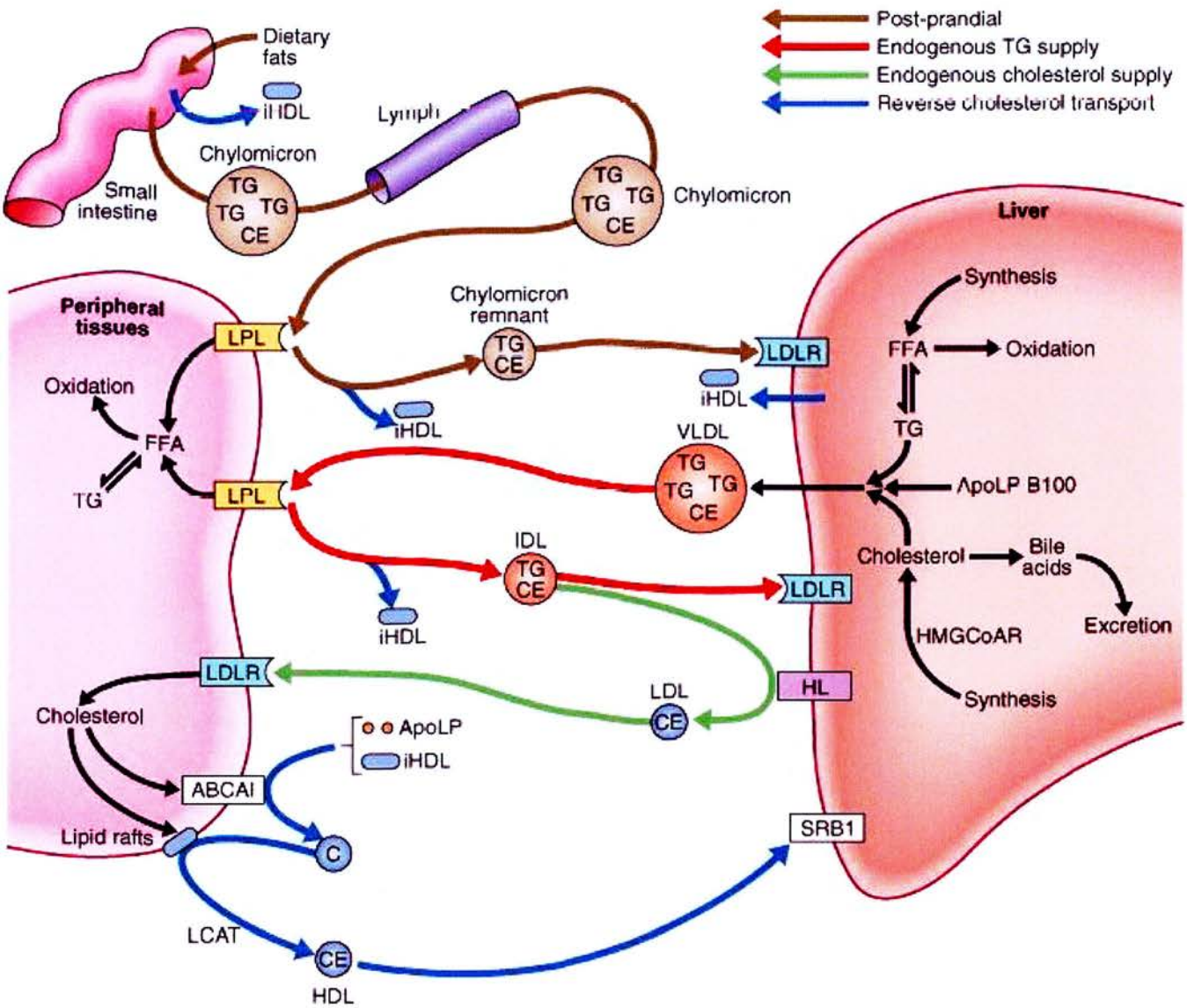
Exogenous lipids

Dietary lipids are insoluble in water and undergo lipolysis before becoming incorporated into mixed micelles which are then absorbed into enterocytes from which they pass into the systemic circulation. This absorption occurs in a step-wise process which includes: i) Fat emulsification by peristalytic movements within the stomach and small intestine. This is aided by salivary and gastric lipases. ii) Lipids cause cholecystokinin to be released from the duodenum and upper jejunum which stimulates the release of pancreatic enzymes including amylase, lipase, co-lipase and proteases. These enzymes allow the major digestive process of lipids to occur whereby triglycerides are cleaved to form fatty acids and monoglycerides. These are solubilised by interacting with bile salts (released from the gallbladder) and phospholipids to form mixed micelles which are considerably smaller than lipid emulsion droplets. iii) Enterocytes absorb the micelles and extract monoglycerides

and free fatty acids which are re-esterified to form triglycerides. Triglycerides (87%) combine with cholesterol (3%) and fat-soluble vitamins, all of which are enveloped in a coat composed of specific apolipoproteins (1%), phospholipids (9%) and free cholesterol to form chylomicrons[28]. iv) Chylomicrons leave the enterocyte by exocytoses and travel to the systemic circulating via lymphatics. They are remodelled by the transfer of additional apolipoproteins and are acted upon by lipoprotein lipase located on endothelium of capillary beds to release fatty acids that can be used for energy or storage. The remnant chylomicron particle is then cleared by the liver as shown in figure 1.9[42].

Endogenous lipids

The liver is the major source of plasma lipids in the fasting state and has the capacity to acquire lipids from uptake, synthesis or conversion from other macronutrients as shown in figure 1.9. These lipids are transported by secretion of triglyceride-rich VLDL which differ from chylomicrons as they contain apolipoprotein B100. Once secreted into the circulation VLDL undergo a similar metabolic process to chylomicrons. Fatty acids are released and the particle is converted to IDL and LDL. The latter being a source of cholesterol used for esters maintaining cell and tissue homeostasis. There is a negative feedback mechanism which controls cholesterol synthesis. Cholesterol down-regulates expression of the LDL receptor gene and reduces the synthesis and activity of the enzyme for cholesterol synthesis, HMGCoA reductase. The intracellular free cholesterol level is maintained within a narrow range due to this negative feedback mechanism and the modulation of cholesterol esterification[42].



ABCA1 - AP-binding cassette A1

Cholesterol ester - CE

Free fatty acids – FFA

HDL - mature high-density lipoprotein

iHDL - immature high-density lipoprotein

HL - hepatic lipase

HMGCoAR - hydroxyl-methyl-glutaryl-coenzyme A reductase

IDL - intermediate-density lipoprotein

LCAT - lecithin cholesterol acyl transferase

LDL - low-density lipoprotein

LDLR - low-density lipoprotein receptor (Apo B100 receptor)

LPL - lipoprotein lipase

SRB1 - scavenger receptor B1

TG – triglyceride

VLDL – very low-density lipoproteins

Figure 1.9 The absorption, transport and storage of lipids[42]

The pathways of lipid transport are shown; in addition cholesterol ester transfer protein exchanges triglyceride and cholesterol ester between VLDL/chylomicrons and iHDL/HDL.

Reverse cholesterol transport

Excessive cholesterol accumulation within peripheral tissue is guarded against by HDL which accepts free cholesterol from cholesterol-rich regions of the cell membranes and then releases them to the liver and other cholesterol-requiring tissues. This process involves a specific membrane tranporter known as ATP-binding cassette A1 transporter and lecithin cholesterol acyl transferase[42].

Atherosclerosis is a process which leads to the narrowing or complete occlusion of the arterial lumen and leads to myocardial infarction, stroke and peripheral vascular disease. Atherosclerotic plaques form as a result of endothelial dysfunction, intimal lipid deposition, and the accompanying inflammatory reaction. This may occur from excess lipoprotein levels, hypertension, diabetes and cigarette smoking[58]. Increased total cholesterol and its major component fraction, LDL cholesterol have been associated to accelerated atherosclerosis and an increased risk of coronary artery disease. In contrast HDL cholesterol appears to have a protective and anti-atherogenic effect because it is involved in the mobilisation of cholesterol from tissues and their transportation back to the liver. The risk of coronary heart disease and other forms of atherosclerotic vascular disease increases with increased plasma cholesterol concentrations, and in particular the ratio of total cholesterol to HDL cholesterol. A much weaker correlation also exists with plasma triglyceride concentration. Large scale studies have demonstrated that reducing total LDL concentration reduces the risk of cardiovascular events including myocardial infarction, stroke and death.

High levels of circulating triglycerides, cholesterol, LDL and Apolipoprotein B (ApoB) are associated with an increased risk of CVD whereas high levels of HDL and Apolipoprotein A-1 (Apo A-1) are protective. HDL is an independent predictor

of cardiovascular risk[57]. Atherogenic ratios can be used to predict cardiovascular risk, often in combination with other variables including age, sex, blood pressure and smoking status. Elevated total cholesterol:HDL, LDL:HDL and ApoB:ApoA-1 are associated with an increased risk of CVD. The ratio of total cholesterol:HDL cholesterol has been shown to be the optimal predictor of cardiovascular risk when incorporated in multiple risk factor equations[59].

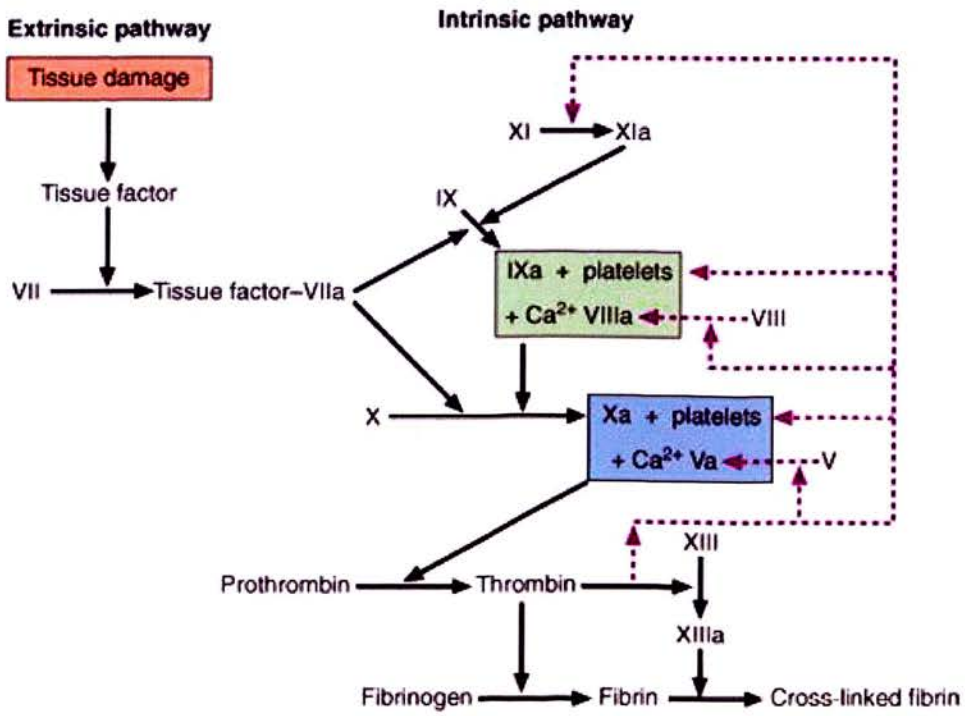
It is known that oestrogen affects hepatic lipoprotein metabolism by increasing the synthesis of VLDL leading to an increase in triglyceride levels. In addition it causes an increased apolipoprotein B receptor to lower LDL and lastly an increased synthesis of apolipoprotein A-1 which causes a high concentration of HDL[60]. Oestrogen therapy is known to have a mixed effect on serum lipid levels resulting in a significant decrease in total cholesterol and LDL, a favourable increase in HDL and an unfavourable increase in triglycerides[61,62]. Premenopausal women have much lower rates of CVD compared to age and risk matched males however this disparity rapidly disappears after the menopause. Additional risk factors include hypertension, hypercholesterolaemia, diabetes, smoking, lack of exercise and obesity and the effect of these risk factors is multiplicative. In addition to increased oestrogen levels, premenopausal women have been shown to have lower systolic and diastolic blood pressures as well as lower pulse pressures and mean arterial pressures than men during the fourth decade of life. By the age of 70 years these differences narrow and, in some cases, reverse as shown in the Framingham Heart Study[63].

Tamoxifen has oestrogenic and anti-oestrogenic properties. It is known to have an overall favourable effect on serum lipoprotein levels and in meta-analyses has a potential cardioprotective effect which reduced the rate of myocardial infarction[64,65,66,67,68]. It exerts oestrogenic effects on lipids and decreases LDL and

increases HDL and triglycerides[69]. The mechanisms underpinning this effect remain unclear[60].

1.5.3 Thromboembolic disease, coagulation and the effects of oestrogen

Thromboembolic disease is a major cause of morbidity and mortality in patients with cancer. Blood circulates around the body transporting blood cells, nutrients, gases, water, metabolic products and chemical messengers between different organs. Complex systems have evolved to maintain its continuity, which prevent excessive bleeding by securing haemostasis, and also prevent obstruction to flow due to thrombosis. Injury to the vessel wall firstly causes vasoconstriction and sets off platelet activation, adhesion and aggregation thus forming a plug which arrests haemorrhage. This is followed by activation of the coagulation cascade which results in the formation of a fibrin network to secure the platelet plug shown in figure 1.10.



The reactions of IXa/VIIIa and Xa/Va (in the green and blue boxes) occur on the platelet surface. The dotted lines represent positive feedback effects from small amounts of thrombin. This greatly enhances the activity of the coagulation network and results in large amounts of thrombin generation and thus fibrin formation in the clot.

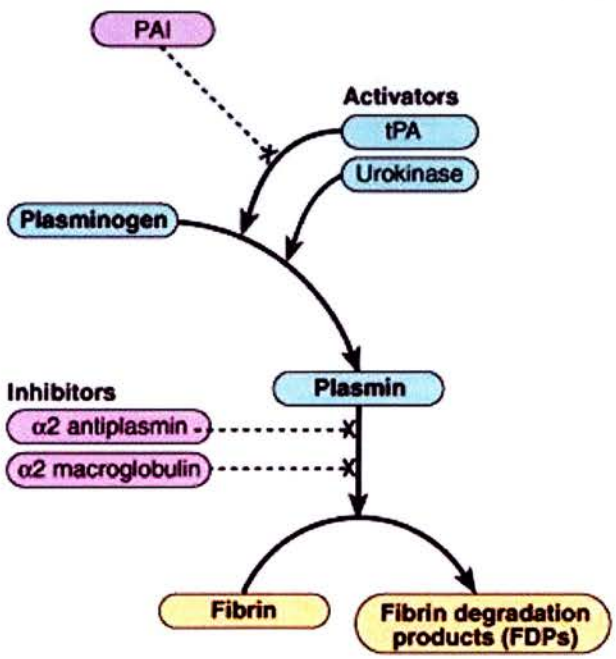
Figure 1.10 Normal haemostatic mechanism: coagulation factors of the intrinsic and extrinsic pathways[42].

The coagulation system consists of a series of soluble inactive zymogen proteins which when proteolytically cleaved and activated, become capable of activating one or more components of the cascade. Coagulation factors are synthesised in the liver and are identified by Roman numerals, activated factors are given the suffix 'a'. Factor V is also produced by platelets and endothelial cells. Two pathways of activation have been identified, the intrinsic and extrinsic although the latter is the main mechanism in vivo. Coagulation is initiated by the extrinsic (or tissue factor) pathway and amplified by the intrinsic pathway. Tissue factor is a transmembrane protein which is expressed during endothelial cell damage, which activates factor VII to VIIa. This in turn activates factor X→Xa which forms a complex with factor V on the surface of platelets which converts prothrombin→thrombin. This in turn converts fibrinogen→fibrin, which polymerises and is cross-linked by factor XIII to form a stable clot. Thrombin plays an important role in what is referred to as the 'final common pathway', it activates factors XI, VIII, V and platelets in a positive feedback loop. Von Willebrand factor (vWF) is a protein synthesised by endothelial cells and megakaryocytes which is also involved with coagulation and platelet function. It acts as a carrier protein to factor VIII and deficiencies cause lower plasma factor VIII levels. It also enables platelets to adhere to damaged vessel walls and reduced levels cause impaired platelet plug formation[42].

To prevent over-activity of the coagulation cascade once haemostasis has been secured, natural anticoagulants are present. These include tissue factor pathway inhibitor (TFPI), antithrombin (AT), protein C and protein S. TFPI inactivates the tissue factor-VIIa complex and factor Xa whereas AT principally inhibits thrombin (IIa) and factor Xa but also inactivates IXa and XIa. Protein C is a vitamin K-dependent factor which, when activated by thrombin along-with its co-factor protein S, degrades and inactivates factor Va and VIIIa. A deficiency in the function of these

natural inhibitors results in a tendency towards thrombus formation (prothrombotic state).

Insoluble blood clots need to be broken down to allow vessel recanalization to occur. This occurs secondary to fibrinolysis as shown in figure 1.11. It is primarily initiated by tissue plasminogen activator (tPA), (released by the endothelium) and also by urokinase (synthesised in the kidney). They convert inactive circulating plasminogen to the active enzyme plasmin which hydrolyses the fibrin clot, producing fibrin degradation products. Within thrombi, both tPA and plasminogen bind to cross-linked fibrin resulting in the production of plasmin which lyses developing thrombi.



PAI – plasminogen activator inhibitor, tPA – tissue plasminogen activator

Figure 1.11 Fibrinolysis[42]

Excessive activity of tPA is prevented by plasminogen activator inhibitor (PAI) which occurs in plasma. Further inhibitors of fibrinolysis include α2 antiplasmin and

α2 macroglobulin which inactivate plasmin. A decreased fibrinolytic capacity is associated with thrombotic disease[42,70].

Thrombosis occurs when haemostasis proceeds 'unchecked' resulting in vascular occlusion. Thrombi may 'break up' into smaller clots and lodge at distant points within the circulation, known as emboli. In arteries thrombosis usually occurs from rupture of an atheromatous plaque which initiates the formation of a platelet nidus on which platelets adhere and aggregate. Predisposing risk factors for arterial thrombosis are related to the development of atherosclerosis and include male sex, positive family history, hyperlipidaemia, hypertension, diabetes mellitus and smoking[71]. Venous thromboses are more common and are associated with Virchow's triad which suggests that there are three components to thrombus formation: disruption of venous endothelium, disruption in the pattern of blood flow and changes in the blood constituents (hypercoagulability). Predisposing risks for venous thrombosis include age >40years, obesity, varicose veins, previous deep vein thrombosis (DVT), oral contraception, pregnancy, hormone replacement therapy (HRT), dehydration, immobility and malignancy.

There are several inherited disorders which are associated with an increased risk of thrombosis. These include factor V Leiden, prothrombin G20210A variant, protein C deficiency, antithrombin deficiency, protein S deficiency and dysfibrinogenaemia. Factor V Leiden is the most common of these disorders and affects 30% of Caucasian factor V allelles. It is associated with failure of activated protein C (APC) when added to plasma to prolong the activated partial thromboplastin time (APTT). It makes factor V less susceptible to cleavage by APC and is called the 'factor V Leiden mutation'. Heterozygous patients have an approximately 5-8 fold increased risk of venous thrombosis compared with the general population whereas those who are homozygous have a 30 – 140 fold risk[71].

The first association between malignancy and thrombosis was reported by Armand Trousseau in 1865[72]. Neoplastic cells are known to activate the coagulation cascade resulting in a hypercoagulable state. Procoagulant mechanisms primarily involve expression of haemostatic proteins on tumour cells, production of inflammatory cytokines and adhesion of tumour cells to endothelium. Tissue factor plays a major role in tumour progression, metastasis and angiogenesis through signaling via its intracellular domain[73].

Increased circulating oestrogen levels are also associated with an increased incidence of thromboembolic events. This has been demonstrated in pregnancy, the puerperium and in women taking oral contraception and HRT. Oestrogen is associated with an increase in plasma levels of factors II, VII, VIII, IX and X and a decrease in levels of anticoagulants AT and tPA in the vessel walls[71]. These changes result in an increased tendency to thrombosis. Tamoxifen has oestrogenic effects on coagulation and is associated with an increased risk of venous thromboembolic events including pulmonary embolism and deep vein thrombosis[74].

1.6 The aetiology of breast cancer

The cause of most breast cancers remains largely unknown however hormonal influences are widely acknowledged as playing a significant constitutional role. There exist several demonstrable risk factors which play varying and sometimes cumulative effects on a person's risk. These include genetic, hormonal, environmental and nutritional influences with the most important risk being attributed to gender, increasing age, lifetime oestrogen exposure and genetic susceptibility.

The most important risk factor is gender: women are 135 times more likely to develop breast cancer compared with males. Only 277 male cases were reported in the UK in 2007. This represents <1% of the total number diagnosed[3].

Age is also an important risk factor which is often overlooked. Half of a woman's cumulative risk occurs after the age of 65 years[75]. The level of risk for younger women is low and in the absence of other risk factors, a woman between the ages of 35 and 55 years has only a 2.5 % chance of developing breast cancer[76]. The incidence doubles approximately every 10 years until the menopause, when the rate of increase slows dramatically[77].

1.6.1 Genetic influences

Epidemiological data has suggested that there is a genetic contribution to the development of breast cancer with certain families having multiple members affected. A family history of breast cancer in a first-degree relative (mother or sister) is associated with a 1.7 increased chance of developing breast cancer. If the relative affected is pre-menopausal there is a three-fold increased risk and if they are postmenopausal the risk is 1.5 fold. Those with a history of bilateral cancers in a first-degree relative are 5 times more likely to develop the disease and if the history includes bilateral cancers in a premenopausal woman then the risk increases further to 9 times. An increasing risk is associated with the larger the number of relatives affected and the closer their genetic relationship[78,79]. Notwithstanding this, most cancers are sporadic and although many patients have a positive family history, this is more likely to be due to the frequent incidence of breast cancer rather than an underlying genetic predisposition which affects <10%.

Several genes are now known to be involved with the development of breast cancer and include BRCA1, BRCA2, p52, PTEN, ATM and CHEK2. The commonest causative genes are BRCA 1 and 2 although these mutations remain rare. Both are inherited in an autosomal dominant pattern and are therefore passed through the generations via the mother or the father. BRCA1 is located on chromosome 17 and gene carriers are at risk of developing other malignancies including ovarian and prostate cancer. Women with this mutation have between 56-86% chance of developing breast cancer and between 15-45% chance of developing ovarian cancer. The BRCA2 gene is located on chromosome 13 and carriers have a similar chance of developing breast cancer compared with BRCA1 carriage. BRCA2 is associated with

an increased risk of developing ovarian, pancreatic, and prostate cancers and melanoma. The Ashkenazi Jewish population are frequent carriers of this mutation.

Li-Fraumeni syndrome is associated with premenopausal breast cancer and other tumours which often appear in childhood. It is rare and is associated with a germline mutation in the p53 gene on chromosome 17.

Women who are heterozygous for mutations in the ATM gene are at increased risk of developing breast cancer, as are those with germline mutations in the PTEN gene located on chromosome 10 which causes Cowden's syndrome and is associated with a lifetime risk of developing breast cancer between 25-50%. Other rare genetic disorders associated with the development of breast cancer include Peutz-Jeghers syndrome and ataxia telangiectasia[9].

Breast cancers are polygenic in nature. In addition to the high and moderate risk genes previously mentioned, there are many other susceptibility alleles associated with lower risk which were discovered by genome wide association studies. The majority of familial breast cancer risk appears to be associated with a combination of multiple lower penetrance alleles. These alleles include single nucleotide polymorphisms (SNPs) in five novel genes including TNRC9, FGFR2, MAP3K1, H19 and lymphocyte-specific protein 1 (LSP1). The effects of the loci appear to combine multiplicatively. The most strongly associated SNP is intron 2 of the FGFR2 gene, a tyrosine kinase receptor, which is amplified and overexpressed in 5-10% of breast cancers. The odds ratio for this allele is estimated to be 1.26. Several of the loci appear to be associated with specific subtypes of breast cancer e.g. the FGFR2 locus is strongly associated with ER positivity. The SNP rs3803662 of the TNRC9 gene appears to be correlated with bone metastases and also ER positivity. The discovery of breast cancer susceptibility loci has led to an improved

understanding of the pathogenesis for breast cancer and may in turn lead to improved diagnostics and treatments in the future[80,81].

The National Surgical Adjuvant Breast and Bowel Project (NSABP) implemented the P-1 Breast Cancer Prevention Trial (appendix F) in 1992 to assess the value of using tamoxifen for the prevention of breast cancer in 13,388 women at increased risk. Results demonstrated that tamoxifen reduced the risk of invasive breast cancer by 49% ($p<0.00001$). It reduced the occurrence of ER+ve tumours by 69%, but showed no difference in the occurrence of oestrogen receptor negative (ER-ve) tumours[82].

Women in the UK deemed likely to have an underlying genetic predisposition to developing breast cancer are referred for genetics counselling and testing.

1.6.2 Hormonal influences

There is a large amount of evidence linking oestrogen with breast development and subsequent tumourogenesis. This is demonstrated by the complete failure of breast development in the absence of ovarian function[83]. Mice studies have confirmed that if the gene for the oestrogen receptor alpha is knocked out, no further breast tissue development occurs and breast cancer development does not occur, even with the addition of oestradiol therapy[84].

The risk of postmenopausal breast cancer increases with higher levels of oestrogen levels. This association is stronger for hormone receptor positive cancers. Oestradiol is considered the most biologically active endogenous oestrogen and circulates 'unbound' or 'bound' to sex hormone-binding globulin or albumin. Unbound oestradiol or that bound weakly to albumin may be more closely associated with an increased risk of breast cancer because it is more readily available to breast tissue as it crosses the plasma membrane of target cells more easily[6]. Adipose tissue is a major source of oestrogen and obese women subsequently have higher levels of endogenous oestrogen and an increased risk of developing breast cancer.

Early age at menarche has been consistently associated with an increased risk of developing breast cancer. Studies suggest that this risk is more closely associated with the development of premenopausal breast cancer. Each year of delay in menarche is associated with a 9% reduction in the development of premenopausal breast cancers and a 4% reduction in postmenopausal breast cancers. The cause of this risk remains to be ascertained however it may be due to lifelong exposure to endogenous hormones or due to the cyclic levels of ovarian hormones.

Shorter menstrual cycles have been associated with a greater risk of breast cancer. This may be explained by an associated increase in the overall number of menstrual cycles.

An early menopause or bilateral oophorectomy at a young age is associated with reduced risk of breast cancer due to decreased endogenous hormone levels and decreased breast cell divisions. Overall there is a marked fall in the incidence of breast cancer in women treated with ovarian ablation or anti-oestrogen therapy[82].

Parity is inversely associated with the risk of breast cancer with nulliparous women being at increased risk. Nonetheless, epidemiological evidence consistently indicates that risk is also increased during the first decade after first pregnancy. A younger age at full term pregnancy is associated with a lower lifetime risk of breast cancer. This risk reduction takes approximately 10-15 years to become evident and is not seen before the age of forty. The first pregnancy is associated with differentiation and permanent changes in breast glandular epithelium. The later the first pregnancy, the more likely mistakes in DNA have been made which may be propagated during the cell proliferation of pregnancy. Each subsequent birth decreases breast cancer risk, probably because of changes in breast morphology. In addition, there is evidence demonstrating that a short time between each pregnancy reduces risk.

Induced abortion has previously been associated with an increased risk of breast cancer in case-control studies however the Nurses' Health Study studied 105,716 women between 1993 and 2003 and concluded that neither spontaneous nor induced abortion is associated with an increased risk of breast cancer[85].

Breastfeeding has been shown to induce a protective effect against breast cancer as it delays the recurrence of the menstrual cycle. This has been shown by the observation between breastfeeding duration and the delayed return of ovulation during the

postpartum period. Suppression of ovarian function has been shown to be associated with a reduced risk of breast cancer compared with normally cycling women. Epidemiological evidence has been inconsistent however a meta-analysis of 40 studies performed in 2000 suggested a small but significant protective effect of breast feeding on the risk of breast cancer in non-menopausal women[86]. Other studies have indicated that there is a 4% overall risk reduction for every year of breast feeding[42].

The relationship between HRT and breast cancer has been extensively studied over the last thirty years. Randomised controlled trials have confirmed the relationship between HRT and an increased risk of breast cancer. Risk is greater for women taking HRT for more than five years and for those who used oestrogen and progestin hormone combinations. Results from the Women Health Initiative Survey and Million Women Study (appendix F) demonstrated a significantly increased incidence of breast cancer in users of HRT. The magnitude of risk was substantially greater for oestrogen-progestagen than for other types of HRT[32,37]. The Breast Cancer Demonstration Project concluded that the risk of breast cancer increased by approximately 1% for every year that women were administered oestrogen alone and about 8% for every year that they took oestrogen plus progestin[87]. Once either type of HRT treatment is stopped, the risk of breast cancer falls[88]. Published results show that since 2003 there has been a decrease in the incidence of breast cancer observed in the Flemish region of Belgium which has been attributed to a fall in the consumption of HRT[89]. HRT has now been classed as a group 1 carcinogen to humans by the International Agency for Research on Cancer[32,37,89,90].

1.6.3 Environmental factors

A high versus low level of physical activity has consistently been associated with a 20-40% reduction in the risk of postmenopausal breast cancer[91]. Proposed mechanisms by which physical activity may act on breast tumour development include reducing the levels of endogenous sex hormones[92], boosting the immune system, modulating insulin and insulin-like growth factors and lowering levels of chronic inflammation[93]. A recent study of 118,899 women demonstrated that >7 hours per week of moderate-to vigorous exercise within the last ten years was associated with a 16% reduced risk of postmenopausal breast cancer[94].

There is a positive association between alcohol consumption and breast cancer. Alcohol is metabolised by the enzyme alcohol dehydrogenase into acetaldehyde, a known carcinogen[95]. One large study concluded that recent consumption of three or more alcoholic drinks per day is associated with a relative risk of 2.2[96]. Alcohol ingestion has been shown to directly increase plasma oestradiol levels in postmenopausal women which may account for these findings[97].

Case-controlled and prospective cohort studies have linked night shift work with breast cancer risk. Melatonin, a hormone produced primarily by the pineal gland, has anti-neoplastic properties including an ability to modulate the immune system with antioxidant, antimitotic and antiangiogenic effects[98]. Melatonin secretion is regulated by the hypothalamus, which receives environmental light signals from the retina via the retino-hypothalamic tract. Artificial light alters the natural release of melatonin, with the highest levels of melatonin physiologically occurring during the dark night phase and relatively low levels throughout the day[99]. Melatonin may block the

oestrogen receptor ERα and affect the enzyme aromatase, which produces oestradiol. Epidemiological studies have shown that night workers have lower nightly melatonin levels. Evidence supporting the association between sleep duration and melatonin production is less clear. Night shift work is likely to become a recognised potential public health hazard in the future[98].

Moderate to high-dose ionising radiation exposure is known to increase the risk breast cancer. Radiation causes DNA damage by interacting directly with DNA molecules and via the production of reaction oxygen species that form as a by-product of water radiolysis. A recent study of 2,882 women demonstrated that low-dose radiation exposure also increases the risk of breast cancer. Women who reported having multiple chest X-rays, 7 or more mammograms and those who had dental X-rays without lead protection before the age of 20 years were at increased risk with an odds ratio of 1.8[100].

1.6.4 Other risk factors for breast cancer

Increased mammographic breast density is strongly associated with an increased risk of breast cancer. A recent meta-analysis demonstrated that the relative risk for women with high breast density ranged from 3.25 – 6.49[101]. This has led to the use of breast density as a biomarker for breast cancer risk[102]. Breast density is correlated with HRT use[103] so breast density may reflect a more oestrogenic environment. It is well established that increased breast density may result in cancers being masked on screening mammograms and it is therefore important that this group are considered for alternative imaging techniques which may result in earlier diagnosis.

The positive relationship between atypical hyperplasia (AH) and breast cancer is well established. AH encompasses both atypical ductal hyperplasia and atypical lobular hyperplasia. Normal breast tissue expresses relatively small amounts of ER on cells yet AH has an abundance of ER. In fact ER increases during the progression from normal to cancerous cells. In the prevention studies, tamoxifen produced a huge reduction in progression from AH to cancer[82] so ER is important in the process. Atypical lobular hyperplasia is associated with the greatest increase in risk. The odds ratio for the development of breast cancer with AH is 4.1[104]. Women with both proliferative breast disease and calcification have an estimated 69% increased risk compared with those without either and women without proliferative disease have no elevation in breast cancer risk compared with women of similar age[105].

In the USA breast cancer is most likely to affect the left breast. There is on average more breast tissue within the left breast however this has not been shown to be the aetiology of the laterality[106]. Other studies have demonstrated similar results and

many explanations for this have been hypothesised however to date there is no supporting evidence demonstrating a cause[106,107].

1.7 Tumourogenesis

The majority of breast cancers arise within the TDLU. Tumourogenesis is thought to occur as a result of highly diverse, multiple genetic changes which appear to develop from pre-existing benign lesions. It involves a spectrum of changes which occur over a long period of time. These include the progression from normal breast epithelium to benign proliferative lesions to atypical proliferative lesions and then to carcinoma in situ and finally invasive neoplasms[108]. The expression of ER increases with this slow progression.

Of the many different types of benign breast conditions, only a few have significant premalignant potential. The most recognised include atypical hyperplasias and carcinomas in situ. Prognosis of pre-malignant conditions has been estimated from epidemiological studies which have shown that women with a history of AHs and in situ carcinomas have approximately 5 – 10 increased relative risk of developing invasive disease. Future therapies may have the potential to target specific genetic defects thus preventing the development of invasive disease[108].

1.7.1 Pre-invasive cancer

There are two main categories of non-invasive cancer which are distinguished by their cell types and histological patterns. Ductal carcinoma in-situ (DCIS) is the commonest and lobular carcinoma in-situ (LCIS) is comparatively rare.

1.7.2 Microinvasion

Microinvasion is defined as an invasive carcinoma with no focus measuring >1mm. It is usually found in DCIS or less often LCIS where small foci of tumour cells invade through the basement membrane into surrounding stroma. It is associated with a good prognosis although the impact of multifocal microinvasive disease remains to be determined[1].

1.7.3 Invasive breast cancer

Infiltrating ductal cancer accounts for the majority (85%) of invasive cancers whereas lobular tumours account for the remainder. Tumour cells arise within the TDLU and invade through the basement membrane into surrounding tissue including lymphatics and blood vessels. The commonest histological type is invasive ductal carcinoma of no special type (NST) however many others exist including tubular, cribriform, mucinous and papillary as shown in table 1.4. Most of these special types express a greater amount of ER and are associated with a much better prognosis compared with NST tumours. In contrast, tumour types that are aggressive such as basal cancers and HER 2*+ve cancers are often ER -ve. Rare cancers include adenoid cystic carcinoma, adenomyoepithelioma and squamous carcinomas[109].

* HER2 (human epidermal growth factor 2 also known as ErbB-2) is a transmembrane growth factor receptor which is expressed in 20% of breast cancers. Breast cancer patients who overexpress HER2 can be treated with the humanised murine monoclonal antibody Transtuzumab which has antitumour activity against cells which overexpress HER2.

In situ carcinomas	Invasive carcinomas
NST (no specific type, not otherwise specified)	NST
Intraductal	Ductal
Intralobular	
Paget's disease	Lobular
	Medullary
	Mucinous
	Papillary
	Tubular
	Cribriform
	Inflammatory
	Secretory
	Undifferentiated
	Paget's disease
	Adenoid cystic
	Squamous cell
	Adenomyoepithelioma

Table 1.4 Histopathological types of breast cancer

1.7.3.1 Tumour grade

All invasive breast carcinomas are graded excluding medullary carcinomas. The Nottingham combined histologic grade is currently recommended. It is the result of the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system. Grade is determined by assessing morphological features including tubule formation, nuclear pleomorphism and mitotic count[109] and is correlated with ER. Grades of 1 (favourable) to 3 (unfavourable) are determined for each feature and are then added together. The resulting combined score of 3-5 is designated grade 1, 6-7 is designated grade 2 and 8-9 is designated grade 3[109].

1.7.3.2 Metastatic disease

Tumour cells spread via direct infiltration into breast parenchyma, along mammary ducts, via tumour emboli to lymphatics and through blood to distal sites. Small deposits of metastatic disease are known as micrometastases and are defined as measuring between 0.2mm and 2mm in size.

Approximately 95% of lymph from the breast drains to the axilla. Axillary lymph node involvement is considered to be the single most prognostic indicator in breast cancer and survival is correlated directly with the number and level of axillary lymph nodes involved. Cancerous cells may also metastasis to the internal mammary lymph nodes but it is uncommon for these to be involved in isolation.

Metastases are often present in women prior to initial diagnosis and treatment. This is demonstrated by the necessity of neoadjuvant and adjuvant systemic therapies compared with tumour excision alone. Bone is the commonest site of first distant relapse in all reported series and affects ¾ of patients with secondary breast cancer. Other sites commonly affected include lung, liver and brain however multiple other sites may be involved. Solitary first relapses to the liver are relatively rare although liver metastases have been reported in >50% of patients who have died of breast cancer following post-mortem examination.

1.8 The role of hormones and hormone receptors in breast cancer

The majority of breast cancers are dependent upon oestrogen and progesterone for their growth. Oestrogen is the major inducement driving the growth of hormone-dependent breast cancer and endocrine therapy is therefore directed towards inhibiting or reducing oestrogen activity.

Changes in breast morphology and physiology are mediated through binding of sex hormones to either intracellular steroid receptors or membrane-bound peptide receptors. Oestrogen and progesterone mediate effects on their target sites via oestrogen receptors (ER) and progesterone receptors (PgR) respectively, both are steroid receptors located in the cell nucleus. They have been demonstrated in approximately 15-25% of normal breast epithelium and are expressed in varying levels throughout the menstrual cycle. Oestrogen promotes the growth and survival of normal and cancerous breast epithelial cells by binding and activating the ER. Both ER and PgR function as transcription factors when they are bound to their respective ligands[6]. The activated receptor in turn binds to gene promoters in the nucleus and activates many other genes responsible for cell division, inhibition of cell death, new blood vessel formation and protease activity. There is an increase in the proportion of cells that express ER found at both the earliest stages of pre-cancerous changes and in approximately 70% of cancers[110].

The expression of ER is inversely correlated with tumour proliferation rates and cancers expressing ER and PgR are more likely to be well differentiated and have lower degrees of proliferation and hence an overall better prognosis.

Initially it was accepted that there was a solitary ER responsible for the effects of oestrogen and its antagonists. In the mid 1990s two distinct and functional ER proteins were identified, ERα and ERβ. Both have been extensively studied and are

now collectively called ER. The amount of ERα is of paramount importance in terms of response to endocrine therapy and subsequent prognosis. ERβ was initially identified from the cDNA of rat prostate and there have now been several isoforms cloned. Similarly, two progesterone receptors have been located which are known as PgR-A and PgR-B, collectively known as PgR.

There is a bimodal distribution of ER with tumours being either predominantly ER negative or strongly positive as shown in figure 1.12. A relatively small number show intermediate values[111].

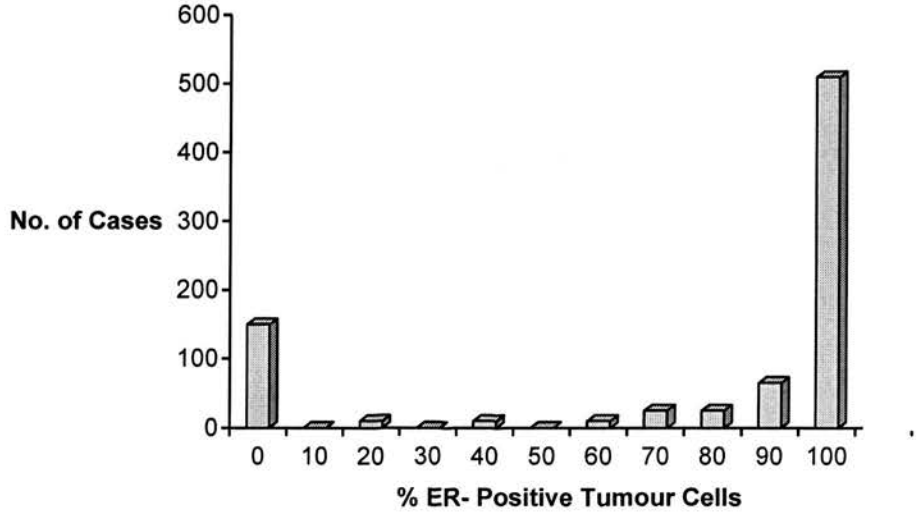


Figure 1.12 Frequency distribution of the percentage of cells showing nuclear staining for oestrogen receptor among 825 primary breast cancers[111]

Numerous assay methods have been available to measure ER and PgR levels. Early studies used multipoint titration analysis using dextran-coated charcoal and sucrose density gradient centrifugation. These methods were replaced in the 1990s with immunohistochemistry assays which are more specific, reliable and are commercially available. They are based on ligand binding methods and the recognition of the receptor protein by specific antibodies. The optimal method to use

is controversial however most pathology laboratories use the manual scoring Allred system or histo score (H score). The semiquantitative Allred scale consists of a proportion score which represents the estimated proportion of positively stained tumour cells (scores ranging from 0 to 5), and an intensity score which represents the average intensity of positive tumour cells (scores ranging from 0 to 3). The proportion and intensity scores are added to obtain a total score resulting in overall scores of 0 or 2 through to 8 as shown in figure 1.13[112]. A score between 3 and 8 is considered ER positive.

The H-score is calculated by multiplying the frequency of positivity and the intensity of staining.

It has been demonstrated that any benefit gained from treatment is directly proportional to the quantity of ER present[113] as shown in table 1.14.

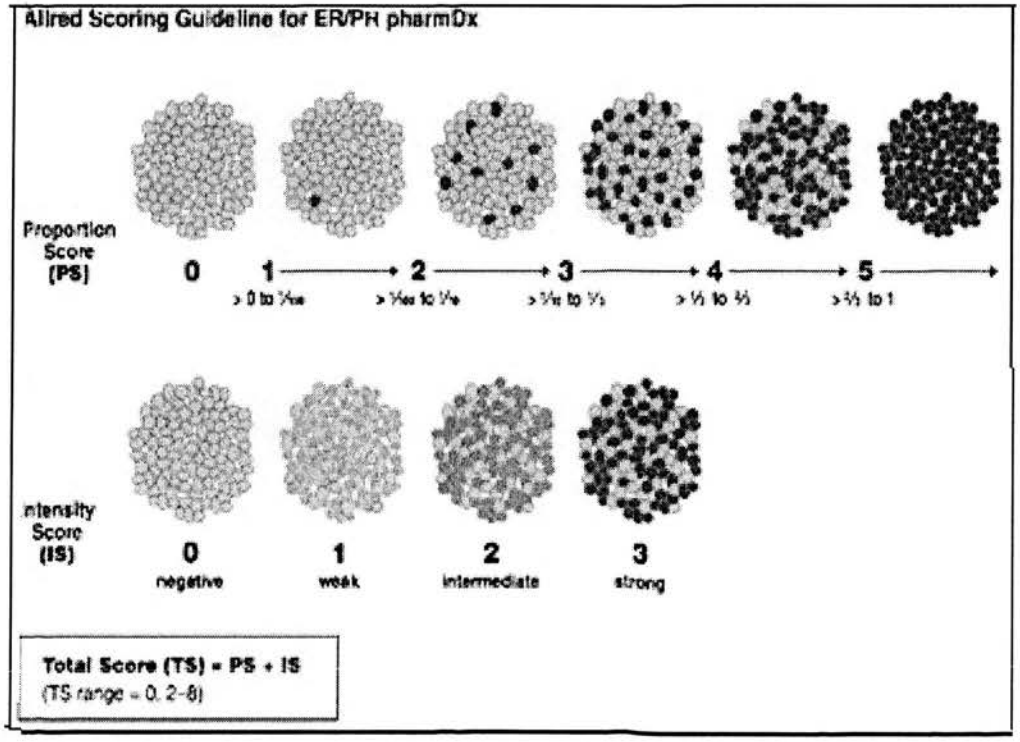


Figure 1.13 Schematic depicting Allred Score[112]

Proportion Score (PS) range 0 to 5, Intensity Score (IS) range 0 to 3

ER score	Clinical responses expected
0	Endocrine treatment will not work
2-3	Associated with a small (20%) chance of response
4-6	Associated with ~50% chance of response
7-8	Associated with a high (75%) chance of response

Table 1.14 Oestrogen receptor scores and related clinical responses

Endocrine treatment is also deemed worthwhile in patients with low oestrogen receptor but high progesterone receptor values[114], although such a receptor profile is rare.

The dominant oestrogen in ER/PgR positive breast cancers is oestradiol. Plasma oestrogen levels can be measured using sensitive assays however the extent to which they reflect absolute tissue oestrogen levels is more complicated to assess. Tissue samples from postmenopausal women with ER+ve tumours have shown that the concentration of oestradiol in the tumour is about 10 fold the concentration measured in plasma. Oestrone sulphate levels within breast cancer tissue have been shown to be much lower compared with plasma oestrone sulphate levels[115].

Despite the fall in plasma oestrogen levels at the time of the menopause, postmenopausal breast tissue has the ability to maintain local concentrations of oestrone and oestradiol at levels that are 2-10 and 10-20 fold higher, respectively than corresponding plasma levels[116,117,118,119]. High levels of intratumoural aromatase and the uptake of oestrogens from the circulation and or in situ oestrogen synthesis by intratumoural aromatase may account for this[120].

There is substantial evidence demonstrating the risk of breast cancer increases with prolonged exposure to oestrogen. Early evidence of the importance of lowering oestrogen levels in breast cancer arose from the observation that a number of patients who developed progression following castration achieved a further response to the suppression of plasma oestrogen following adrenalectomy[121,122]. Nowadays clinicians target endocrine treatment at those women with breast cancer who are likely to respond i.e. those whose tumours express ER and PgR receptors. This is accomplished by measuring the quantity of these steroid hormone receptors present within tumours. Endocrine therapies target the ER signalling pathway either by strategies which act on the receptor or by approaches which deprive the receptor of oestrogen. Adjuvant endocrine therapy is effective only in patients who have ER +/- PgR positive breast cancer. ER is the best predictor of tamoxifen benefit[123] and large studies have confirmed that both ER and PgR are important predictors of the benefits of endocrine therapy. The incidence of response to hormone therapy increases with increasing ER levels and those found to be ER, PgR negative respond rarely if at all to hormonal manipulation. Approximately two thirds of all patients with breast cancer have cancers which are ER+ve[114,120] and can therefore be treated using oestrogen suppression. The levels of ER expression vary between patients and change throughout the course of the disease and also in response to endocrine therapies. ERs are present more often in tumours of postmenopausal women compared with those of premenopausal women and are contained within 90% of male breast carcinomas. PgR is present in 45% to 60% of primary or metastatic breast tumours.

ER and PgR should therefore be assessed in all tumour specimens as they correlate with the response of systemic endocrine therapy. The absence of oestrogen and

progesterone receptors and the overexpression of HER2 are related to a worse prognosis. Paradoxically, it is important to note that not all ER +ve cancers will respond to endocrine therapy. It is becoming increasingly clear that ER positivity is a good prognostic marker whereas PgR positivity may be a marker of a more aggressive tumour type[6]. The absence of ER in a breast tumour correlates well with an increased response to cytotoxic chemotherapy.

There are large-scale molecular differences between ER+ve and ER–ve breast cancers[124]. Research into the biology of the ER and resistance mechanisms are of considerable interest as these results may hold the key to future therapies targeting resistance and therefore improving outcomes. Despite the successes of endocrine treatment, there are many patients who develop de novo or acquired resistance and subsequently die.

1.9 The enzyme aromatase

In postmenopausal women, aromatase is the key enzyme that catalyses oestrogen synthesis by converting androstenedione to oestrone and testosterone to oestradiol. Circulating androgens, mainly androstenedione, are secreted by the adrenal gland and, to a minor extent, probably the postmenopausal ovary[125,126]. They are synthesised from cholesterol via a number of different transformations as previously shown in figure 1.5. The last step is catalysed by the action of the aromatase enzyme (product of CYP19 gene). After the menopause, aromatase is present in peripheral tissues including adipose tissue, liver, muscle, skin, brain and breast tumours. It is also identified in the epithelial and stromal components of breast tissue. Breast cancers may be able to produce hormones or growth factors which can modulate the local environment to support tumours. The majority of post-menopausal breast cancers express significant levels of ERα, the nuclear transcription factor that is activated by oestrogen binding and will respond to endocrine treatment[19].

The human aromatase gene (CYP19; P450arom) is expressed in many postmenopausal tissues and is comprised of a 30-kb coding region containing nine exons and a 93-kb regulatory region containing 10 untranslated exons. The large regulatory region contains ten tissue-specific promotors that are alternatively used in various cell types. Each individual promotor is controlled by regulatory sequences in the DNA and transcription factors that bind to these specific sequences[127]. Promoters II, 1.3 and 1.7 are particularly active in breast cancer[128]. These regions are promoted by various growth factors and interleukins known to be synthesised in breast tumours, probably contributing to the high local oestrogen concentrations. Tissue

oestrogen concentrations appear to be much higher in tumours of ER+ve women compared with those who are ER-ve.

Circulating oestrogens are thought to arise from passive diffusion, leakage from tissue following metabolism and excretion by the liver and kidney, respectively[129]. The use of tracer techniques to measure the total amount of body aromatisation is an estimation of the amount of oestrogen produced in the peripheral tissues and can therefore be used as a surrogate marker. There is marked variation in tissue oestrogen levels between postmenopausal breast tumours and this is most likely to be due to the different degrees of expression of the aromatase enzyme. Additionally, differences may also be secondary to local oestrogen metabolism.

In postmenopausal breast cancer the intratumoural concentrations of 17B-oestradiol are much higher than that in the plasma due to high levels of tumour aromatase production and a combination of tumour synthesis and selective uptake from the plasma. The amount of ER expressed in the physiological state is higher in those with breast cancer. Inhibiting aromatase reduces circulating oestrogen levels and inhibits local synthesis. Studies have shown that tissue oestrogen levels in breast cancer tissue are significantly decreased after the introduction of the third generation aromatase inhibitors, anastrozole[130] and letrozole[131].

1.10 The management of breast cancer

1.10.1 Diagnosis

Patients with breast cancer may present with a range of symptoms for example, a palpable lump(s), asymmetrical nodularity, inflammatory changes, pain, nipple discharge and changes in breast or nipple skin or contour. Less commonly they present with symptoms of metastatic disease including abdominal pain, bone pain and spinal cord compression. In the UK patients are commonly referred to specialist breast centres and it is widely recognised that those treated at larger units with dedicated specialists working as part of a multidisciplinary team have the best outcomes[132]. It is recommended that patients attending breast clinics should receive all necessary investigations and be informed of the results in a single clinic visit i.e. 'one stop'.

A thorough history is obtained prior to 'triple assessment' which includes clinical examination, imaging and biopsy to aid diagnosis. Clinical examination includes identifying the location and size of any lump or abnormality and determining if there is evidence of involvement of adjacent tissues including skin and muscle. The axillae, supraclavicular fossae and neck are palpated to detect possible lymph node metastases however the correlation between clinical examination and lymph node involvement is <50%[133]. Any signs of metastatic disease are sought.

Imaging includes breast ultrasound in patients under the age of 35 years and mammography in those over the age of 35 years. Ultrasound is also used to guide core biopsies in those lumps/opacities which are impalpable, small or difficult to localise. Core biopsy is used to assess malignancy and remains the current standard

of care for evaluating breast lumps. It provides a higher level of specificity when compared with fine needle aspiration cytology (FNAC), (92% and 88% respectively) as it provides more tissue and histologic architecture to better classify pathological types but takes longer to process. It also results in higher positive predictive values of suspicious lesions (100% and 78% respectively) and atypia (80% and 18% respectively)[134]. Biopsies are obtained using a size 14 gauge disposable core biopsy instrument which yields on average 100mg tissue per core. Multiple biopsies are obtained where possible and subsequently placed in 4% buffered formalin to allow histological examination. The results are usually available within a few days and provide information on tumour type and grade (degree of differentiation), ER and PgR positivity, HER2 status, the presence of lymphatic or vascular invasion and if there is in situ cancer present.

Several pre-operative investigations are routinely performed after diagnosis. They assess the patient's overall fitness for treatment and facilitate staging. They include blood tests (full blood count, urea and electrolytes, liver function tests, calcium), a chest x-ray and electrocardiogram. Other investigations may be performed if there is a clinical suspicion of metastatic disease e.g. liver ultrasound and bone isotope scan. The results of all diagnostic procedures should be discussed with a multi-disciplinary team to facilitate the most appropriate management plan.

Nowadays an increasing number of breast cancers are being identified by routine screening mammography. Breast screening is targeted at women at risk e.g. those > 50 years of age and those in high risk groups e.g. younger women with a significant genetic predisposition. The aim is to reduce morbidity and mortality by detecting disease at an early stage and treating it before metastases have a chance to occur. At

present, mammography is routinely used for screening however magnetic resonance imaging (MRI) is used in younger high risk women, particularly those who carry the BRCA1 gene[135] and also when imaging women with breast implants.

In the UK, women are routinely invited to attend mammography every three years between the ages of 50 to 70 inclusive. Those at higher risk may attend more frequently and at a younger age. Two mammographic views are obtained: craniocaudal and mediolateral oblique. Double reading of mammograms improves the overall sensitivity. Studies have shown that screening can significantly reduce absolute mortality from breast cancer by up to 40% in those who attend. Drawbacks include anxiety associated with attendance and potential recall although only a small number of women recalled for biopsy are found not to have cancer[136]. The risk of radiation induced breast cancer following mammography is small with one extra cancer per year after ten years in women receiving a single mammogram[137].

1.10.2 Prognosis

Prognostic factors put individual patients into risk categories and indicate the likely future behaviour of the disease although this is not absolute. They are useful in guiding therapeutic decisions. The most powerful prognostic factors include the presence of disease spread or 'stage' at presentation and on the aggressiveness of the tumour. The most widely used prognostic index in the UK is the Nottingham Prognostic Index (NPI) which is based on three factors using the following formula:

NPI = pathological tumour size (cm) x 0.2 + lymph node stage (1,2,3) + histological grade (1,2,3)

Patients are divided into six prognostic groups depending on their overall score as depicted in table 1.5.

NPI	Prognostic groups
≤ 2.4	Excellent prognostic group
2.5 – 3.4	Good prognostic group
3.5 – 4.4	Moderate I prognostic group
4.5 – 5.4	Moderate II prognostic group
5.5 – 6.4	Poor prognostic group
>6.4	Very poor prognostic group

Table 1.5 Nottingham Prognostic Index

Several other factors are taken into consideration when assessing prognosis including: age at diagnosis with younger women being associated with a poorer prognosis; hormone receptor positivity; and HER2 status.

Other tools used to assess prognosis include Adjuvant! Online (www.adjuvantonline.org) which is a simple-to-use computer program designed to produce prognostic estimates of outcome with and without therapy, based on the estimates of individual patient prognosis. It is aimed at patients with early breast cancer who are considering chemotherapy, hormonal therapy or both after surgery and estimates the cancer related mortality, the reduction in risk afforded by therapy, and the risks of side effects of the therapies[138].

The Oncotype Dx breast cancer assay is a validated commercially available multigene expression test which predicts the likelihood of chemotherapy benefit as well as recurrence in early stage breast cancer. It is a RT-PCR assay which measures the expression of 21 genes and is intended to be used by women with early stage, node negative, ER positive invasive breast cancer who will be treated with tamoxifen. It is included in the American Society of Clinical Oncology (ASCO) 2007 and the National Comprehensive Cancer Network (NCCN) guidelines as an option to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy[139].

1.10.3 Staging

When breast cancer is diagnosed, the extent of disease should be assessed and the tumour staged. Staging is determined using information related to the tumour (T), regional nodes (N) and metastases (M) whereby cases with similar prognosis are grouped together. The TNM staging system for breast carcinoma was developed in 1959. During this era it reflected the risk of distant recurrence and death subsequent to local therapy which included radical mastectomy and adjuvant radiation to the chest wall. At this time there was no effective systemic therapy and the system provided prognosis for those with newly diagnosed cancer. Its main use was to preclude ineffectual therapies for patients who were going to die rapidly despite aggressive treatments which would certainly impede their quality of life[1].

Staging may be 'clinical' or pre-surgery and 'pathological' or postsurgical. The system can be applied to both invasive and in situ carcinomas. The complete TNM staging system depicted in table 1.6 is determined using information sought prior to neoadjuvant therapy or surgery. Pathological staging is determined using information identified following surgery and is depicted using 'p'. Post-therapy pathologic staging is described using 'yp'. The system is updated annually by the American Joint Committee on Cancer (AJCC) to reflect any advances in technology and or clinical advance.

The TNM classification permits scientists and clinicians to communicate with one another using a common 'language'. It also holds some value in determining future outcome although other variables including hormone receptor and HER2 status and the availability of multigene expression assays should be taken into account.

Additionally, the TNM system can be used to differentiate between prognostic groups by assigning patients into stages from 0 – IV as depicted by the International

Union Against Cancer (UICC) system shown in table 1.7 The stages correlate with outcome e.g. increasing tumour size and the presence of lymph node involvement have a negative impact on prognosis.

Neither the TNM nor UICC classifications are well suited to breast cancer as the clinical measurements and clinical assessment of lymph node status are often inaccurate[140].

T stage	
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	In situ disease only (*DCIS, LCIS and Paget's disease of the nipple with no invasive cancer)*
T1	≤ 2cm
T1mic	≤ 0.1cm
T1a	> 0.1 – 0.5cm
T1b	> 0.5 – 1cm
T1c	> 1 – 2cm
T2	>2 – 5cm
T3	> 5cm
T4	Any size of tumour with involvement of chest wall or skin
T4a	Direct extension into chest wall
T4b	Direct extension into skin, with oedema, ulceration or nodules
T4c	Both chest wall and skin involvement
T4d	Inflammatory breast cancer
N stage	
Nx	Lymph node status has not been assessed
N0	No regional lymph node metastases
N1	Mobile axillary lymphadenopathy
pN1mi	Micro-metastasis, > 0.2mm ≤2mm
pN1a	1-3 positive axillary nodes
pN1b	Internal mammary nodes with micro-metastasis
pN1c	1-3 positive axillary nodes and internal mammary micro-metastasis
N2a	Fixed axillary lymph nodes
pN2a	4-9 positive axillary lymph nodes
N2b	Internal mammary nodes clinically apparent
pN2b	Internal mammary nodes positive, clinically apparent, negative axillary nodes
N3a	Infraclavicular lymphadenopathy
pN3a	≥ 10 positive axillary nodes or positive infraclavicular node
N3b	Internal mammary and axillary lymphadenopathy
pN3b	Positive axillary and internal mammary nodes
N3c	Supraclavicular lymphadenopathy
pN3c	Supraclavicular node positive
M stage	
M0	No evidence of metastasis
M1	Evidence of distant metastasis

DCIS – ductal carcinoma in situ, LCIS – locular carcinoma in situ.

Table 1.6 Classification of breast cancer using the TNM Staging (2009)[77]

Prognostic Groups				10 year survival
Stage 0	Tis	N0	M0	>95%
Stage IA	T1	N0	M0	75-95%
Stage IB	T0	N1mi	M0	75-95
	T1	N1mi	M0	
Stage IIA	T0	N1	M0	45-85%
	T1	N1	M0	
	T2	N0	M0	
Stage IIB	T2	N1	M0	40-80%
	T3	N0	M0	
Stage IIIA	T0	N2	M0	10-60%
	T1	N2	M0	
	T2	N2	M0	
	T3	N1	M0	
	T3	N2	M0	
StageIIIB	T4	N0	M0	0-35%
	T4	N1	M0	
	T4	N2	M0	
Stage IIIC	Any T	N3	M0	0-30%
Stage IV	Any T	Any N	M1	<5%

Mi = micrometastases

Table 1.7 Breast cancer staging. Prognostic groups with treatment outcomes[17]

1.10.4 Surgery

Surgery is determined by tumour size and margins, location and multicentricity. Patients with early breast cancer require complete removal of the primary tumour by either wide local excision or mastectomy. The original mastectomy performed by Halsted until the latter half of last century has been replaced by more conservative surgery. Wide local excision is now the most common operation performed for those with early breast cancer and involves the removal of the tumour mass with a surrounding margin of normal tissue (0.5-1cm). This is combined with breast irradiation for the majority of those with T1-2 tumours.

Breast reconstruction is discussed with patients requiring mastectomy. Reconstruction can be performed at the time of surgery or at a later date. Latissimus dorsi and transverse rectus abdominus myocutaneous flaps are used for reconstruction often in combination with a silicone implant or tissue expander.

Breast lymphatic drainage is of paramount clinical significance as it is the most common site of local metastases. For surgical purposes, the axilla can be divided into three levels depending on their relationship with the pectoralis minor muscle. Level I contain the majority of nodes and, by definition, lies below and lateral to the inferolateral border of the pectoralis minor muscle. Level II contains 4 or 5 nodes and is located posterior to the pectoralis minor muscle. There are usually only 2-3 modes located in level III which lie superior to the upper border of the pectoralis minor. Tumour metastasises to the axilla in a progressive manner from levels I to III.

Axillary surgery may involve axillary clearance or more commonly sentinel lymph node biopsy. The advantage of the former is that it obviates the need for adjuvant radiotherapy however associated morbidity includes arm stiffness, pain,

lymphoedema and potential effects on quality of life. Sampling involves taking a minimum of four nodes from the lower axilla. If these are positive for tumour, patients require axillary radiotherapy or less commonly axillary surgical clearance. The technique of sentinel lymph node biopsy assumes that breast cancer spreads in a progressive manner from level I – III nodes. It involves identifying the first node which drains the tumour. This is usually obtained using a combination of blue dye which is infiltrated around the nipple area and also the injection of radiolabelled colloid injection around the nipple. A small incision is made over the axillary hairline and the first blue node or one with a radioactivity count >10 times greater than the background level is excised.

1.10.5 Early breast cancer

Early breast cancer is defined as disease which can be removed by surgery, i.e. T1-3, N0-1, M0 tumours. Local treatment is used to control local disease and involves breast conserving surgery/mastectomy and radiotherapy. Systemic treatment is used to combat any micrometastases and includes chemotherapy, endocrine therapy and HER 2 treatment. The choice is determined by the risk of recurrence which involves both patient and tumour factors: menopausal status, tumour size, type, grade, lymph node involvement, the presence of tumour markers including ER, PgR, HER2 and gene expression. Discussion between the multidisciplinary team (surgeon, pathologist, oncologist, radiotherapist and nursing staff) enables the optimum treatment regimen to be selected after discussion with the patient.

Wide local excision is the most common type of surgery performed for early breast cancer as previously discussed. Unless contraindicated, patients receive adjuvant radiotherapy which uses ionising radiation to destroy cancer cells. Energy released within the tissues from X-ray beams cause tissue damage to DNA molecules. Patients attend a treatment planning consultation and at this stage the treatment area is pre-marked. Regimens vary between departments however a common course includes 2 Grays of radiotherapy given daily over a five week period. Irradiation of lymph nodes is recommended if there is nodal tumour involvement. An effort is made to reduce the amount of radiation delivered to lung and cardiac tissues and the overall aim of radiotherapy is to deliver sufficient treatment intensity whilst reducing any adverse effects.

Endocrine therapy includes the use of tamoxifen and the newer AIs, anastrozole, letrozole and exemestane which are licensed for use in women with postmenopausal tumours only. These treatments will be discussed in depth in section 1.10. The prognosis for patients with early breast cancer is excellent following treatment, with <10% developing local recurrence after 10 years of follow-up[3].

1.10.6 Locally advanced breast cancer

Locally advanced breast disease is characterised by signs suggesting skin or chest wall infiltration or clinically involved matted axillary nodes. It arises due to tumour position within the breast, as a result of neglect or due to biological aggressiveness which includes inflammatory cancer and the majority of those with peau d'orange.

The mainstay treatment has been radiotherapy as surgery in the form of mastectomy is often not possible and results in higher rates of local recurrence. Intensive neoadjuvant chemotherapy and endocrine therapy can cause shrinkage of the tumour rendering it operable and often breast conservation is possible at this stage.

Standard chemotherapy regimens have increased the initial rate of control and result in a reduction in local recurrence. Hormonal treatment reduces the risk of locoregional failure, distant metastases and mortality in those with hormone receptor positive tumours.

The prognosis for patients with locally advanced breast cancer depends on the biology of the underlying disease. Overall five year survival is approximately 50%[141].

1.11 Endocrine treatment of breast cancer

There have been several significant advances in the management of breast cancer over the last decade. The emergence of hormonal treatments since Beatson's historic Lancet publication in 1896 reporting breast cancer regression after oophorectomy, has revolutionalised the options available to patients however their side effects remain a major challenge. The clinical rationale behind endocrine therapy is to deprive tumour of oestrogen, which is the major established mitogen for human breast cancer in vivo[142].

Endocrine therapy can be used in the premenopausal and postmenopausal setting and is commonly used in adjuvant, neoadjuvant and metastatic care. Tamoxifen and other anti-oestrogens competitively block the binding of oestrogen to its receptor whereas oophorectomy, gonadotrophin-releasing hormone (GnRH) agonists and AIs reduce the level of serum oestrogen[137]. Table 1.8 lists common endocrine treatments.

Endocrine treatments of breast cancer	
Ovarian ablation	surgical oophorectomy, radiation ablation, GnRH agonists
Selective oestrogen receptor Modulators	tamoxifen, toremifene
Aromatase inhibitors	anastrozole, letrozole, exemestane
Oestrogen down-regulators	fulvestrant
Oestrogens	oestradiol, diethylstilboestrol
Progesterones	medroxyprogesterone acetate, megesterol acetate

Table 1.8 Endocrine therapies[137]

1.11.1 Tamoxifen

Tamoxifen is a synthetic, non-steroidal, triphenylethylene which has been used to treat women with breast cancer for the last forty years. Because of its agonist and antagonist effects, it is known as a SERM. It is converted to its active metabolite 4-hydroxy-N-desmethyltamoxifen (endoxifen) by the enzyme cytochrome P450. Tamoxifen competes for the binding of oestradiol to the ER, but still allows the dimerisation of tamoxifen-receptor complexes, which can interact with the oestrogen responsive elements at the nuclear level[143]. This results in a block in the G_1 phase of the cell cycle and a decrease in tumour growth[144]. It acts as a competitive antagonist of oestrogen in breast tissue and inhibits the growth of ER+ve breast cancer. It is also a partial oestrogen agonist and stimulates endometrium explaining its association with endometrial thickening, polyps, fibroids, hyperplasia and an increased rate of endometrial cancers. This agonist property is also responsible for the increase in thrombogenicity and favourable impact on bone mineralisation and lipids (as a result of a decrease in the atherogenic fractions of cholesterol)[145]. It is known to activate the coagulation system with an associated significant increased risk of venous thromboembolic events, pulmonary emboli and stroke[82,146,147]. Tamoxifen is generally a well tolerated drug but is associated with additional side effects including hot flushes, vaginal dryness, vaginal bleeding, weight gain and loss of libido which may have a considerable impact on quality of life. Overall, menopausal symptoms, a modest increase in blood clots and endometrial cancer in postmenopausal women are the most important side effects noted in all studies.

Tamoxifen remains an important component of endocrine therapy despite clinical trials which demonstrate that AIs are more effective and better tolerated alternatives

in postmenopausal women. Over the last few decades, tamoxifen was known as the standard adjuvant endocrine treatment for ER+ve breast cancer and remains so for hormone sensitive premenopausal women. Patients have been traditionally prescribed five years of adjuvant tamoxifen (20mg daily). It has been shown to offer both substantive and persistent benefits in terms of reducing disease recurrences and breast cancer-related mortality in women with early ER+ve disease[67]. Research has shown that extended treatment with tamoxifen beyond five years does not yield any great benefit in disease free survival or reduction in time to recurrence[148,149,150,151,152]. This may be explained by breast cancer cells becoming resistant to the anti-oestrogenic effects of tamoxifen over time.

The need to explore alternative endocrine treatments for breast cancer is driven by the toxicity profiles of treatment and also the emerging resistance to tamoxifen. Therapy is commonly given as a sequence of treatments to minimise the development of resistance to one particular drug.

Pharmacogenomics addresses the relationship between a patient's genetic makeup and their response to an individual drug. Recent studies have shown significant variations in the enzyme cytochrome P450 which converts tamoxifen to its active compound.

Despite the effects of anti-oestrogen therapies, intrinsic (de novo) or acquired resistance to the drugs inevitably develops. Future research will hopefully predict which patients may or may not benefit from specific therapies and may facilitate the development of new approaches for the treatment of hormone receptor positive breast cancers[153].

1.12 Aromatase inhibitors

AIs were initially used in the treatment of postmenopausal women with metastatic breast cancer and were intended to have the benefits of tamoxifen without the adverse side-effects[154]. The superiority of the third-generation AIs in advanced stage disease led to their efficacy in the adjuvant setting being proven for women with early stage disease. They have additionally been investigated as neoadjuvant and preventative treatments.

AIs interfere with the body's ability to convert androgens into oestrogen in postmenopausal women by suppressing the cytochrome P450 aromatase enzyme and thereby prevent oestrogen biosynthesis. This mode of action differs from that of tamoxifen which competes with the natural ligand for binding to the ER[155]. AIs may be divided into two classes, steroidal (type 1) and non-steroidal (type 2), based on their chemical structure. Anastrozole and letrozole are non-steroidal drugs which bind reversibly to the enzyme at the cytochrome P450 moiety. Both drugs are triazoles which have a flat aromatic ring, enabling a good fit with the substrate-binding site of the enzyme. Exemestane is a steroidal AI which competes with the natural substrate for aromatase and binds irreversibly to the enzyme at the active site, acting as a suicide inhibitor. It leads to irreversible inhibition of aromatase by covalently binding to the enzyme[156] and produces metabolites which mimic androstenedione[157].

Aromatase inhibitors and inactivators differ in their mechanism of action. The classes and structure of AIs are shown in figure 1.14.

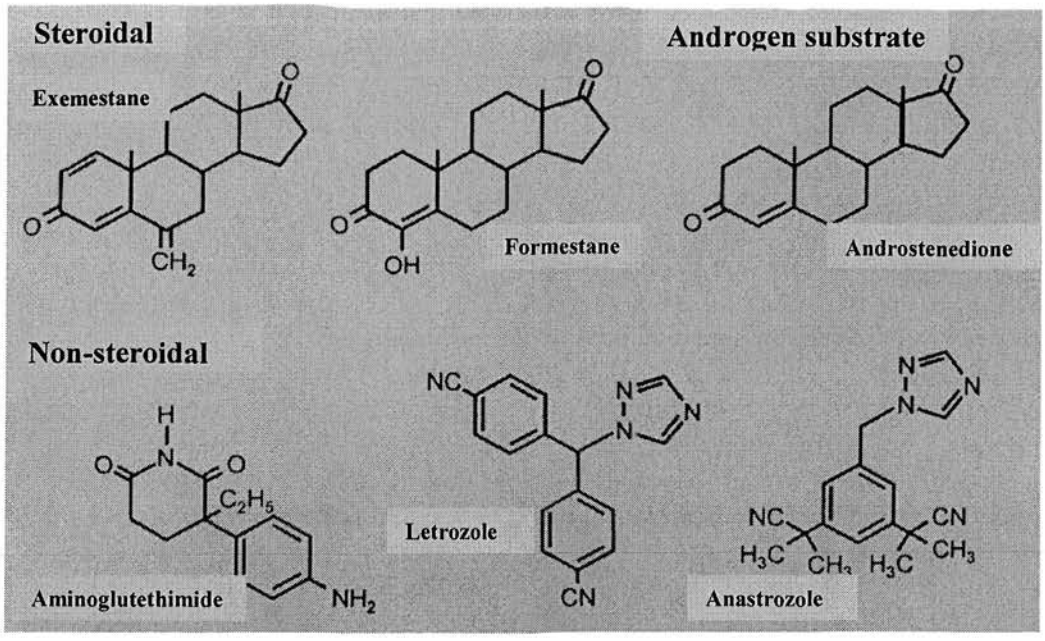


Figure 1.14 Structure and classes of aromatase inhibitor[19]

The antiepileptic drug and first generation AI aminoglutethimide was shown to inhibit the peripheral conversion of adrenal androgens to oestrogens by the enzyme aromatase, using radioisotope techniques[158]. To demonstrate this, tracer compounds including ^{3}H-labelled androstenedione and ^{14}C-labelled oestrone were administered by intravenous injection. Thereafter, urine was collected for four days with measurement of the isotope ratio in the oestrogen metabolites[159].

The first and second generation AIs inhibit up to 84–92% of aromatase in vivo. Moreover, the third-generation drugs achieve >97% aromatase inhibition[14,160,161,162] as shown in table 1.9.

Aminoglutethimide attained 81.4% of plasma oestrogen suppression compared with letrozole which suppressed levels to 98.0% as shown in table 1.7[159,163,164]. Letrozole has been shown to be the most potent suppressor of oestradiol, the most bioactive

oestrogen, compared with other AIs[163,165]. The clinical significance of these findings remains to be defined[165].

Each successive generation of AI has been developed with higher specificity for the aromatase enzyme, greater suppression of aromatase activity, and fewer adverse events[166].

Drug	Dose *(daily unless indicated)*	Aromatase inhibition %	Plasma oestrogen %
First generation			
Aminoglutethimide	250mg qid	90.6	81.4
Second generation			
Fadrozole	2mg bd	92.6	n/a
Formestane	250 mg x 2 weekly	84.8	51.8
Third generation			
Letrozole	2.5mg od	98.9	98.0
Anastrozole	1mg od	97.3	93.5
Exemestane	25mg od	97.9	93.2

Abbreviations: od- once daily, bd- twice daily, qid- four times daily

Table 1.9 Efficacy of aromatase and oestrogen suppression by three generations of AIs[164]

Preclinical pharmacology may not always be translated to the in vivo setting in humans and the effects of an individual drug depend on several factors including pharmacokinetics, tissue penetration and cellular uptake. AIs act on the aromatase enzyme CYP19 and genetic variability in the expression of the CYP19 gene may be related to the activity of the aromatase enzyme[167]. The effects of AIs at the tissue

level, in particular with respect to local effects in the normal breast as well as breast tumour tissue, is still incompletely understood[168].

Third-generation AIs can reduce levels of circulating oestrogens to undetectable values (with standard assays) in postmenopausal women, thereby removing very efficiently the growth stimulus for hormone-sensitive tumours[169].

Anastrozole

Anastrozole was the first 3rd-generation AI to enter into clinical trials and was launched in 1995. The dosing is 1mg daily and it is rapidly absorbed after oral administration with peak plasma concentrations reached after approximately two hours. Its estimated half life is approximately 40-50 hours[170]. Plasma oestrogen levels may need up to four weeks to recover following termination of treatment[171]. It is currently recommended as adjuvant early and advanced breast cancer treatments in postmenopausal women. It can also be used for two to three years following two to three years of tamoxifen.

Letrozole

Letrozole dosing is 2.5mg daily and is administered orally. It is rapidly absorbed and its estimated half life is approximately 48 hours[170]. Plasma oestrogen levels may need up to four weeks to recover following termination of treatment[171]. It is recommended as either upfront or extended adjuvant therapy for postmenopausal women have already received tamoxifen as first or second line treatment of adjuvant disease. It may also be used neoadjuvantly in an attempt to reduce the size of tumours enabling breast conserving surgery.

Exemestane

Exemestane dosing is 25mg daily and is also administered orally. It is rapidly absorbed, with maximum plasma concentrations reached within two hours of a single dose. It undergoes extensive metabolism and has a mean half life of approximately 24 hours[170,172]. Unlike anastrozole or letrozole, it induces aromatase degradation in a dose responsive manner without affecting mRNA levels[173]. Exemestane is used for adjuvant treatment of early breast cancer or after completion of two or three years of tamoxifen treatment or in postmenopausal women with advanced breast cancer whose disease has progressed following treatment with tamoxifen.

All three of these drugs may also be used to treat postmenopausal patients in whom other anti-oestrogen therapy has failed.

The two classes of AIs demonstrate a lack of complete cross-resistance[171,174]. Patients who fail to respond to one type of drug may still have a 25-30% chance of achieving clinical benefit from the other. This may be explained by the differing pharmacological characteristics between steroidal and non-steroidal compounds. In addition, the binding kinetics vary, with non-steroidal compounds binding reversibly, while the steroidal compounds bind irreversibly. Other possible causes include the differing endocrine efficacy which is seen in vivo between the non-steroidal compounds[175].

AIs are not co-administered with tamoxifen because tamoxifen in a low oestrogen environment may be seen as oestrogenic[176,177] and there is no greater efficacy when the agents are used together. Previous studies demonstrated a 27% reduction of plasma anastrozole levels when it was administered concomitantly with tamoxifen[178].

1.12.1 Pre-menopausal use of AIs

AI therapy is currently not recommended for premenopausal women with breast cancer because it is generally ineffective. It causes a reduced feedback of oestrogen to the hypothalalmus and pituitary which in turn results in an increase in gonadotropin secretion. This stimulates the ovary, leading to an increase in androgen substrate and aromatase[179]. Ovarian suppression may be achieved using GnRH agonists, LHRH (luteinsing hormone releasing hormone) agonists, oophorectomy or radiation. These modalities may be used to prevent the increased hormone levels caused by AIs in pre-menopausal women. Interestingly, results from the ABCSG-12 trial (appendix F) suggested no benefit to the use of an AI plus ovarian suppression when compared with tamoxifen plus ovarian suppression[180].

Other phase III clinical trials include SOFT (Suppression of Ovarian Function Trial) (appendix F) which aims to evaluate the benefits of tamoxifen plus ovarian suppression, tamoxifen alone and exemestane plus ovarian suppression in premenopausal women with hormone positive breast cancer after surgery. The IBCSG 25-02 (TEXT) trial (appendix F) aims to assess the role of tamoxifen and exemestane plus GnRH analogue as adjuvant therapy for premenopausal women with breast cancer. The results of these trials are awaited.

1.12.2 Neoadjuvant use of AIs

Neoadjuvant endocrine therapy is emerging as an alternative to neoadjuvant chemotherapy in postmenopausal women with large operable or locally advanced breast cancers. In this group, the likelihood of breast conservation with a cosmetically acceptable outcome is often limited and neoadjuvant endocrine treatment improves the chances of breast conservation by downstaging tumours. It also treats any micrometastases in a timely manner. Initially, small non-randomised studies suggested that third generation AIs might be more effective than tamoxifen as first-line endocrine agents in postmenopausal women with advanced and early breast cancer[181,182,183].

Letrozole

Larger, randomised trials include the P024 study which studied postmenopausal women with large, operable or locally advanced ER and/or PgR+ve untreated breast cancers. Patients were randomised to letrozole or tamoxifen. Objective response rates by palpation, mammography and ultrasound were all significantly higher in the letrozole group. Overall objective response rate was 55% for letrozole compared with 36% for tamoxifen $p<0.001$. There was also a higher rate of breast conserving surgery for patients randomised to letrozole (45% versus 35% $p=0.022$)[182]. A further multicentre trial demonstrated that letrozole improved operability and breast conservation surgery however there was considerable variability in responsiveness[184].

Anastrozole

Two large randomised trials compared anastrozole with tamoxifen prior to surgery: the IMPACT and PROACT studies (appendix F). The objective response rates in IMPACT showed no significant differences between the groups (anastrozole vs tamoxifen vs combination). Nevertheless, a significantly higher number of women experienced sufficient tumour regression to be suitable for breast conservation following treatment with anastrozole (46%) compared with tamoxifen (26%) p=0.03[185]. The PROACT study randomised a small subgroup of patient to concomitant chemotherapy. Only women treated with endocrine therapy alone who required mastectomy or had locally advanced disease at the onset received a significantly better objective response rate in the anastrozole group (49%) versus the tamoxifen group (36%) p=0.04[186].

Exemestane

Only one study randomised postmenopausal women with ER+ve breast cancer to receive neoadjuvant exemestane or tamoxifen alone. Results demonstrated that exemestane was superior in terms of clinical overall response (76% versus 40%) p=0.05) and also breast conservation surgery (36% versus 20%) p=0.05[187].

The ACOSOG Z1031 study (appendix F) demonstrated no difference in clinical response or breast conservation rate between women randomised to anastrozole, letrozole or exemestane. Each were effective at promoting breast conservation[188].

1.12.3 AIs as preventative therapy

AIs have shown encouraging results when used in breast cancer prevention. The ATAC trial (shown in table 1.8) demonstrated a further reduction of 40% in the frequency of de novo primary breast cancers in those taking anastrozole compared with tamoxifen[189]. Anastrozole and exemestane are currently under investigation as preventative agents in phase III randomised trials. The IBIS II study[190] was set up in 2003 and aims to compare anastrozole versus placebo in postmenopausal women at increased risk of developing breast cancer and who are not taking hormone replacement therapy. Additional data is expected from the NSABP B-35 trial which will test the preventative effects of anastrozole in women with DCIS[191]. The MAP.3 trial randomised women at increased risk of breast with exemestane plus placebo or exemestane plus celecoxib for five years. The results are not yet available[191].

It is expected that third-generation AIs will provide better protection against breast cancer compared with the two SERMS, tamoxifen and raloxifene which are currently approved. However until further results become available they are not yet routinely used as primary prevention. Newer agents including bisphosphonates and metformin have shown promise in observational studies[192].

1.12.4 AIs in the adjuvant setting

Several large, multicenter, randomised phase 3 trials have compared the efficacy and toxicity of AIs and tamoxifen. They include trials whereby postmenopausal women were randomly assigned a drug after a new diagnosis of breast cancer i.e upfront adjuvant therapy and an AI after previous tamoxifen therapy as shown in tables 1.10 – 1.11. These trials demonstrated that AIs are superior to tamoxifen in a number of settings:

(1) Used upfront as first-line adjuvant treatment (ATAC, BIG 1-98, TEAM, MA.27)

(2) After 2-3 years of tamoxifen (IES, ITA, ARNO)

(3) As extended adjuvant treatment after 5 years of tamoxifen (MA-17)

A recent meta-analysis of randomised trials of AIs compared with tamoxifen either as initial monotherapy or after 2-3 years of tamoxifen demonstrated that AIs produce significantly lower recurrence rates compared with tamoxifen, either as initial monotherapy or after 2-3 years of tamoxifen[193].

Upfront AIs	Phase 3 Multicenter Trials								
Trial	**Treatment arms**	**No. of patients**	**Median follow-up**	**Disease-free survival (DFS)**	**Overall survival (OS)**		**Musculoskeletal adverse events**	**Cardio-vascular events**	**Venous thromboembolic events**
ATAC [176,194]	1. anastrozole 5 yrs or tamoxifen 5 yrs 2. anastrozole + tamoxifen 5 yrs	9, 366	100 months	HR=0.85 [95% CI 0.76-0.94] p=0.003	No difference HR=1.00 [95% CI 0.89-1.12] p=0.99	**Adverse events**	***Arthralgia*** A=35.6% vs T=29.4% OR 1.32 [95% CI 1.19-1.47] p<0.0001 ***Fracture rate*** A=11.0% vsT=7.7% OR 1.49 [95% CI 1.25-1.77] p<0.0001	***Ischaemic CV events*** A=4.1% vs T=3.4% OR 1.23 [95% CI 0.95-1.60] p=0.1	***DVT*** A=1.6% vs T=2.4% OR 0.64 [95% CI 0.45-0.93] p=0.02 ***VTE*** A=2.8% vs T=4.5% OR 0.61 [95% CI 0.47-0.80] p=0.0004
BIG1-98 [195]	1. letrozole 5 yrs or tamoxifen 5 yrs 2. letrozole 2 yrs→ tamoxifen 3 yrs 3. tamoxifen 2 yrs→ letrozole 3 yrs	8, 010	25.8 months	HR=0.81 [95% CI 0.70-0.93] p=0.003	HR=0.86 [95% CI 0.70-1.06] p=0.16		***Arthralgia*** L=20.3% vsT=12.3% p<0.001 ***Fracture rate*** L=5.7% vs T=4.0% p<0.001	***Cardiac event*** L=4.1% vs T=3.8% p=0.61	***Thromboembolic event*** L=1.5% vs T=3.5% p<0.001
TEAM [196]	1. exemestane 5 yrs 2. tamoxifen → exemestane 5 yrs	9,779	61 months	HR=0.97 [95% CI 0.88-1.08] p=0.60	No difference HR=1.00 [95% CI 0.89-1.14] p>0.99		***Joint disorders*** E=36% vs T→E=31% p<0.0001 ***Fracture rates*** E=5% vs T→E=3% p<0.0001 ***Osteoporosis*** E=10% vs T→E=6% p<0.0001	***Hyperlipidaemia*** E=5% vs T→E=3% p<0.0001 ***Myocardial ischaemia /infarction*** E=2% vs T→E=1% p=0.171	***Venous thrombosis*** E=1% vs T→E=2% p<0.0001
MA.27 [197]	1. exemestane 5 yrs 2. anastrozole 5 yrs	7,576	61 months	No difference	No difference OS=91% for each arm		***Osteoporosis*** Less common with E	***Hypercholesterolaemia*** Less common with E ***Hypertriglyceridaemia*** Less common with E	Similar

ATAC = the Arimidex, Tamoxifen Alone or in Combination trial (the combination arm of anastrozole and tamoxifen was discontinued after 33 months as it was found to be equivalent to tamoxifen alone and inferior to anastrozole monotherapy), BIG 1-98 = the Breast International Group 1-98 Trial, TEAM = the Tamoxifen Exemestane Adjuvant trial, MA.27 = National Cancer Institute study comparing the non-steroidal and steroidal AIs (results not yet published)
A – anastrozole, CV – cardiovascular, DVT – deep venous thromboembolic events, E – exemestane, HR – hazard ratio, L – letrozole, OR – odds ratio, T – tamoxifen, vs – versus, VTE – venous thromboembolic events, yrs - years

Table 1.10 Clinical trials of upfront adjuvant therapy

(i) Sequential therapy with tamoxifen followed by an AI compared to 5 years of tamoxifen									
Trial	**Treatment arms**	**No. of patients**	**Median follow-up**	**Disease-free survival (DFS)**	**Overall survival (OS)**		**Musculoskeletal adverse events**	**Cardio-vascular events**	**Venous thromboembolic events**
ITA[198]	2-3 yrs of tamoxifen, then randomised to anastrozole for 2-3 yrs or to continue tamoxifen. Total duration 5 yrs	448	64 months	HR=0.57 [95% CI 0.38-0.85] p=0.005	HR=0.56 [95% CI 0.28-1.15] p=0.1		***Musculoskeletal disorders and bone fractures*** A=9.9% vs T=6.7% p=0.2	***Cardiovascular disease*** A=7.6% vs T=6.2% p=0.6	***Venous disorders*** A=2.2% vs T=4.4% p=0.2
ARNO95 / ABCSG8[199]	2yrs of tamoxifen, then randomised to anastrozole or tamoxifen for 3 yrs. Total duration 5 yrs	3, 224	28 months	HR = 0.60 [95% CI 0.44-0.81] p = 0.0009	OS higher (97%) in A group vs T group (96%) p=0.16		***Bone pain*** A=16% vs T=19% OR 1.25 [95% CI 1.00-1.56] p=0.0546 ***Fracture rate*** A=2% vs T=1% OR 2.14 [95% CI 1.14-4.17] p=0.015	***Myocardial infarction*** A<1% vs T<1% OR 1.50]95% CI 0.17-17.9] p=1.0	***Thromboses*** A<1% vs T<1% OR 0.25 [95% CI 0.04-0.92] p=0.034 ***Embolism*** A<1% vs T<1% OR 0.22 [95% CI 0.02-1.07] p=0.064
IES[200,201]	2-3 yrs of tamoxifen, then randomised to exemestane for 2-3 yrs or continue with 2-3 yrs of tamoxifen. Total duration 5 yrs	4, 724	30.6 months	HR=0.68 [95% CI 0.56-0.82] p<0.001	HR=0.88 [95% CI 0.67-1.16] p=0.37		***Arthralgia*** E=5.4% vs T=3.6% p=0.005 ***Osteoporosis*** E=7.4% vs T=5.7% p=0.023	***CVD other than myocardial infarct*** E=42.6% vs T=39.2% p=0.016	***Thromboembolic disease*** E=1.3% vs T=2.4% p=0.007
(ii) Extended therapy									
MA.17[202]	5 yrs of tamoxifen, then randomised to letrozole for 5 yrs or placebo for 5 yrs	5, 187	30 months	HR = 0.58 [95% CI 0.45-0.76] p<0.001	HR = 0.82 [95% CI 0.57-1.19] p=0.3		***Arthralgia*** L=25% vs placebo=21% P<0.001 ***Fracture rate*** L=5.3% vs placebo=4.6% p=0.25	***Myocardial infarction*** L=0.3% vs placebo=0.4% There were no significant differences between rates of cardiovascular disease in each group p=0.76	***Thromboembolic event*** L=0.4% vs placebo=0.2% p-value not given

Adverse events

ARNO 95 trial / ABCSG trial 8 = ARimidexNOIvadex / Austian Breast and Colorectal Cancer Study Group, IES = Intergroup exemestane study, ITA = Italian tamoxifen anastrozole study, MA.17 = National Cancer Institute study

A – anastrozole, CVD – cardiovascular d isease, E – exemestane, HR – hazard ratio, L – letrozole, OR – odds ratio, T – tamoxifen, vs – versus, yrs - years

Table 1.11 (i) Sequential Therapy: Effects of sequential therapy with tamoxifen followed by an AI
(ii)Extended therapy

Upfront adjuvant therapy in women with newly diagnosed postmenopausal breast cancer:

ATAC trial

The ATAC trial was the first large, double-blind, randomised controlled, multicenter trial of an adjuvant AI to be published. It compared anastrozole with tamoxifen in women diagnosed with early breast cancer that had completed primary treatment (surgery +/- radiotherapy +/- chemotherapy) and were suitable to receive adjuvant endocrine therapy. The main objectives were to establish whether anastrozole is at least as effective and as well tolerated as tamoxifen. It ran between July 1996 and March 2000. Patients were randomised to receive 5 years of anastrozole plus placebo, tamoxifen plus placebo or anastrozole and tamoxifen combined. Eighty three per cent of patients were ER+ve, 7% were ER-ve and 10% were unknown. After 68 months follow-up, anastrozole significantly prolonged disease-free survival, reduced distant metastases and reduced contralateral breast cancers. In addition, fewer withdrawals occurred with anastrozole[189]. Follow-up at 100 months showed no difference in overall survival[203].

BIG 1-98 Collaborative Group Study

The purpose of this double-blind study was to compare letrozole with tamoxifen in the adjuvant setting. The analysis compared two groups assigned to receive letrozole initially with the group assigned to receive tamoxifen initially. The study included ER+ve postmenopausal women with breast cancer and ran between March 1998 and May 2003. Letrozole reduced the risk of recurrent disease, especially at distant sites[195].

TEAM study

The TEAM study compared adjuvant tamoxifen and exemestane in early postmenopausal ER+ve breast cancer. Patients were assigned treatment between January 2001 and January 2006. There were two experimental arms, exemestane alone and sequential therapy (tamoxifen followed by exemestane). The primary endpoint was DFS and no difference was seen between the groups[196].

MA.27 study

The MA.27 study is the first ever head-to-head comparative trial of anastrozole versus exemestane. Exemestane was not superior to anastrozole but comparable in its anticancer effects and likely better in terms of its side-effect profile. The DFS rate was 91% in both arms. The results suggest that exemestane may be a better choice of AI at risk of osteoporosis and dyslipidaemias[197].

Sequential adjuvant therapy with AIs following tamoxifen in women with postmenopausal breast cancer

ITA trial

The Italian Tamoxifen/Arimidex trial assessed switching patients from tamoxifen to anastrozole. Women randomised included ER+ve postmenopausal women with breast cancer. Primary endpoints included DFS and secondary endpoints included safety and overall survival. The study ran between March 1998 and December 2002. Results confirmed a significant benefit in the switch group however overall survival was not significantly different. Results suggested switching to an AI is a valuable option for women being treated with tamoxifen[198].

ARNO 95 and ABCSG 8 trials

This was a combined analysis of two studies. Patients had to complete 2 years' of adjuvant tamoxifen prior to being randomised to anastrozole. The trial randomised patients between January 1996 and August 2003. Results demonstrated a 40% decrease in the risk of an event in the anastrozole group as compared with the tamoxifen group[199]. A meta-analysis of these studies combined with the ITA demonstrated significant improvements in DFS and overall survival in the anastrozole switch group compared with patients randomised to tamoxifen alone. These results suggested that postmenopausal women should be switched to anastrozole after completing 2-3 years of adjuvant tamoxifen[204].

IES study

This trial investigated the idea that switching to exemestane after 2-3 years of tamoxifen is more effective that continuing on tamoxifen for the duration of adjuvant therapy. The trial randomised patients between April 2000 and February 2006. DFS. The mature 55.7 month follow-up results confirmed an absolute benefit of 3.3% in favour of exemestane and also that switching to exemestane resulted in improved survival relative to women remaining on 5 years of tamoxifen[200,201,205].

Extended adjuvant therapy with tamoxifen or AIs

MA-17 study

Most breast cancer recurrences in women receiving five year of adjuvant tamoxifen occur after five years. This study assessed extending tamoxifen therapy beyond 5 years using an AI verus placebo. The study randomised patients between August 1998 and September 2002. Letrozole significantly improved DFS[202,206].

Adjuvant third generation AI treatment has been shown to be superior at decreasing breast cancer recurrence risk compared with adjuvant tamoxifen treatment. They have proven to be between 15-25% more effective than tamoxifen in terms of reducing the relative risk of breast cancer recurrence[65,176,207]. ASCO released guidelines in 2004 recommending that AIs be used as adjuvant therapy for postmenopausal women with ER+ve, invasive breast cancer[208]. The major clinical trials to date have indicated a significant benefit of adjuvant AI treatment over tamoxifen in terms of disease-free survival (DFS), distant DFS and contralateral breast cancers[65,176,207]. However they have failed, up to this point, to show any advantage in terms of overall survival[177]. ASCO recommend that postmenopausal women with receptor-positive breast cancer receive an AI in order to lower the risk of tumour recurrence. Optimal adjuvant hormonal therapy for postmenopausal women with hormone receptor +ve breast cancer should include AI therapy at some point during adjuvant treatment, either as up-front therapy or as sequential treatment after tamoxifen according to their 2010 guidelines. The optimal timing and duration of endocrine treatment remain unresolved. Careful consideration of adverse effect profiles and patient preferences in deciding whether and when to incorporate AI therapy is advised[209]. As previously stated, the side-effect profiles of tamoxifen and AIs differ. To date, the long term consequences of AI therapy are not well established but they have been shown to increase the risk of developing osteoporosis. There is currently no data on the use of tamoxifen after an aromatase inhibitor in the adjuvant setting.

1.12.5 AIs in the advanced breast cancer setting

Third generation AIs are now replacing tamoxifen as first-line agents for advanced breast cancer (ABC), as they have superior efficacy and in some cases, tolerability advantages. They were first assessed as second-line agents against megestrol acetate in the treatment of metastatic breast cancer and thereafter they were compared with tamoxifen in the first-line setting. Large scale trials demonstrated an advantage in the objective response rates, clinical benefit rate and overall survival for third generation AIs[210,211]. The median time to progression was similar in the AI groups when compared with megestrol acetate except for exemestane which showed a significant benefit (4.7 months for exemestane versus 3.8 for megestrol acetate)[212]. In a study comparing the effectiveness of letrozole with anastrozole in ABC, no significant differences were seen in overall response rate, time to disease progression, or median overall survival in the ER+ve group, suggesting that the two non-steroidal AIs are clinically equivalent[213]. The EFECT trial demonstrated that fulvestrant and exemestane were equally active and well-tolerated in postmenopausal women with ABC who had experienced progression or recurrence during treatment with a non-steroidal AI. These results reinforced the idea that there is incomplete resistance between the non-steroidal and steroidal AIs[174].

All metastatic patients receiving endocrine therapy eventually progress but often they will benefit from a new second-line endocrine agent that lacks the cross-resistance with first-line agents[214].

1.13 Long-term safety and tolerability of aromatase inhibitors

Postmenopausal women are not only at risk of dying from breast cancer, they are also susceptible to other major health problems which affect this age group including bone fractures and CVD[215]. Third generation AIs may alter these risks and may translate into different safety and tolerability profiles. This is of great important given the number of patients who will receive AIs in the future and due to the long duration of treatments. Adverse events associated with AIs include hot flushes, vaginal dryness, loss of libido, fatigue, arthralgias, joint stiffness and loss of bone mineral density (BMD) with subsequent increased risk of fractures[208].

1.13.1 Effects of AIs on bone

Concern over the long-term effects of adjuvant AI therapy on bone health is well established. Third generation AIs reduce oestrogen levels by >90% and this results in an increase in the rate of bone remodeling. This results in an overall acceleration of bone loss and probably to an increase in the rate of fractures.

Findings from a subgroup of 249 patients in the ATAC trial demonstrated that the biochemical markers of bone formation and resorption increased during treatment with anastrozole and this increase in bone remodeling was also reflected by a decrease in BMD measurements from lumbar spine and hip X-rays[216]. Factors associated with fractures in the ATAC trial included older age, the use of anastrozole, geographical location (higher incidence in the US and Scandinavia) and the absence of use of statins[216]. Other studies have found increased frature rates after letrozole[217] and exemestane[196,218] as shown in tables 1.10 and 1.11.

There may be a difference between changes in the rates of bone turnover caused by the different third generation AIs which may reflect differences in potential fracture risk.

1.13.2 Effects of AIs on quality of life related to bone health

Oestrogen deprivation is associated with arthralgias. The incidence of arthralgia has been reported to affect more women treated with AIs compared to those treated with tamoxifen[219]. It is not surprising that women treated with AIs often experience joint pain and musculoskeletal aches, given the extent to which they suppress oestrogen, and this may lead to cessation of treatment.

The term arthralgia encompasses a wide range of symptoms. Joint pain may emanate from a wide variety of locations including bone, ligaments, joint capsule, articular surfaces and periosteum. If inflammation is present there may be a surge of inflammatory markers including prostaglandins and bradykinins which activate peripheral nociceptor receptors, which in turn, increase the sensitivity to pain. This may result in normal day-to-day stimuli causing painful symptoms[220]. Oestrogen influences inflammation and neural processing of nociceptive input and do not have any specific known effects on articular surfaces[221]. In particular oestrogen has tissue-specific effects on inflammatory cytokines. The effects of oestrogen deprivation caused by aromatase inhibition may therefore explain the exaggerated nociception which occurs in many of these women[222].

Oestrogen deficiency has been shown to be associated with an increase in pain sensitivity. Oestrogen is known to affect nociceptive input at the levels of the central nervous system which may be related to symptoms such as arthralgia and breast pain described by patients taking AIs[221]. It has direct effects on opioid pain fibres in the central nervous system[223]. The specific effects of aromatase depletion on nociceptive fibres are less well established compared with the effects of oestrogen. However aromatase has been located in dorsal horn cells in some species[224] which may act to convert androgens to oestrogen. In addition, there is data suggesting that there is an

inverse link between the proportion of circulating oestrogen and the sensitivity to pain e.g. during pregnancy when oestrogen levels are elevated, women have exaggerated pain thresholds.

Clinical trials have consistently demonstrated a link between AIs and higher rates of musculoskeletal complains versus patients taking placebo or tamoxifen. Table 1.13 demonstrates the results of recent large scale trials comparing the musculoskeletal effects of AIs compared with placebo or tamoxifen.

Clinical Trial	*N*	Follow -up	AI	Type of musculo-skeletal toxicity	Rate of musculo skeletal toxicity	Rate of musculoskeletal toxicities with control
ATAC[176]	9,366	5 yrs	anastrozole	arthralgia	35.6%	tamoxifen 29.4%
MA.17[202]	5,187	5 yrs	letrozole	bone pain arthralgia myalgia	5% 25% 15%	placebo 6% placebo 21% placebo 12%
IES[205]	4,724	5 yrs	exemestane	musculoskeletal pain arthralgia joint stiffness	21.0% 18.6% 1.9%	tamoxifen 16.1% tamoxifen 11.8% tamoxifen 1.0%

Table 1.13 Clinical trials demonstrating the musculoskeletal effects of AIs

A diagnosis of breast cancer, together with treatment, is likely to affect the quality of life (QOL) of most patients which in turn may affect drug compliance.

QOL can be assessed using a number of methods. Firstly any adverse events (AEs) can be reported by the clinician or alternatively the patient can self report events using validated QOL instruments. There are several questionnaires which have been developed for use in oncology. The validated Functional Assessment of Cancer Therapy-Breast + Endocrine Subscale (FACT-B+ES) questionnaire is tailored for

patients with breast cancer and includes specific questions relevant to endocrine therapy including menopausal symptoms. The questionnaire includes 49 questions which are graded on a 5-point scale from 0 (not at all) to 4 (very much). It was validated by Fallowfield et al in 1999 in 268 women with breast cancer who received adjuvant endocrine therapy and has been shown to be reliable and demonstrated consistency and sensitivity[225].

Adjuvant hormonal treatment is currently recommended for a minimum duration of 5 years which can be extended for another 3-5 years in those treated with sequential therapy following 2-3 years of tamoxifen. It is vital that the QOL of that survival is maximised, given the long duration of treatment. In published clinical trials and in clinical practice, AEs constitute the main reason for non-adherence to endocrine treatments[226]. Some toxicities are resolved by simply switching to another drug whereas others may resolve with conservative treatment or with the addition of further agents. This may allow the patient to remain on therapy without compromising their QOL[226].

It is hypothesised that different AIs will result in different toxicities due to the varied mechanisms of action of each drug. It is understood that musculoskeletal symptoms may be related to bone loss and it is of great interest to determine if increases in bone turnover markers are associated with increased rates of musculoskeletal side-effects.

1.13.3 Effects of AIs on lipids

AIs do not improve lipid profiles as tamoxifen does, and there has been a suggestion that there is an increased risk of CVD with their use[227]. This is of critical importance as women diagnosed with postmenopausal breast cancer are likely, at the time of diagnosis, to have a significant risk of developing CVD which may then be added to by the direct and indirect effects of breast cancer treatments[228]. Studies comparing the incidence of cardiovascular events between women treated with AIs versus tamoxifen have reported a small increase in the incidence in the AI group as shown in tables 1.10 and 1.11. It is not clear whether this finding is simply due to the tamoxifen-associated cardioprotective effects which are lost or if AI therapy directly causes an adverse effect. Obesity is also an established risk factor for CVD and studies have associated the use of adjuvant endocrine therapy for breast cancer with weight gain[229,230,231]. In addition, whether any increased CVD risk is related to lipid dysfunction is unclear. The BIG-98 study showed elevated lipid levels in the 43.6% of the letrozole group versus 19.2% of those taking tamoxifen, p-value not available. Lipid disorders affected 8.1% of those taking anastrozole versus only 1.4% on tamoxifen in the ITA study. Letrozole is the only AI that has placebo-controlled cardiovascular data from a large trial, MA.17. It demonstrated no significant differences between the groups. Studies so far have shown differences between the classes of AIs and their effects on lipid metabolism and it has been suggested that these differences are a function of the steroidal nature of exemestane. Exemestane and its metabolites mimic androstenedione and may have a protective effect on lipids, especially triglycerides, by reducing serum hormone-binding protein and ApoA levels[227].

1.13.4 Effects of AIs on coagulation

For the individual patient with breast cancer, the absolute risk of venous thromboembolism (VTE) is dependent upon the interaction between a number of patient and cancer specific factors. These include age, obesity, immobility, medical co-morbidities, site and stage of cancer, chemotherapy regimens, and hospitalization[232]. In addition, the use of oestrogen-related compounds such as tamoxifen is known to have pro-thrombotic effects[233] and between 1-2% of women taking tamoxifen develop DVT as a result of tamoxifen's oestrogenic properties. Thrombosis is currently the second leading cause of death in patients with malignancy[232] and underlying malignancy is responsible for up to 25% of all cases of symptomatic venous thromboemboli[73].

In theory, AIs should have either no significant effect or reduce the risk of thrombembolism as they significantly reduce circulating oestrogen levels. Large scale clinical trials have shown that AIs cause less thromboembolic events in postmenopausal women compared with tamoxifen. Both anastrozole and exemestane are associated with significantly fewer venous and arterial vascular events when compared with tamoxifen[189,200].

SECTION 2: ALIQUOT STUDY

2.1 ALIQUOT INTRODUCTION

There is concern over the long-term effects of adjuvant AI therapy on bone and cardiovascular health. Studies have shown a higher fracture rate when AIs are compared with tamoxifen therapy and an increased incidence of cardiovascular events when compared with a healthy population.

The aim of this study was to compare whether there is a significant difference between the nonsteroidal AIs in their effects on bone turnover, quality of life and lipid parameters in a series of healthy postmenopausal women with ER+ve operable breast cancer.

2.2 ALIQUOT MATERIALS AND METHODS

2.2.1 Study design

ALIQUOT (Anastrozole versus Letrozole, an Investigation of Quality Of Life and Tolerability) was a prospective, open-label, randomised pharmacodynamic study involving 185 postmenopausal women with invasive ER+ve breast cancer. Patients were randomised as part of their adjuvant endocrine therapy to receive either 3 months of letrozole followed by 3 months of anastrozole or 3 months of anastrozole followed by 3 months of letrozole. Some patients received adjuvant AI therapy immediately following surgery, while other patients had already received adjuvant tamoxifen and then received AIs in the extended adjuvant setting.

All patients were treated in the Edinburgh Breast Unit, UK. Following informed consent, each patient was randomised to receive 3 months of anastrozole (1 mg) or letrozole (2.5 mg) orally once daily in this crossover study as shown in figure 2.1. Each drug was administered for 3 months, and after completion of the study period, patients in the immediate adjuvant group were either switched to tamoxifen or continued on anastrozole or letrozole. All those having extended adjuvant therapy continued on letrozole unless they expressed a specific preference for anastrozole, as letrozole is approved for extended therapy.

The cross-over design was chosen to remove interpatient variability. The drug carryover effect after 3 months is minimal because the half-life of both anastrozole and letrozole is between 40 to 50 hours only[234,235]. Within one month of stopping an AI, little drug remains. Thee months is certainly more than adequate to ensure that the results obtained for each drug represent values for that drug and that there is no

significant carryover effect[165]. Randomisation for ALIQUOT was 1:1, anastrozole: letrozole.

The primary endpoints were the effects of anastrozole and letrozole on bone turnover markers and lipid parameters. Secondary endpoints included the effects of these drugs on quality of life with particular reference to bone health.

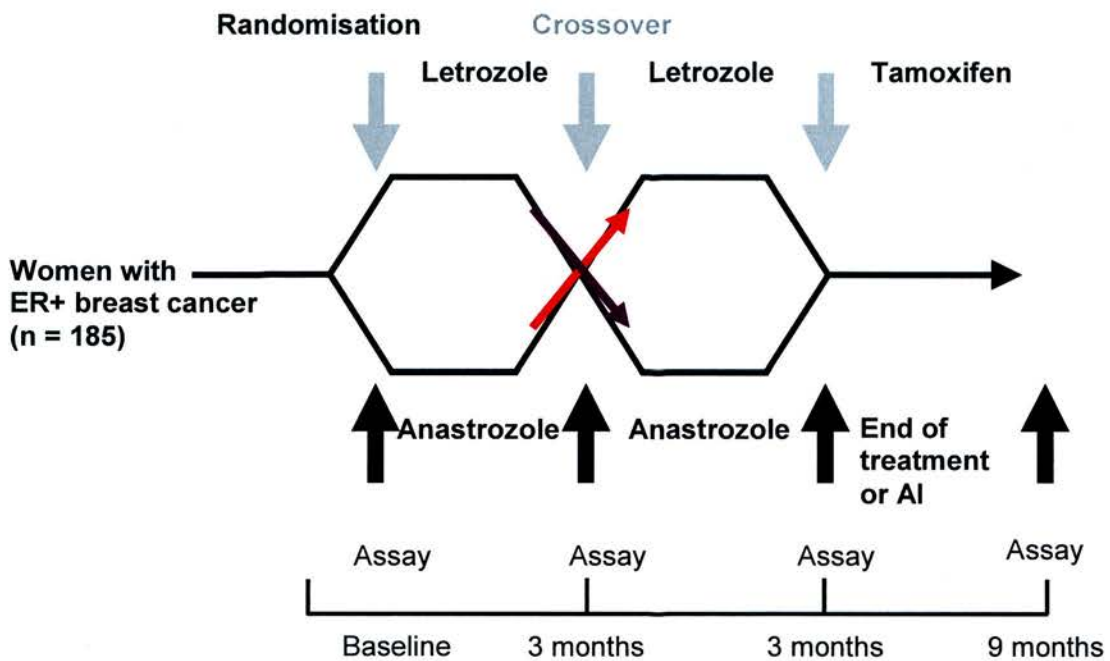


Figure 2.1 ALIQUOT Cross-over study design

2.2.2 Ethical Approval

The study was approved by the Lothian Research Ethics Committee and the Lothian NHS Trust Research and Development Department. It was carried out in accordance with the Declaration of Helsinki and in keeping with Good Clinical Practice.

2.2.3 Patient selection

Patients were recruited from the Edinburgh Breast Unit, Western General Hospital, Edinburgh. Postmenopausal women who had recently undergone surgery and been identified as having ER+ve breast cancer suitable for adjuvant treatment with tamoxifen alone were identified at the multi-disciplinary team meeting. Study participation was discussed at this meeting alongside all aspects of future management. Each patient had to satisfy the inclusion / exclusion criteria as shown in appendix A. Entry into the study was then discussed with potential patients at the time they received their pathology results and proposed management. Patient information sheets were given to patients as shown in appendix B. Patients were given plenty of time to ask any questions during and at the end of the clinic consultation. If they were interested in participating, a checklist to ensure suitability was performed. All patients were given a minimum of 24 hours to consider entering the trial prior to recruitment. Patients were asked to return to a specialised research clinic if they wished to participate in the study. At this time, informed consent was obtained using a standard consent form which was approved by the local ethics committee. Baseline QOL questionnaires and blood and urine samples were collected for analysis.

A smaller number of patients were identified at the long-term follow-up clinic when patients were approaching the completion of five years of adjuvant tamoxifen therapy and were adjudged to be appropriate for extended adjuvant therapy with an AI.

2.2.4 Oestrogen receptor testing

All patients eligible for the study were ER+ve. For trial entry, each patient had to score >4 on the ALLRED scoring chart however the majority were oestrogen rich and scored ≥7. A standard immunohistochemical scoring system was used to assess the quantity of oestrogen and progesterone receptor present.

2.2.5 Collection of clinical data

A clinical research folder (CRF) was completed for every patient. The following data were collected: date of entry into study; patient demographics; tumour characteristics including ER status; date and type of surgery; adjuvant treatments received; randomisation drugs and start date; previous hormone treatment; past medical history; current medications; height, weight and body mass index.

In addition the person who initially discussed the trial was recorded. Copies of the consent forms and pathology reports were filed in each CRF. QOL questionnaires and adverse events were recorded at each visit. All data were collected prospectively.

2.2.6 Collection and storage of blood and urine specimens

Blood and urine samples for bone marker measurements and blood samples for lipid measurements were collected following an overnight fast, at the same time of day and on the same day of the week at the beginning and end of each 3 month period of drug treatment. Further samples were obtained after 9 months. The timing of samples was scheduled to minimise diurnal and diet-related effects. Plasma samples were separated by centrifugation and stored at -80°C until analysed. The second voided urine of the day was collected for measurement of urinary bone markers and stored at -80°C.

Patient details were encoded to ensure anonymity. A detailed chronological log book of all samples collected was recorded, ensuring easy and accurate identification of specimens.

2.2.7 Bone turnover marker analysis

Several bone turnover markers were measured as shown in table 2.1. Procollagen type 1 N-terminal propeptide (PINP) and bone specific alkaline phosphatase (ALP) are markers of bone formation. The cross linked C and N telopeptides of type 1 collagen, serum C-terminal telopeptides (sCTX) and urinary N-terminal telopeptides (uNTX) are markers of bone resorption. Increases in uNTX and sCTX indicate bone resorption, while increases in PINP and ALP indicate bone formation. Parathyroid hormone (PTH) is a calcium regulating hormone and is also referred to as a regulator of bone remodelling[46]. It was previously described as having bone resorbing properties but has since been shown to have anabolic effects too. The normal reference ranges for postmenopausal women are shown for the bone turnover markers in table 2.1. No available postmenopausal ranges are available for PTH and therefore only the normal range has been given in table 2.1.

Blood and urine samples were analysed at the Academic Unit of Bone Metabolism, Metabolic Bone Centre, Sorby Wing, Northern General Hospital, Sheffield. Urinary NTX was measured by an automated Vitros Eci chemiluminescence immunoassay (Ortho Clinical Diagnostics) and was expressed as a ratio to urinary creatinine which was measured by a dry slide method (Citros 250, Ortho Clinical Diagnostics). Serum CTX was measured by an enzyme-linked immunoassay (Crosslaps®, Nordic Bioscience Diagnostics). Intact PINP was measured by radioimmunoassay (Orion Diagnostics Oy). Bone ALP was measured by the Ostase® assay, a paramagnetic chemiluminescent method on an Access® autoanalyser (Beckman Coulter Inc.). Serum PTH was measured by enzyme linked immunoassay (Biomericalnc).

Bone markers associated with bone formation	normal reference ranges / unit
PINP[236]	16 - 96 ng/ml
Bone ALP[237]	3.8 - 22.6 µg/l
Bonemarkers associated with bone resorption	
sCTX[238]	0.104 - 1.008 ng/ml
uNTX[239]	26 - 124 nmolBCE/mmolCr
Regulator of bone remodelling	
PTH[238]	8.3 - 63.0 pg/ml

s - serum u - urinary BCE – bone collagen equivalents Cr - creatinine
Reference ranges shown are for postmenopausal women with the exception of PTH which is the adult reference range.

Table 2.1 Markers of bone turnover and bone metabolism hormone

The PINP interassay CV was 5.7% and the intra-assay CV was 5.2%. The bone ALP interassay CV was 2.3% and the intra-assay CV was not calculated as bone ALP was run on an autoanalyser. Intra-assay sCTX CV was 3.2% and interassay CV was 3.0%. Interassay uNTX coefficient of variations (CV) was 6.2 % and the intra-assay CV was not calculated as NTX was run on an autoanalyser. The serum PTH interassay CV was 7.2% and the intra-assay CV was 3.7%.

2.2.8 Quality of life assessment

FACT-B+ES (version 4) questionnaires were completed by patients prior to hormonal treatment and at regular intervals both during and after treatment i.e baseline, 3, 4 and 12 months, as shown in appendix C. Any adverse events or side effects offered by patients on direct questioning were also recorded. It is well known that there is a weak correlation between the aforementioned methods and it is for this reason that we chose to record both. Due to the different mechanisms of actions of each AI class, it is expected that there would be associated different toxicities. AIs are known to result in a significantly higher incidence of musculoskeletal symptoms (including arthralgia, bone loss and fractures) compared with tamoxifen[177]. This study therefore set out to identify if there was any differences in the degree of musculoskeletal side-effects between the AIs. In addition a correlation between bone turnover markers and bone/joint side-effects was sought.

2.2.9 Lipid analysis

The lipid transport system has evolved to transport fat from sites of origin to sites of utilisation via plasma. The major types of lipids that circulate in plasma were measured including cholesterols, triglycerides and lipoproteins. The proteins that mediate this circulation are apolipoproteins which were also measured. The lipids and lipoproteins measured are shown in table 2.2 alongside their normal reference ranges. LDL increases and HDL decreases after the menopause but these changes have not been taken into account when formulating the normal reference ranges.

Atherogenic lipids (associated with an increased risk of atherosclerosis	**Normal reference ranges / unit**
Triglycerides[30]	< 1.7 mmol/l
Cholesterol[30]	< 5.0 mmol/l
Low-density lipoprotein cholesterol (cLDL)[30]	< 3.0mmol/l
Apolipoprotein B (ApoB)[218]	0.63 - 1.14 g/l
Atheroprotective lipids (associated with cardioprotective Properties)	
High-density lipoprotein cholesterol (cHDL)[30]	> 1.0 mmol/l
Apoprotein A1 (ApoA1)[240]	1.20 - 1.76 g/l

Table 2.2 Plasma lipids

Cholesterol acts as the substrate for steroid hormones. Triglycerides are hydrolysed by lipases to generate free fatty acids used for energy utilisation. Lipoproteins transport triglycerides from intestine and liver to sites of utilisation, they also transport cholesterol to peripheral tissues.

Triglycerides, total cholesterol, low-density lipoprotein (LDL), apolipoprotein B (ApoB), high-density lipoprotein (HDL), and apoplipoprotein A1 (ApoA1) were measured at baseline and after 3 and 6 months of AI. At this point patients who had not received any other form of hormone therapy were switched to tamoxifen and further lipid measurements obtained at 9 months. High levels of circulating

triglycerides, cholesterol and LDL are associated with an increased risk of CVD whereas high levels of HDL and apoA-1 lipoproteins are protective against CVD (due to their ability to facilitate transportation of lipids from the intima).

Plasma lipids were collected in EDTA tubes. Patient number 1 – 60 specimens were immediately centrifuged after collection. They were then transported on ice to the Centre for Cardiovascular Science, Queen's Medical Research Institute, Royal Infirmary of Edinburgh where they were stored at -80°C until analysis. Patient number 61–182 specimens were immediately centrifuged and stored at the Breast Research Facility, Western General Hospital, Edinburgh. They were subsequently transported to the Centre for Cardiovascular Science at completion of the study where they were analysed alongside the samples from patients 1-60. Lipid parameters were analysed at the Centre for Cardiovascular Science, Queen's Medical Research Institute, Edinburgh.

Total cholesterol, triglyceride and HDL cholesterol were measured on an Olympus AU2700 automated analyser using the manufacturer recommended reagents (Beckman Coulter Ltd, High Wycombe, UK). The percentage coefficient of variation for all three analyses was <5% across the working range.

Calculated LDL cholesterol was determined using the Friedewald Equation:

LDL cholesterol = total cholesterol – HDL cholesterol – (0.45 × triglycerides)
All units mmol/l[241]

ApoA1 and ApoB concentrations were measured using immunoturbidimetric immunoassay kits (A.Menarini Diagnostics, Winnersh-Wokingham, UK) adapted for

use on a Cobas Fara Centrifugal Analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). These methods are based on the reaction of a sample containing either ApoA1 or ApoB and the appropriate specific antiserum to form an insoluble complex which can be measured turbidimetrically at 340nm. The between batch percent coefficient of variations for the ApoA1 assay for low medium and high quality control material was 4.02, 3.66 and 4.64 respectively. While the between batch percent coefficient of variations for the ApoB assay for low, medium and high quality control material was 4.00, 3.68 and 3.53 respectively. Analyses were carried out under blinded conditions.

Atherogenic ratios can be used to predict cardiovascular risk, often in combination with other variables including age, sex, blood pressure and smoking status. The ratios of total cholesterol:HDL, LDL:HDL and ApoB:ApoA-1 were calculated. These are associated with an increased risk of cardiovascular disease when elevated.

2.2.10 Adverse event monitoring

The incidence, type and grade of adverse events (AEs) and serious AEs were recorded during treatment in accordance with the National Cancer Institute's common terminology criteria for adverse events (version 3.0). Side effect profiles and other adverse events after initiation of anastrozole and letrozole have been published elsewhere[242].

2.3 ALIQUOT RESULTS

2.3.1 Bone turnover markers

2.3.1.1 Patients

Ninety-four postmenopausal women with ER+ve breast cancer were suitable for adjuvant or extended adjuvant treatment with an AI and were eligible to provide blood and urine samples for analysis. Very few patients enrolled were on drugs likely to have an effect on bone metabolism. Patients were either due to start endocrine therapy as their first treatment after surgery and were therefore tamoxifen-naïve (n=52) or were finishing 5 years of tamoxifen and beginning extended adjuvant therapy (n=42).

All patients included had histologically confirmed invasive cancer that was ER+ve (ALLRED score $\geq$4). Postmenopausal status was defined as amenorrhea for 1 year and/or luteinising hormone with follicular stimulating hormone levels in the postmenopausal range. Postmenopausal women who had early invasive breast cancer (T1-3, N0-1, M0) and who were able to give informed consent were considered eligible. Of note, the only post chemotherapy patients were those who had completed 5 years of tamoxifen. All premenopausal women; women receiving concurrent or previous chemotherapy (within the last four years); women taking concomitant hormonal therapy, including hormone replacement therapy; women taking drugs likely to affect bone metabolism, including steroids and bisphosphonates; and patients unable to give informed consent were excluded from entry into the study.

Each patient was randomised (1:1) to receive 6 months of AI therapy, which included 3 months of anastrozole followed by 3 months of letrozole or 3 months of

letrozole followed by 3 months of anastrozole. Patients with no prior exposure to tamoxifen were thereafter commenced on 20mg tamoxifen daily for four and a half years.

Eighty four patients had complete sample measurements and were included in the analysis. Patient disposition is shown in the CONSORT diagram in appendix G. Forty-six patients were randomised to letrozole followed by anastrozole, and 48 were randomised to anastrozole followed by letrozole. Ten patients were excluded from analysis due to technical problems with samples. One patient was later removed from the analysis, as she was found to be taking medication likely to affect bone turnover.

The median age of patients was 63 years (range, 40 to 87). Table 2.3 shows additional demographics and it can be seen that both groups were similar except for a difference between the median ages and for the number of patients who had undergone hysterectomy. Patients who received five years of prior tamoxifen were slightly younger and there were a larger number of women who had undergone hysterectomy in this group. The type of statistical analysis performed on the data is included in table 2.3. Baseline variables were accounted for.

	Post 5 years of tamoxifen (n=42)	**No prior tamoxifen (n=52)**	**p-value**	**Statistical test perfomed**
Median age at entry, years (range)	61 (40-78)	65 (44-87)	**0.015**	Mann-Whitney
Mean baseline height, cm (SE)	161.7 (1.21)	161.9 (0.88)	0.85	ANOVA
Mean baseline weight, kg (SE)	72.0 (2.13)	74.9 (2.24)	0.36	ANOVA
Prior HRT (n)	17 (40.5%)	20 (38.5%)	0.84	χ^2 – test
Hysterectomy (n)	11 (26.2%)	5 (9.6%)	**0.03**	χ^2 – test
Bilateral oophorectomy (n)	3 (7.1%)	0 (0%)	0.09	Fisher's Exact Test

Table 2.3 Bone marker patient demographics

2.3.1.2 Statistical analysis

The statistical analysis was conducted by an independent statistician. Hormone therapy for each patient was coded to maintain the blind assessment and avoid bias.

A repeated measures analysis of variance was conducted, with baseline variables accounted for, since measurements were of the same patient over a series of time points. Whether the patients had taken tamoxifen for 5 years was also included in the model. Bone ALP and PTH were log transformed to achieve Normality prior to analysis.

2.3.1.3 Effect of prior tamoxifen treatment

Baseline results

There were significant differences at baseline between the tamoxifen-naïve group and patients who had received prior tamoxifen for PINP levels (47.94 versus 37.3ng/l, p=0.005) and sCTX levels (0.67 versus 0.49ng/l, p=0.0003) as shown in table 2.4. Lower baseline levels of bone turnover markers were seen in all parameters for those patients who received prior tamoxifen although only the aforementioned were statistically significant.

Changes over time

Results are presented as mean percentage change from baseline for each group as shown in figures 2.2 – 2.6. Patients who received prior tamoxifen had a greater increase in levels of PINP (figure 2.2), ALP (figure 2.3), sCTX (figure 2.4) and uNTX (figure 2.5) after 3 and 6 months of an AI compared with the tamoxifen naïve patients. PTH (figure 2.6) is an indirect measure of overall bone turnover, and greater reductions between the two groups reflect the increased bone turnover in patients previously treated with tamoxifen, compared with those with no prior tamoxifen exposure.

	Mean baseline absolute values (95% CI)		p - value	Mean baseline absolute values (95% CI)		p - value
	Study drug			Tamoxifen for 5 years		
	anastrozole	letrozole		No (n = 41)	Yes (n = 42)	
PINP (ng/l)	42.10 (36.55, 47.65)	42.61 (37.26, 47.96)	0.89	**47.94** (42.86, 53.02)	**37.30** (32.09, 42.50)	**0.005**
ALP[log transformed] (µg/l)	2.45 (2.35, 2.56)	2.57 (2.47, 2.68)	0.11	2.54 (2.43, 2.64)	2.50 (2.39, 2.61)	0.62
sCTX (ng/ml)	0.57 (0.49, 0.65)	0.56 (0.48, 0.64)	0.81	**0.67** (0.61, 0.74)	**0.49** (0.42, 0.56)	**0.0003**
uNTX (nmolBCE/mmolCr)	46.05 (39.84, 52.26)	43.30 (37.46, 49.14)	0.52	48.77 (42.84, 54.69)	40.51 (34.66, 46.36)	0.052
PTH[log transformed] (pg/ml)	4.10 (3.96, 4.24)	4.03 (3.89, 4.16)	0.42	4.07 (3.94, 4.20)	4.06 (3.92, 4.19)	0.91

Table 2.4 Bone marker results - baseline absolute values

The bold results show where there is a significant change from baseline.

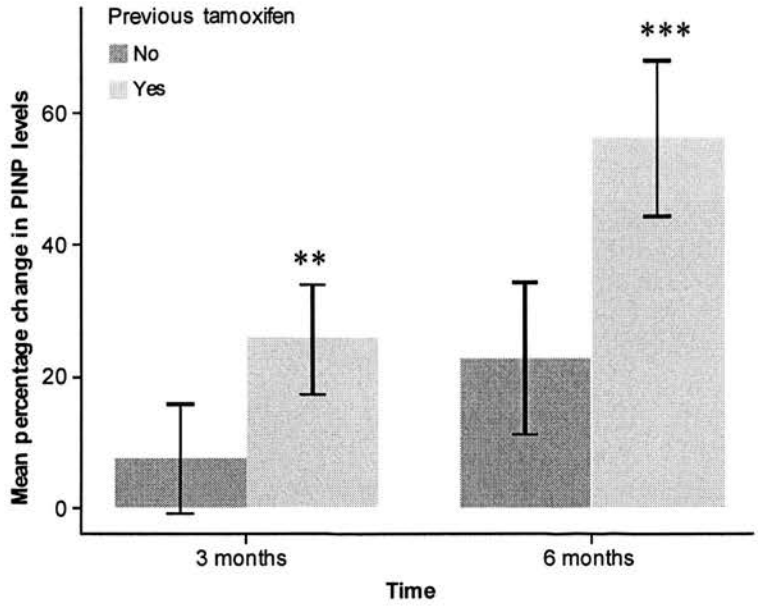


Figure 2.2 Mean percentage change in PINP from baseline
Difference between no previous tamoxifen and previous tamoxifen at 3 months p=0.0044**
Difference between no previous tamoxifen and previous tamoxifen at 6 months p=0.0003***

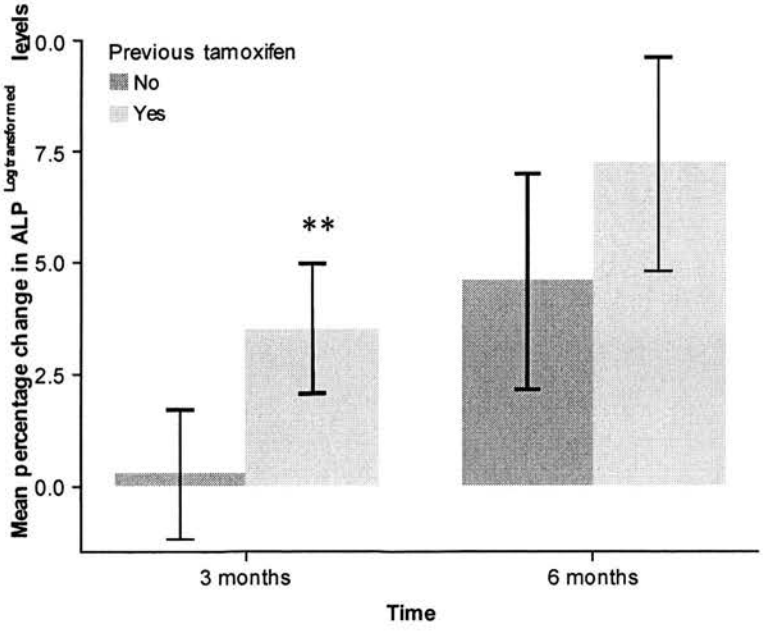


Figure 2.3 Mean percentage change in ALP from baseline
Difference between no previous tamoxifen and previous tamoxifen at 3 months p=0.004**
Difference between no previous tamoxifen and previous tamoxifen at 6 months p=0.16

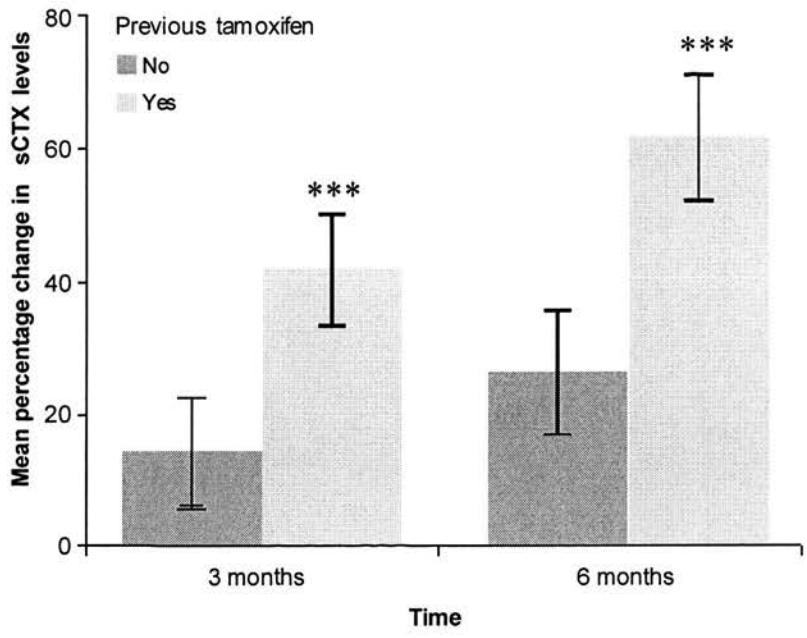


Figure 2.4 Mean percentage change in sCTX from baseline
Difference between no previous tamoxifen and previous tamoxifen at 3 months p<0.0001***
Difference between no previous tamoxifen and previous tamoxifen at 6 months p<0.0001***

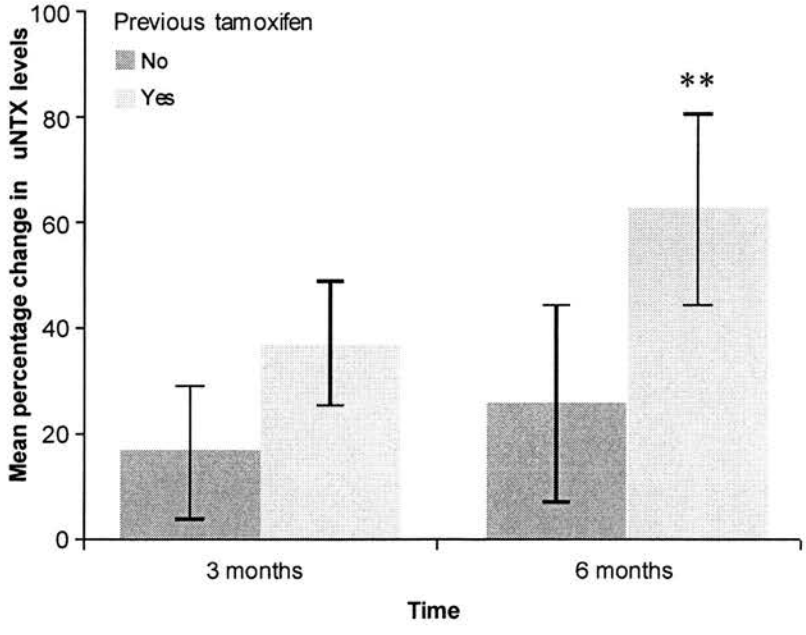


Figure 2.5 Mean percentage change in uNTX from baseline
Difference between no previous tamoxifen and previous tamoxifen at 3 months p=0.03
Difference between no previous tamoxifen and previous tamoxifen at 6 months p=0.006**

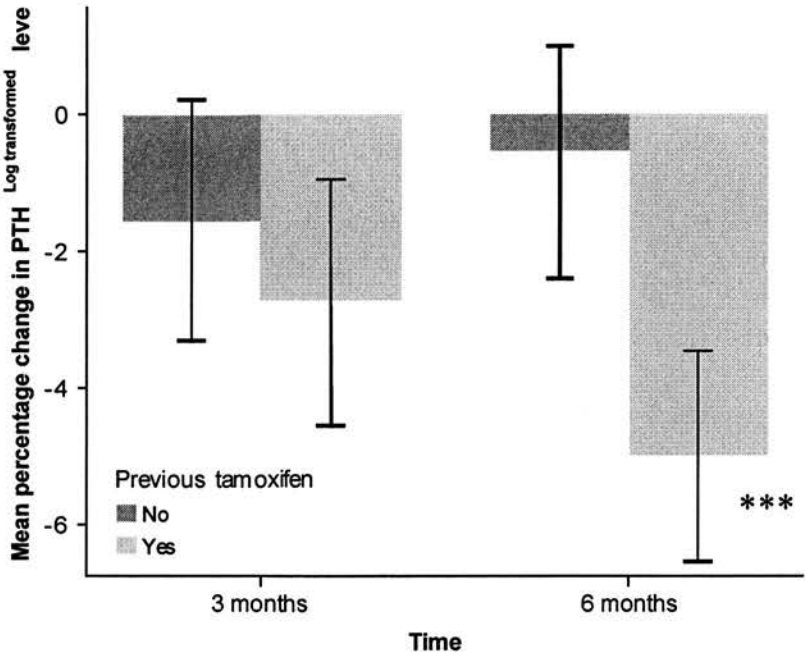


Figure 2.6 Mean percentage change in PTH from baseline
Difference between no previous tamoxifen and previous tamoxifen at 3 months p=0.45
Difference between no previous tamoxifen and previous tamoxifen at 6 months p=<0.0001***

2.3.1.4 Differences between anastrozole and letrozole

There were no significant differences at baseline between those patients who initially received anastrozole compared to those who received letrozole as seen in figure 2.4. Both AIs had major effects on all bone markers, although there were no significant differences between the drugs at the 3- or 6-month time points for any of the parameters measured (all $p>0.1$). This was independent of the drug sequencing.

Absolute changes from baseline as shown in table 2.4 and mean percentage changes from baseline are shown in figures 2.7 - 2.11. Both anastrozole and letrozole markedly increased bone turnover markers.

Changes over time

There were significant increases in both bone formation and bone resorption between baseline and 3 months and 3 and 6 months for PINP, bone ALP and sCTX (all $p<0.0001$), and uNTX ($p=0.04$). PTH showed no change. The group who had previously received tamoxifen had significantly greater increases (all $p <0.0006$) in markers of bone resorption together with significantly larger rises in markers of bone formation at all time points compared with the tamoxifen-naive group. The differences for PTH were also significant, with smaller rises in the prior tamoxifen group ($p=0.0004$).

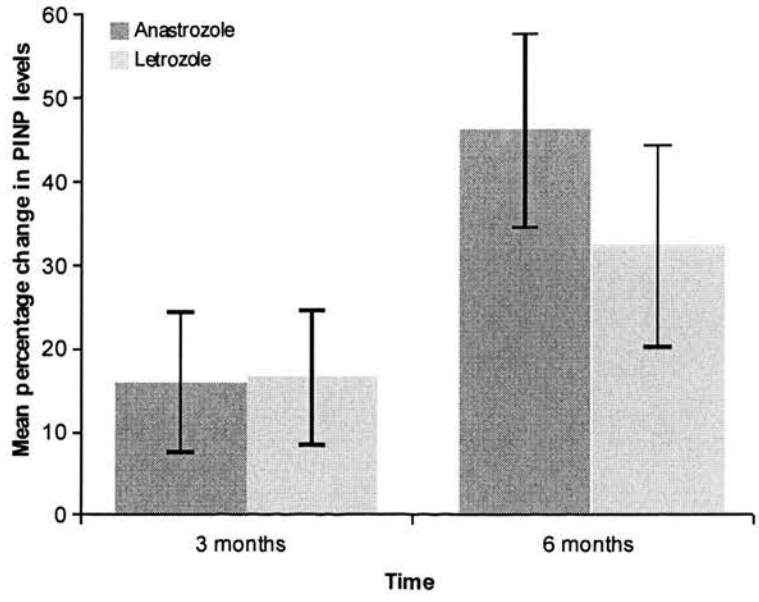


Figure 2.7 Mean percentage change in PINP over time
Difference between anastrozole and letrozole at 3 months p=0.97
Difference between anastrozole and letrozole at 6 months p=0.14

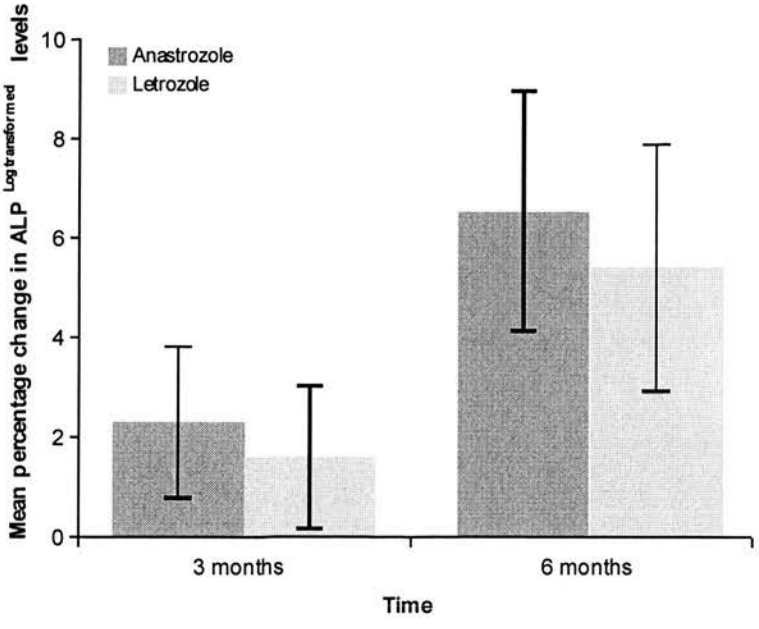


Figure 2.8 Mean percentage change in ALP over time
Difference between anastrozole and letrozole at 3 months p=0.53
Difference between anastrozole and letrozole at 6 months p=0.51

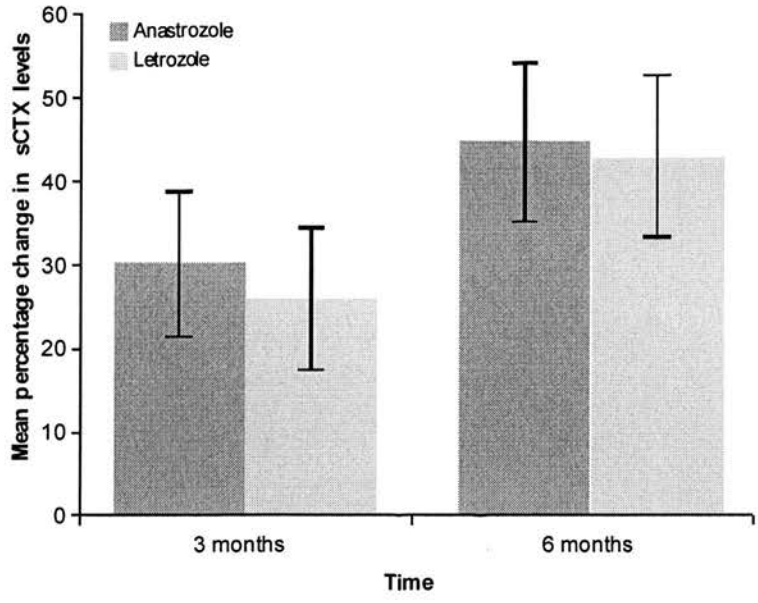


Figure 2.9 Mean percentage change in sCTX over time
Difference between anastrozole and letrozole at 3 months p=0.48
Difference between anastrozole and letrozole at 6 months p=0.90

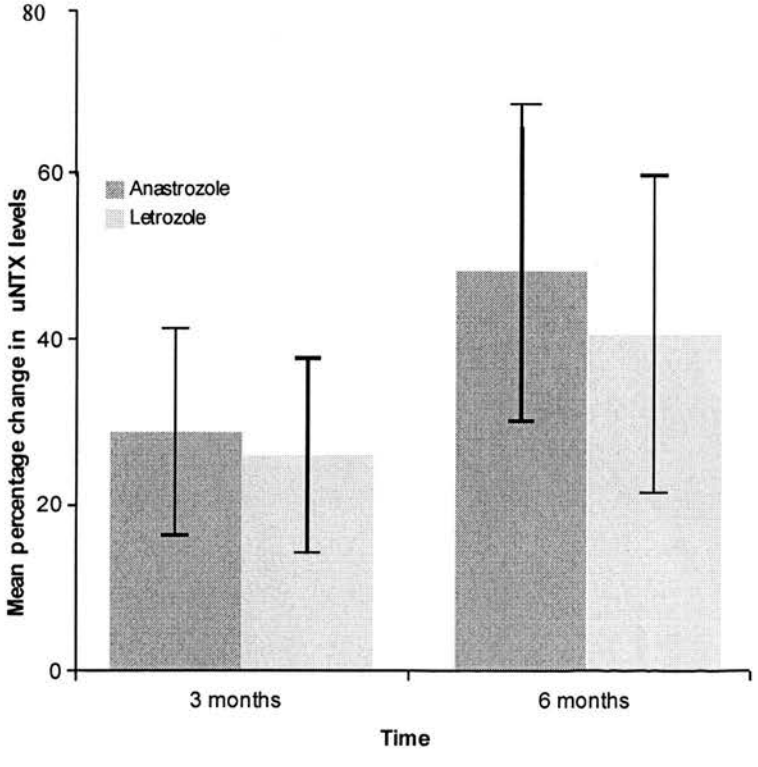


Figure 2.10 Mean percentage change in uNTX over time
Difference between anastrozole and letrozole at 3 months p=0.75
Difference between anastrozole and letrozole at 6 months p=0.57

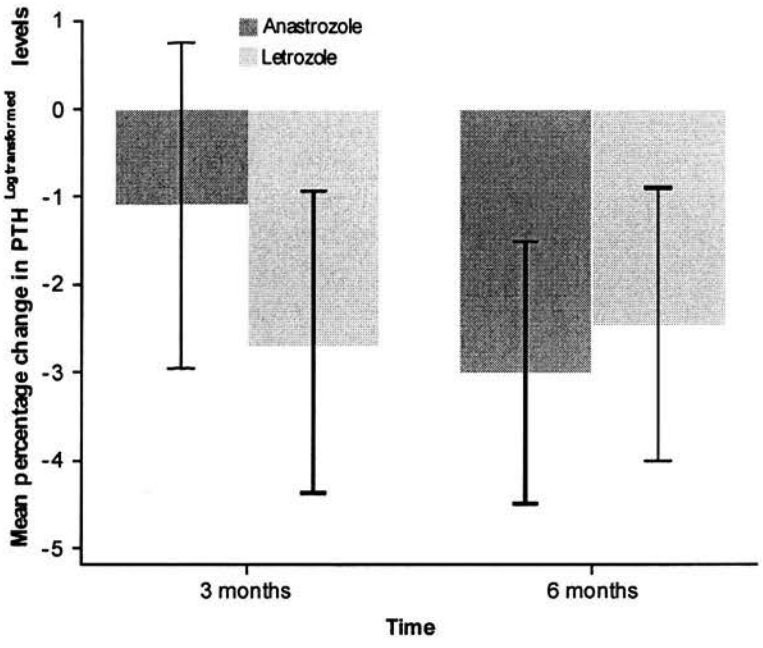


Figure 2.11 Mean percentage change in PTH over time
Difference between anastrozole and letrozole at 3 months p=0.25
Difference between anastrozole and letrozole at 6 months p=0.74

2.3.1.5 Effects of AIs followed by tamoxifen

Tamoxifen had major effects on the markers of bone resorption when administered after AIs, sCTX (p=0.0004) and uNTX (p=0.0009), as shown in table 2.5. In both cases, no difference was seen between the anastrozole and letrozole group. Only a limited number of patients had data for this analysis, so some degree of care must be taken in the interpretation of the results. There was no influence on mean percentage change following tamoxifen by the sequence of the previous AIs (all p>0.5).

	Drug (mean percentage change, 95% CI)			
	Anastrozole	**Letrozole**	**Tamoxifen (following 6 months of AI)**	***p* value**
PINP ng/l (*n* = 31)	11.12 (-9.20 to 31.43)	8.98 (-11.94 to 29.90)	-4.18 (-20.30 to 11.93)	0.51
$ALP^{transformed}$ µg/l (*n* = 31)	0.79 (-0.88 to 2.45)	0.71 (-1.08 to 2.50)	-0.03 (-2.93 to 2.87)	0.82
sCTX ng/ml (*n* = 38)	-6.39 (-13.00 to 0.22)	-7.56 (-14.05 to -1.08)	-20.00 (-26.03 to -13.97)	**0.0004**
uNTX nmolBCE/ mmolCr (*n* = 30)	9.96 (-1.31 to 21.23)	7.61 (-3.44 to 18.66)	-10.20 (-21.69 to 0.87)	**0.0009**

Table 2.5 Comparison of anastrozole, letrozole and tamoxifen following aromatase inhibitor

The bold results show where there is a significant change from baseline.

2.3.1.6 Bone turnover discussion and conclusion

Anastrozole and letrozole are potent third-generation AIs that cause profound suppression of plasma oestrogen levels in postmenopausal women[173,243]. There is evidence that letrozole is a more potent inhibitor of aromatase, and that at clinically used doses, letrozole reduces oestrogen levels to a greater degree than anastrozole[165,244]. These results demonstrate that six months of treatment with letrozole and anastrozole induces a significant increase in bone turnover, and that this is further augmented in patients who have already received 5 years of adjuvant tamoxifen therapy. Despite the greater ability of letrozole (2.5 mg) to lower circulating oestrogen levels compared with anastrozole (1 mg)[165], the effects on bone metabolism are similar at clinically used doses. These effects increase with time, and greater bone turnover is evident at 6 months compared with 3 months, although there is no difference between the drugs. There is therefore unlikely to be any difference between these drugs in fracture rate or the rate of osteoporosis. In postmenopausal women, AI therapy has been associated with increases in bone turnover and bone loss at an average rate of 1% to 3% per year[245]. Consequently, an increase in fracture incidence is also observed when compared with that seen during tamoxifen use[245]. In the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) trial, the fracture rate for anastrozole was 11% at a median follow-up of 68 months, versus 7.7% for tamoxifen[176]. This increased fracture rate with anastrozole did not continue after treatment had stopped, suggesting that this is a short-term effect that could be managed with DEXA scans and bisphosphonates when needed[177]. Similarly, in the subset analysis restricted to the monotherapy arms of the Breast International Group (BIG) 1-98 trial, the fracture rate for letrozole was 8.6% at a median follow-up of 51

months, compared with 5.8% for tamoxifen[246]. The fracture rates increased in both groups with the length of follow-up as shown in table 1.8.

Oestradiol levels have been reported to be virtually undetectable in patients taking AIs, and this partly explains the high rate of bone loss and greater risk of fractures. Deterioration in bone health is a major concern for patients taking AIs long-term. In our study, there was no difference between letrozole and anastrozole with regard to their effects on bone metabolism. Our findings are similar to those in the Letrozole, Exemestane and Anastrozole in healthy Postmenopausal women (LEAP) study, which also reports that the third-generation AIs, whether steroidal or nonsteroidal, are similar with respect to their effects on bone[247].

Postmenopausal women with hormone-sensitive breast cancer receiving adjuvant AI therapy are at risk of AI-associated bone loss, but it is important to note that this bone loss is manageable, and AI therapy should not be withheld for fear of bone loss. The American Society of Clinical Oncology （ASCO）recommends BMD assessments in all women beginning adjuvant AI therapy. While most women will have normal BMD, routine screening may identify women who are at increased risk for bone loss and are candidates for bisphosphonate therapy[248]. Bisphosphonates are synthetic analogues of pyro-phosphate that adsorb on to bone surfaces and become incorporated within the bone matrix. The drug is released when osteoclasts resorb bone containing this compound. It inhibits signalling pathways that are necessary for osteoclast function and therapy therefore results in a fall in bone resorption[35]. Randomised clinical trials such as the Zometa-Femara Adjuvant Synergy Trial (Z-FAST in the United States and ZO-FAST in Europe) support the use of zoledronic acid (4 mg), a bisphosphonate, every 6 months to prevent AI-associated bone loss[249,250]. In addition to calcium and vitamin D supplementation, oral bisphosphonates are recommended to patients who start AI therapy with a T-score

<−2.0. Bisphosphonates can also be recommended for any patient who is receiving AI therapy and has any two of the following risk factors: T-score <−1.5, age >65 years, family history of hip fracture, personal history of fragility fracture after age 50, or oral corticosteroid use >6 months[251].

It is evident that both anastrozole and letrozole cause a significant increase in the bone turnover markers studied here, and that these effects increase over time. Since both drugs have similar effects on bone metabolism and turnover, there is a clear AI class effect on bone health in postmenopausal women with hormone-sensitive breast cancer. Tamoxifen has been reported to have some beneficial effects on bone turnover and fracture risk in postmenopausal women[252,253,254,255] but the results here suggest that these positive effects do not carry over once tamoxifen therapy is discontinued. Patients who had previously received tamoxifen showed significantly greater increases in the bone turnover markers PINP, bone ALP, sCTX and uNTX and when switched to an AI, compared with patients who had not been treated with tamoxifen. These results are in keeping with those from the ATAC trial, which suggested that bone resorption and formation are likely to be suppressed by about 30% and 15%, respectively, in patients treated with tamoxifen compared with an untreated population[216]. Tamoxifen inhibits bone turnover, explaining its bone-preserving properties. Bone turnover rates increased in patients who had recently stopped tamoxifen as a result of a 'rebound' increase in their rate of bone turnover. The introduction of an AI is likely to further increase this rate of bone turnover and this explains our results.

Bone remodelling is regulated by hormones including PTH. PTH causes increased bone resorption and increased bone formation. PTH levels were lower in patients treated with prior tamoxifen because tamoxifen has a beneficial effect on bone turnover levels and PTH levels will be suppressed as a result of negative feedback.

Prior tamoxifen therapy has also been shown to have a major effect on how AIs affect bone. This study has shown that prior treatment with tamoxifen followed by treatment with an AI results in a major increase in bone turnover markers which is likely to result in bone loss. Major increases in bone turnover are likely to occur when tamoxifen is withdrawn and then added to this are the effects that occur when anastrozole or letrozole is started. These findings are similar to those seen in the IES bone substudy: in those patients who were on tamoxifen for 2 to 3 years and then switched to exemestane, there was a significant decrease in BMD compared with baseline within 6 months at both the lumbar spine (2.7%; p<.0001) and hip (1.4%; p<.0001)[201]. Thus, any benefit that tamoxifen has on bone density appears to be lost rapidly after tamoxifen treatment ends and AIs begin. This is not significantly different from the effects of withdrawal of HRT. Therefore, patients who take anastrozole or letrozole after tamoxifen need the same bone monitoring as any patient taking anastrozole or letrozole alone.

In conclusion, these results indicate that the effects of anastrozole and letrozole on bone turnover are similar and increase with time. However prior tamoxifen treatment has a major effect on how AIs affect bone. The effects of AIs are amplified when introduced after previous tamoxifen treatment. This suggests a rebound increase in bone turnover resulting from tamoxifen withdrawal and the introduction of AIs. Any benefits on bone health from tamoxifen appear to be rapidly lost after introducing AIs.

2.3.2 Quality of life and bone health

Oestrogen is an important component of bone homeostasis and causes stimulation and inhibition of bone proliferation and resorption. Oestrogen deprivation is associated with increased bone turnover and overall bone loss. It is suggested that any bone or joint symptoms might be related to bone loss and it is of interest to determine if an increase in bone turnover is associated with an increased rate of musculoskeletal side-effects. Any side-effects which compromise QOL are important.

2.3.2.1 Patients

A total of 166 patients were eligible for analysis of joint pain/stiffness symptoms. Forty two patients reported joint pain or stiffness as shown alongside bone turnover parameters in table 2.6.

The mean PINP marker for patients with joint symptoms was 55.6ng/l compared with 50.2ng/l for those with no symptoms. These results were statistically significant p=0.03. The mean sCTX bone turnover marker was 0.76ng/l for the 42 women who reported joint symptoms. This figure was lower at 0.70ng/l for those who did not report joint symptoms. These results showed borderline statistical significance p=0.05.

When the patients were separated into groups depending on which drug they had taken, there was no statistically significant difference demonstrated, although the size of the measurements were very similar (tables 2.8 and 2.9) This is explained by the

smaller numbers in each group which resulted in an increase in the size of the confidence intervals.

These results suggest that PINP and sCTX are increased in patients who report increased joint symptoms. There was no significant difference between the other bone turnover parameters (ALP, uNTX or PTH) when comparing the group who reported joint pain/stiffness with the group who reported no joint symptoms.

	No report of joint pain/ stiffness (n=124)	Report of joint pain/ stiffness (n=42)	p-value of difference
PINP (ng/l)	50.2 (47.8, 52.6)	55.6 (51.4, 59.8)	**0.03**
ALP ^{transformed} (µg/l)	2.60 (2.57, 2.63)	2.62 (2.57, 2.68)	0.47
sCTX (ng/ml)	0.70 (0.67, 0.73)	0.76 (0.71, 0.82)	**0.05**
uNTX (nmolBCE/mmolCr)	56.2 (52.9 59.5)	57.3 (51.6 63.0)	0.74
PTH ^{transformed} (pg/ml)	3.96 (3.91, 4.00)	3.98 (3.91, 4.05)	0.57

Note that patients appear twice in this comparison (once per drug).
Mean and 95% CI given, results adjusted for baseline. Repeated measures analysis performed to take into account repeated patient measurements.

Table 2.6 Comparison of bone markers by presence of joint pain/stiffness

2.3.2.2 Comparison of bone markers and joint symptoms by drug

There was no evidence to suggest that that the presence of joint symptoms was affected by the type of drug or interaction of drug (χ^2 test p = 0.48) as shown in table 2.7.

	Anastrozole n (%)	Letrozole n (%)	Total
No report of joint pain/stiffness	64 (77.1%)	60 (72.2%)	124
Report of joint pain/stiffness	19 (22.9%)	23 (27.8%)	42
Total	83	83	166

Table 2.7 Reports of joint pain/stiffness by drug

The bone turnover marker PINP was significantly increased in patients who reported joint symptoms compared with those who did not (60.9ng/l versus 50.1ng/l p=0.004) in the anastrozole group as shown in table 2.8. There was no difference in those taking letrozole as shown in table 2.9 and no significant difference between the two drugs (p=0.10).

Anastrozole	No report of joint pain/stiffness (n=64)	Report of joint pain/stiffness (n=19)	p-value of mean difference
PINP (ng/l)	50.1 (46.9, 53.3)	60.9 (54.6, 67.1)	**0.004**
ALP ^{transformed} (µg/l)	2.61 (2.57, 2.65)	2.65 (2.57, 2.72)	0.45
sCTX (ng/ml)	0.71 (0.67, 0.75)	0.77 (0.69, 0.85)	0.19
uNTX (nmolBCE/mmolCr)	57.5 (53.4, 61.7)	58.6 (50.9, 66.3)	0.82
PTH^{transformed} (pg/ml)	3.95 (3.89, 4.02)	4.04 (3.93, 4.15)	0.17

Table 2.8 Comparison of bone markers by presence of joint pain/stiffness while on anastrozole

Letrozole	No report of joint Pain/stiffness (n=60)	Report of joint Pain/stiffness (n=23)	p-value of mean difference
PINP (ng/l)	49.9 (46.7, 53.1)	52.4 (47.2, 57.6)	0.42
ALP $^{\text{transformed}}$ (µg/l)	2.59 (2.54, 2.64)	2.61 (2.53, 2.68)	0.67
sCTX (ng/ml)	0.69 (0.65, 0.73)	0.76 (0.69, 0.83)	0.10
uNTX (nmolBCE/mmolCr)	55.2 (50.5, 59.9)	55.1 (47.4, 62.81)	0.99
PTH $^{\text{transformed}}$ (pg/ml)	3.96 (3.91, 4.01)	3.93 (3.84, 4.02)	0.56

Table 2.9 Comparison of bone markers by presence of joint pain/stiffness while on letrozole

2.3.2.3 Effects of prior tamoxifen

There was no evidence to suggest any relationship between the reporting of joint symptoms and prior treatment with tamoxifen as demonstrated in table 2.10.

	No prior tamoxifen n (%)	Prior tamoxifen n (%)	Total N
No report of joint pain/stiffness	62 (75.6%)	62 (73.8%)	124
Report of joint pain/stiffness	20 (24.3%)	22 (26.2%)	42
Total n	82	84	166

Table 2.10 Reports of joint pain/stiffness by prior tamoxifen
χ^2 test p=0.79

PINP was elevated in patients who reported joint symptoms and had not been exposed to prior tamoxifen (56.2ng/l) when compared to those who did not report joint symptoms (44.9ng/l) p=0.002 as shown in table 2.11.

There was a significant interaction between the report of joint symptoms and prior tamoxifen (p=0.009), suggesting that the behaviour of PINP is not consistent with regards to each variable, such that where there is no prior tamoxifen, the PINP measure is higher when there is a report of joint symptoms than without, but the PINP measure is lower when there are joint symptoms than without, where there is prior tamoxifen.

sCTX was significantly lower in patients who were tamoxifen naïve as shown in Tables 2.12 and 2.13 (p=0.006). This had no bearing on the report of joint pain/stiffness in those previously exposed to tamoxifen, however there was a difference between those who were tamoxifen naive (no symptoms 0.63ng/l versus symptoms 0.74ng/l p=0.016).

uNTX levels were significantly different between those with previous tamoxifen and those without (p=0.01), but this has no bearing on the report of joint pain/stiffness.

No prior tamoxifen	No report of joint pain /stiffness (n=62)	Report of joint pain /stiffness (n=20)	p-value of difference
PINP ng/l	44.9 (41.8, 48.1)	56.2 (50.2, 62.2)	**0.002**
ALP ^{transformed} µg/l	2.57 (2.52, 2.61)	2.61 (2.52, 2.69)	0.41
sCTX ng/ml	0.63 (0.59, 0.67)	0.74 (0.66, 0.82)	**0.016**
uNTX nmolBCE/mmolCr	51.7 (47.1, 56.3)	53.2 (45.1, 61.3)	0.74
PTH ^{transformed} pg/ml	4.02 (3.96, 4.08)	3.99 (3.89, 4.09)	0.61

Table 2.11 Comparison of bone markers by presence of joint pain/stiffness without prior tamoxifen

Prior tamoxifen	No report of joint pain /stiffness (n=62)	Report of joint pain /stiffness (n=22)	p-value of difference
PINP ng/l	55.9 (52.6, 59.1)	54.6 (49.3, 59.8)	0.67
ALP ^{transformed} µg/l	2.64 (2.59, 2.68)	2.64 (2.57, 2.71)	0.95
sCTX ng/ml	0.78 (0.73, 0.82)	0.77 (0.70, 0.84)	0.60
uNTX nmolBCE/mmolCr	60.7 (56.1, 65.3)	60.8 (53.1, 68.6)	0.98
PTH ^{transformed} pg/ml	3.88 (3.82, 3.94)	3.97 (3.87, 4.07)	0.13

Table 2.12 Comparison of bone markers by presence of joint pain/stiffness with prior tamoxifen

2.3.2.4 Effects by visit intervals

More patients reported joint symptoms at visit 2 (month 6) compared with visit 1 (month 3) as shown in table 2.13. This showed borderline statistical significance χ^2 test, p=0.07.

	Visit 1 (month 3)	**Visit 2 (month 6)**	**Total**
No report of joint pain/stiffness **n (%)**	67 (80.7%)	57 (68.6%)	124
Report of joint pain/stiffness **n (%)**	16 (19.2%)	26 (31.3%)	42
Total	83	83	166

Table 2.13 Reports of joint pain/stiffness by order

The bone turnover markers PINP, sCTX and ALP were significantly increased at visit 2 (p=0.0025, 0.01, 0.003 respectively) as shown in tables 2.14 and 2.15. There was no suggestion that this was related to the presence or absence of joint symptoms.

Visit 1	**No report of joint pain /stiffness (n=67)**	**Report of joint pain /stiffness (n=16)**	**p-value of difference**
PINP ng/l	46.6 (43.6, 49.6)	51.6 (45.4, 57.8)	0.15
ALP ^{transformed} µg/l	2.55 (2.51, 2.59)	2.58 (2.50, 2.66)	0.56
sCTX ng/ml	0.67 (0.64, 0.71)	0.70 (0.62, 0.79)	0.52
uNTX nmolBCE/mmolCr	54.0 (49.7, 58.3)	54.78 (46.2, 63.4)	0.87
PTH ^{transformed} pg/ml	3.96 (3.91, 4.02)	4.03 (3.92, 4.14)	0.27

Table 2.14 Comparison of bone markers by presence of joint pain/stiffness after visit 1

Visit 2	**No report of joint pain /stiffness (n=77)**	**Report of joint pain /stiffness (n=26)**	**p-value of difference**
PINP ng/l	54.5 (51.2, 57.7)	57.9 (53.0, 62.8)	0.25
ALP ^{transformed} µg/l	2.66 (2.62, 2.70)	2.65 (2.59, 2.72)	0.81
sCTX ng/ml	0.73 (0.69, 0.78)	0.80 (0.74, 0.86)	0.09
uNTX nmolBCE/mmolCr	58.8 (54.1, 63.4)	58.8 (51.9, 65.8)	0.98
PTH ^{transformed} pg/ml	3.95 (3.89, 4.01)	3.95 (3.86, 4.04)	0.97

Table 2.15 Comparison of bone markers by presence of joint pain/stiffness after visit 2

2.3.2.5 Correlation between bone markers and frequency of joint pain (BRM1)

The FACT-ES questionnaire investigates physical wellbeing, social and family wellbeing, emotional and functional wellbeing, endocrine symptoms and additional concerns. The endocrine section as shown in appendix C asks the patient to indicate how often they experience pains in their joints. This is referred to as BRM1.

There was a statistically significant correlation between PINP in women who had not been exposed to tamoxifen prior to commencing an AI and BRM1. Women taking letrozole showed a significant correlation between ALP and BRM1 as shown in table 2.16.

		Joint pain /stiffness		Drug		Visit		Prior Tamoxifen	
	Over-all	No	Yes	A	L	1	2	No	Yes
PINP	-0.11	-0.02	0.03	-0.16	-0.07	-0.16	-0.04	**-0.28**[3]	0.12
ALP[transformed]	**-0.20**[1]	-0.17	-0.04	-0.18	**-0.23**[2]	-0.19	-0.20	-0.13	-0.20
sCTX	-0.12	-0.01	-0.01	-0.18	0.07	-0.13	-0.09	-0.15	0.03
uNTX	0.003	0.04	0.04	-0.05	0.04	-0.03	0.03	-0.08	0.10
PTH[transformed]	-0.10	-0.11	-0.05	-0.05	-0.16	-0.04	-0.18	0.07	-0.25

A = anastrozole L = letrozole
[1] p=0.01 [2] p=0.05 [3] p=0.02
The bold results show where there is a significant change

Table 2.16 Correlation of BRM1 and bone markers

Due to the large number of hypothesis tests in the section on joint pain and bone turnover markers, multiple testing may be an issue. There are several ways to deal with this problem, one of which is to calculate a per comparison significance level across the entire section. In this section, there are 83 significance tests, 45 alone in table 2.16. The adjusted p-value calculated using Bonferroni's correction is less than 0.0006, so that for any test to be considered significant, the p-value would need to be

less than 0.0006. In this section, no test reaches this threshold, implying that there is no significant difference in any analysis however larger sample sizes may demonstrate statistical significance in future trials.

2.3.2.6 Bone turnover markers and musculoskeletal symptoms Discussion and conclusions

In summary, the bone turnover markers PINP and sCTX were increased in women with an increased incidence of joint symptoms suggesting that women with increased joint pain may have increased bone turnover. There was no evidence to suggest that the type of AI had any effect on the presence of joint symptoms. As mentioned in the results section, it is important to acknowledge that multiple tests were performed and therefore the nominally significant p-values need to be considered as possibly spurious when multiple testing is taken into account. The combination of QOL with bone markers had too few cases to provide clinically meaningful information and this is a major limitation of the study which was not powered to investigate any relationship between these two variables. These results correlating bone turnover markers and bone and musculoskeletal pain should therefore be considered as hypothesis generating and it will be interesting to see if these results are confirmed in future larger scale trials.

Musculoskeletal symptoms have been shown to affect 47% of women taking AIs in early stage breast cancer[256] however the aetiology of these symptoms remains unclear. Arthralgias and myalgias may result from increased bone turnover and could potentially be a marker of oestrogen suppression. Increased bone turnover results from a complex process of cytokine and growth factor production, some of which are involved with inflammation and may cause musculoskeletal symptoms.

Oestrogen has direct anti-nociceptive effects and therefore its withdrawal may in addition increase pain sensation to underlying musculoskeletal pathologies. This may be independent of the effects of increased bone turnover resulting from oestrogen deprivation. The absence of circulating oestrogen binding to ER in joint tissue may also induce pathophysiological changes leading to joint pain. Previous studies have

not shown an association between systemic inflammatory cytokine levels and AI associated musculoskeletal symptoms. One study did however detect a lower concentration of multiple cytokines before AI initiation in women who developed musculoskeletal symptoms compared with controls[48]. Patients with metastatic breast cancer treated with AI therapy report a much lower incidence of AI associated musculoskeletal symptoms (16%) and this may be explained by patients with 'active cancer' having higher levels of circulating cytokines before initiation of therapy[257] MRI studies have demonstrated the development of tenosynovitis in patients treated with AIs[258], suggesting that localised inflammation may be involved with musculoskeletal symptoms.

These current studies have suggested an association between bone turnover markers and musculoskeletal symptoms. One explanation is that multiple oestrogen-dependent inflammatory mediators resulting in increased bone turnover may be associated with the development of a localised inflammatory process resulting in pain although the precise mechanism may be multi-factorial.

One major limitation of this study is that the patients recruited had cancer and potentially other conditions rather than well patients with no other conditions such as those included in the LEAP study. Other limitations include the relatively small number of subjects investigated especially the group included in QOL and musculoskeletal symptoms. In addition, there was no control/placebo group therefore we are unable to state that the changes detected were definitely a result of tamoxifen/AI. The groups were not aged-matched however there was no significant difference in ages in each group. Compliance may also have been a limitation as there is no way of knowing whether the patients adhered strictly to the regimen. Both studies would have benefitted from having age-matched and cancer-free control groups.

Clinical trials have shown that AIs generally have more favourable adverse events compared to tamoxifen. Results from ATAC demonstrated that discontinuation rates due to adverse events were significantly higher in the tamoxifen compared with anastrozole group (14.3% vs. 11.1% respectively p=0.0002)[176]. The MA.17 trial demonstrated that more patients discontinued letrozole compared with the placebo group (4.9% vs. 3.6% p = 0.019[202]. However a 138 patients taking exemestane in the IES study discontinued therapy compared with 121 taking tamoxifen. A further 164 patients taking exemestane refused to continue therapy compared with 116 in the tamoxifen group[200]. AI associated musculoskeletal symptoms respresent an important toxicity and results from the major trials shown in table 1.12 demonstrate that there is an increased incidence compared with tamoxifen. This in turn may affect drug non-compliance and early cessation of treatment.

2.3.3 ALIQUOT Lipids

2.3.3.1 Patients

Ninety-four postmenopausal women with ER+ve breast cancer were suitable for adjuvant or extended adjuvant treatment with an AI and were eligible to provide samples for analysis. Patient disposition is shown in the CONSORT diagram in appendix G. Very few patients enrolled were on drugs likely to have an effect on lipid metabolism.

2.3.3.2 Statistical analysis

The statistical analysis was conducted by an independent statistician. Hormone therapy for each patient was coded to maintain the blind assessment and avoid bias. Baseline data were analysed using analysis of variance methods. For the post-treatment data, analysis of the variables was conducted using mixed models and repeated measures. Estimates of effect size (and 95% CI) have been calculated from the least square means, with baseline values entered into the model as covariates. Triglycerides were log transformed to achieve Normality at baseline.

2.3.3.3 Effect of treatment on lipid profile

Results are presented as percentage change from baseline for each group.

Anastrozole versus letrozole:

There was little evidence to suggest a difference between the two drugs. Only cLDL at 6 months appears to show any difference. Letrozole caused a significant increase of cLDL from baseline compared to anastrozole. This is shown in table 2.17.

Atherogenic lipids / lipoproteins	Period	Drug: mean percentage change (96% CI)		p-value (drug)
		Anastrozole	Letrozole	
Triglyceride[log transformed]	3 months	-5.52	+4.74	0.21
mmol/l	6 months	+5.39	-0.32	0.51
	p-value	0.20	0.55	
Cholesterol	3 months	+0.64	+5.06	0.19
mmol/l	6 months	-0.83	+4.17	0.18
	p-value	0.67	0.90	
cLDL	3 months	+4.74	+6.87	0.69
mmol/l	**6 months**	**-2.71**	**+8.60**	**0.04**
	p-value	0.17	0.75	
ApoB	3 months	-1.17	+5.05	0.27
g/l	6 months	-0.83	-2.27	0.80
	p-value	0.95	0.20	
Atheroprotective lipids/lipoproteins				
HDL	3 months	+1.36	+0.92	0.92
mmol/l	6 months	+4.45	+3.20	0.76
	p-value	0.93	0.62	
ApoA1	3 months	-6.74	-1.90	0.27
g/l	6 months	+0.24	-3.27	0.37
	p-value	0.09	0.75	

Table 2.17 Mean percentage changes in lipids from baseline

The bold results show where there is a significant change from baseline. A positive mean with all positive confidence limits indicates a significant percentage rise from baseline, while a negative mean with all negative confidence limits indicates a significant fall from baseline

2.3.3.4 Effects of prior tamoxifen

Prior tamoxifen versus no tamoxifen in system anastrozole versus letrozole:

Tables 2.18 show a comparison of anastrozole versus letrozole in relation to prior tamoxifen use (absolute values).

	Drug	Prior tamoxifen	No prior tamoxifen	p-value (drug)
Atherogenic lipids		**Mean (95% CI)**		
Triglyceride$^{\text{logtransformed}}$	**anastrozole**	**-0.02 (-0.24, 0.20)**	**0.51 (0.37, 0.66)**	**0.0001**
mmol/L	**letrozole**	**0.08 (-0.13, 0.29)**	**0.52 (0.38, 0.67)**	**0.001**
p-value (tamoxifen)		0.20	0.86	
Cholesterol	**anastrozole**	5.56 (5.00, 6.12)	5.66 (5.28, 6.03)	0.78
mmol/L	**letrozole**	5.72 (5.17, 6.27)	5.95 (5.57, 6.32)	0.50
p-value (tamoxifen)		0.52	0.11	
cLDL	**anstrozole**	3.37 (2.86, 3.88)	3.36 (3.01, 3.71)	0.96
mmol/L	**letrozole**	3.54 (3.04, 4.05)	3.66 (3.32, 4.01)	0.70
p-value (tamoxifen)		0.44	0.07	
ApoB	**anastrozole**	**0.78 (0.64, 0.91)**	**0.94 (0.85, 1.03)**	**0.04**
g/L	**letrozole**	**0.84 (0.71, 0.97)**	**0.95 (0.86, 1.04)**	**0.19**
p-value (tamoxifen)		0.23	0.90	
Atheroprotective lipids				
HDL	**anastrozole**	**1.68 (1.49, 1.86)**	**1.40 (1.28, 1.52)**	**0.0155**
mmol/L	**letrozole**	**1.66 (1.47, 1.84)**	**1.43 (1.31, 1.55)**	**0.0453**
p-value (tamoxifen)		0.74	0.52	
ApoA1	**anastrozole**	1.52 (1.37, 1.66)	1.36 (1.26, 1.46)	0.08
g/L	**letrozole**	1.48 (1.34, 1.63)	1.40 (1.29, 1.49)	0.32
p-value (tamoxifen)		0.53	0.37	

Table 2.18 Comparison of final measurements by tamoxifen status

The bold results show where there is a significant change from baseline. A positive mean with all positive confidence limits indicates a significant percentage rise from baseline, while a negative mean with all negative confidence limits indicates a significant fall from baseline

Triglycerides (mmol/l)

Both anastrozole and letrozole resulted in a fall in the levels of triglycerides in patients who had recently stopped tamoxifen as shown in figure 2.12. Anastrozole =-0.02 (-0.24, 0.20) and letrozole =0.08 (-0.13, 0.29) compared with those with no recent tamoxifen exposure. Anastrozole =0.51 (0.37, 0.66) and letrozole =0.52 (0.38, 0.67), p-values 0.0001 and 0.001 respectively.

ApoB (g/l)

There were significantly lower levels of ApoB in patients who had recently stopped tamoxifen and were then treated with anastrozole, than those with no recent tamoxifen exposure (0.78 (0.64, 0.91) versus 0.94 (0.85, 1.03), p=0.04), as shown in figure 2.13.

HDL (mmol/l)

Patients treated with anastrozole had significantly higher levels of HDL in those who had recently stopped tamoxifen as shown in figure 2.14. Anastrozole: 1.68 (1.49, 1.86) versus letrozole 1.40 (1.28, 1.52), p=0.02. Levels on letrozole were also higher for patients who had recently stopped tamoxifen than for those with no recent tamoxifen exposure (letrozole: 1.66 (1.47, 1.84) versus 1.43 (1.31, 1.55) (p=0.045).

ApoA1 (g/l), cholesterol (mmol/l) and cLDL (mmol/l)

There were no significant changes for ApoA1, cholesterol or cLDL as shown in figures 2.15 – 2.17.

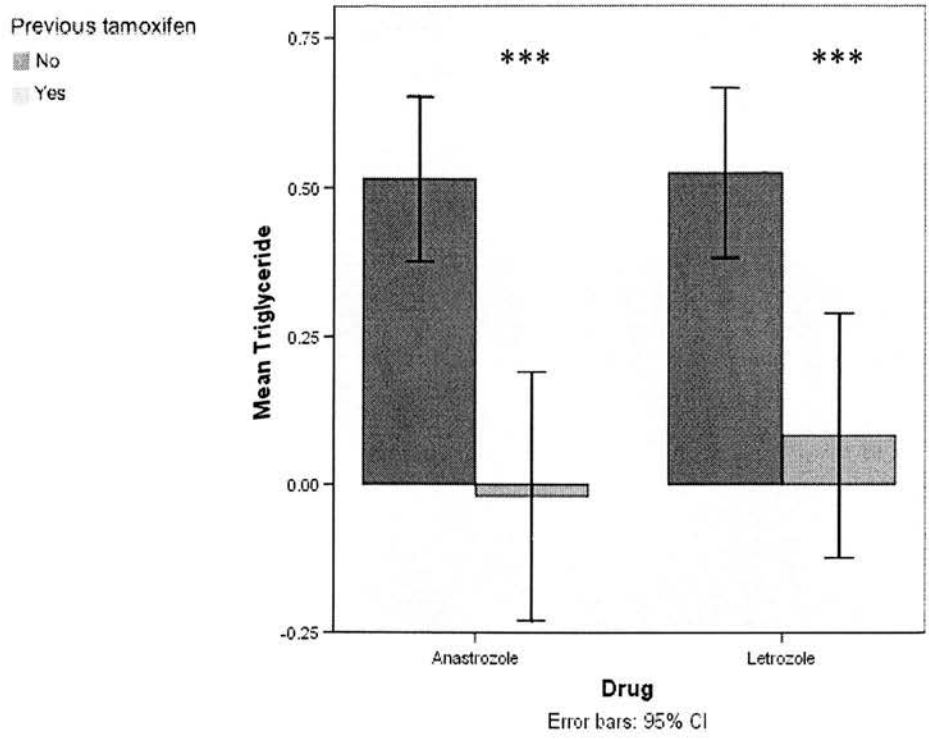


Figure 2.12 Triglycerides (mmol/L)
No previous tamoxifen versus previous tamoxifen
Anastrozole p<0.001***, letrozole p<0.001***

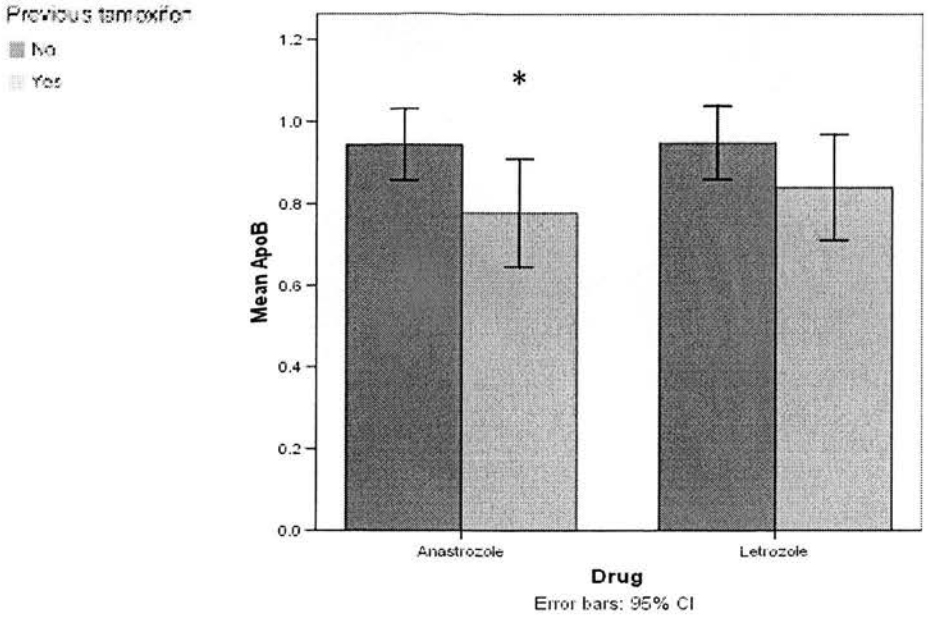


Figure 2.13 ApoB (g/L)
No previous tamoxifen versus previous tamoxifen
Anastrozole p=0.04*, letrozole p=0.19

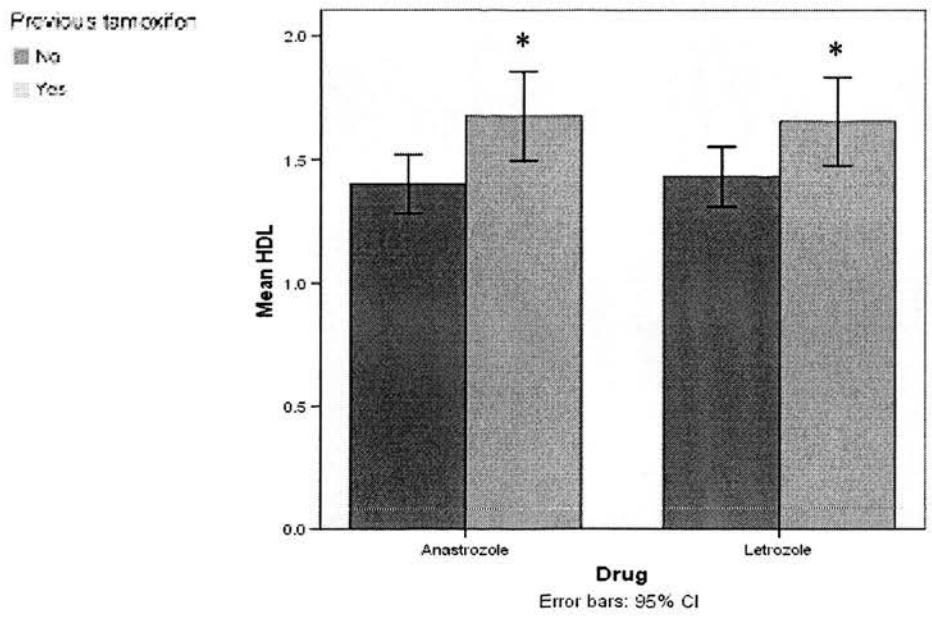


Figure 2.14 HDL (mmol/L)

No previous tamoxifen versus previous tamoxifen
Anastrozole p=0.02*, letrozole p=0.045*

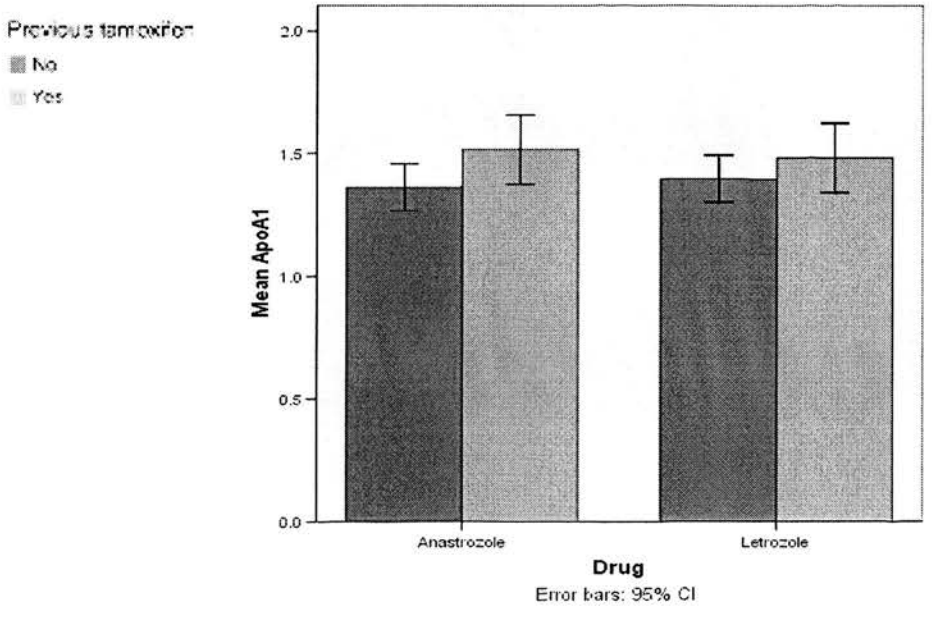


Figure 2.15 ApoA1 (g/L)
No previous tamoxifen versus previous tamoxifen

Anastrozole p=0.08, letrozole p=0.32

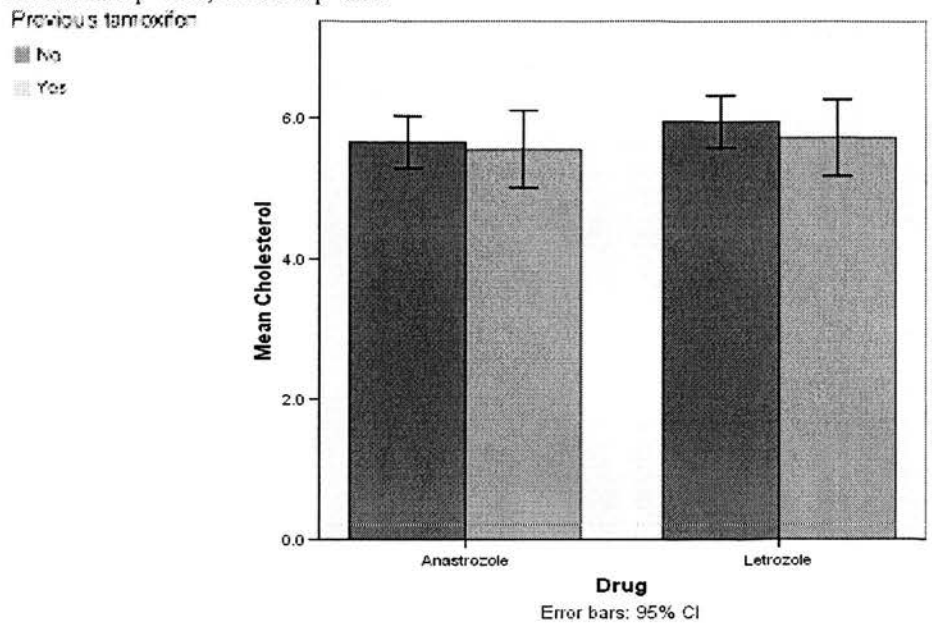


Figure 2.16 Cholesterol (mmol/L)
No previous tamoxifen versus previous tamoxifen
Anastrozole p=0.78, letrozole p=0.5

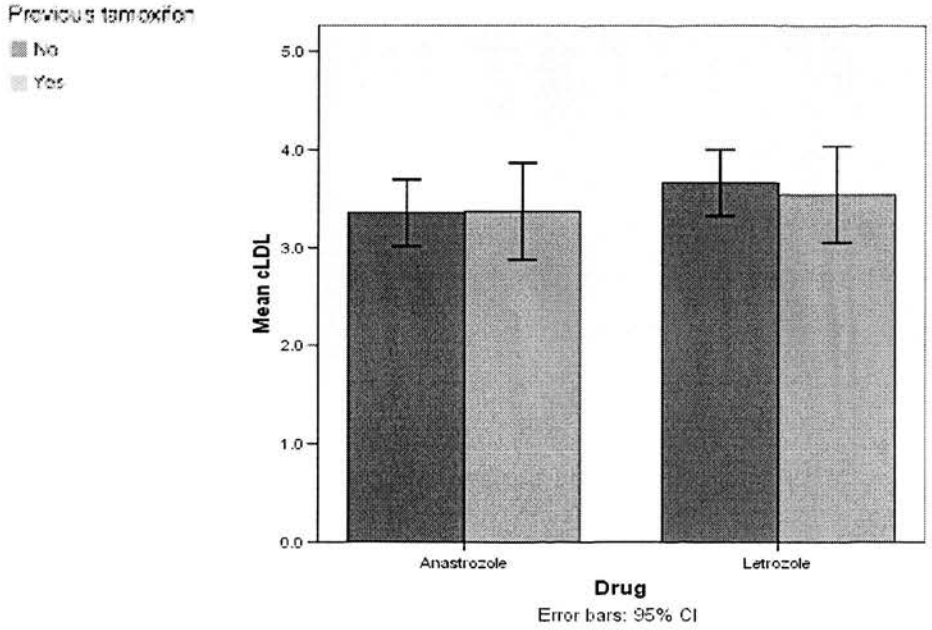


Figure 2.17 cLDL (mm/L)
No previous tamoxifen versus previous tamoxifen
Anastrozole p=0.96, letrozole p=0.7

2.3.3.5 ALIQUOT Lipids Discussion and conclusions

The mechanisms by which tamoxifen and AIs affect lipids and lipoproteins are unclear. It is known that oestrogen affects hepatic lipoprotein metabolism by increasing the synthesis of very low-density lipoproteins, leading to an increase in triglyceride levels. In addition it causes an increased apolipoprotein B receptor to lower LDL and lastly an increased synthesis of Apo A-1 which causes a high concentration of HDL[60].

The effects of long term AI treatment on lipid metabolism are a concern. Previous studies have suggested an unfavourable effect of letrozole on serum lipid profiles resulting in an increase in total cholesterol, cLDL and ApoB levels as well as similar unfavourable changes in the atherogenic risk ratios cholesterol:HDL cholesterol and LDL cholesterol:HDL cholesterol[259]. Studies investigating the effects of anastrozole on lipids have shown beneficial increases in HDL and favourable decreases in triglycerides repeatedly. The effects on total cholesterol or cLDL are variable[260,261,262].

This study demonstrated no major differences between these two drugs although letrozole increased cLDL at 6 months p=0.04 compared with anastrozole. This could be a spurious result given the number of analyses however anastrozole did reduce cLDL after letrozole so there is some consistency. This may relate to the lower oestradiol levels caused by letrozole.

Patients treated with tamoxifen who then switch to anastrozole or letrozole had a significant beneficial change in their lipid profile. This may be of value when planning drug sequencing. Triglycerides and ApoB levels (anastrozole only) were significantly lower in patients who had recently stopped tamoxifen. HDL levels were

significantly greater in those with no recent tamoxifen exposure. Tamoxifen has oestrogen-like properties and is known to favourably affect lipid profiles. Oestrogen has been reported to reduce total cholesterol and cLDL and to increase HDL, while the effects on triglycerides are unclear[263]. The ATENA substudy studied the duration of the beneficial tamoxifen effects on lipidaemic profiles following deprivation of treatment. Long-term tamoxifen results in a significant decrease in total cholesterol and LDL. These lipids could therefore be expected to rise adversely after tamoxifen therapy is completed. Tamoxifen withdrawal demonstrated an overall trend for increasing cholesterol and LDL and decreasing triglycerides, as early as 6-12 months after tamoxifen withdrawal[264]. These changes were also demonstrated in our study however they were not statistically significant.

One major limitation of this study is that the patients recruited had cancer and potentially other conditions rather than well patients with no other conditions such as those included in the LEAP study. Other limitations include the relatively small number of subjects investigated. In addition, there was no control/placebo group therefore we are unable to state that the lipid changes detected were definitely a result of tamoxifen/AI. The groups were not aged-matched however there was no significant difference in ages in each group. Compliance may also have been a limitation as there is no way of knowing whether the patients adhered strictly to the regimen. Both studies would have benefitted from having age-matched and cancer-free control groups.

Although lipid parameters are important risk factors, the relationship between hormone-related changes in these factors and the development of CVD is unclear[260]. The ATAC study demonstrated no statistical difference in the incidence of ischaemic cardiovascular events[176] although ischaemic cardiovascular events were reported more frequently with anastrozole compared to tamoxifen. i.e. a similar incidence of

ischaemic cardiovascular events. The ITA trial reported a statistically significant difference between the effects of anastrozole versus tamoxifen on lipid metabolism. Dyslipidaemia was detected in 9.3% of patients treated with anastrozole versus only 4.0% receiving tamoxifen[198]. The LEAP trial directly compared the safety profiles between exemestane and the non-steroidal AIs anastrozole and letrozole in 90 healthy postmenopausal women. Results demonstrated no significant differences between anastrozole and letrozole on their effects on LDL:HDL ratios, triglycerides and non-HDL concentrations however exemestane significantly decreased total cholesterol at 3 months (-5.5%)[247]. While oestrogen withdrawal has the potential to alter lipid profiles detrimentally, studies have shown that this does not occur with anastrozole[261]. Perhaps therefore anastrozole is less potent and certainly the clinical evidence points to anastrozole being less effective as seen in ATAC compared with BIG1-98[246]. The beneficial effects of tamoxifen may explain the higher incidence of lipid disorders demonstrated in the ITA trial which showed that switching from adjuvant tamoxifen to anastrozole was associated with a higher incidence of lipid disorders[198]. Studies have shown that anastrozole has fewer thromboembolic and ischaemic cerebrovascular events compared with tamoxifen and does not demonstrate androgenic, progestogenic or oestrogenic effects. The BIG 1-98 study reported 5.4% of hypercholesterolaemia in the letrozole arm compared with 1.2% in the tamoxifen arm in patients with baseline values within normal limits, who then had an increase of 1.5 times the upper limit of normal[65]. These were not fasting samples and the subsequent lipid substudy did not confirm the numbers of hypercholesterolaemia seen in those who had fasted. More women in the letrozole group had grade 3,4 or 5 cardiac events (2.1% vs. 1.1%) $p<0.001$ compared to tamoxifen however this may reflect the favourable effects on lipid parameters of tamoxifen which are well known. Big 1-98 also reported an increased number of

cardiovascular-related deaths with letrozole compared to tamoxifen[74,265]. The MA-17 trial compared letrozole with a placebo after prior tamoxifen therapy. It showed that after 36 months of letrozole there was no significant change in lipid profile[266]. This raises the possibility that changes seen in lipids are a result of previous tamoxifen treatment.

Women diagnosed with early breast cancer are at risk from cardiovascular disease and nearly all adjuvant treatments are associated with a degree of cardiovascular toxicity e.g. radiotherapy, chemotherapy, HER-2 therapy and tyrosine kinase inhibitors (lapatinib). In addition to these is often a decrease in exercise and concomitant weight gain which may accompany the diagnosis of breast cancer. As cardiovascular disease is the highest cause of mortality in postmenopausal women, it is vital to minimise the cardiotoxic effects of adjuvant endocrine therapy. Recommendations to reduce major cardiovascular risk factors will help to reduce morbidity in this patient group. Hypercholesterolaemia can be successfully treated using lipid lowering drugs. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) reduce cholesterol synthesis, especially in the liver. They are more effective than other lipid-lowering drugs at lowering low-density lipoprotein cholesterol <100mg/dl. They are known to reduce cardiovascular disease events and total mortality irrespective of the initial cholesterol concentration[267]. Other recommendations to reduce risk include lifestyle modifications such as beneficial changes to diet, exercise and weight management. Regular exercise may have a positive effect on hepatic fat metabolism. Alcohol consumption and smoking cessation are other areas which can be targeted. Hypertension can be specifically treated using beta-blockers +/- angiotensin-converting enzyme inhibitors and diabetic patients can be successfully treated using sulfonylureas or biguanides[228]. In addition, preclinical studies have demonstrated that lipophilic statins, ACE inhibitors and

metformin exhibit antineoplastic activity in several experimental models of breast carcinogenesis[268,269,270]. Statins (inhibitors of HMG-CoA reductase) are widely used to lower lipid concentrations for prevention of coronary artery disease. Recent observational studies have suggested a reduction in breast cancer risk associated with statin use. The evidence is however inconsistent and further evidence is required before statins can be included in large-scale prevention studies[192].

These results demonstrate little evidence to suggest a difference between the two available non-steroidal AIs and their effects on lipids. Patients who had recently completed tamoxifen and commenced anastrozole or letrozole showed beneficial changes in their lipid profile compared to patients with no recent exposure to tamoxifen.

SECTION 3: ALEX STUDY

3.1 Introduction

There are two distinct groups of AIs: non-steroidal and steroidal. Each have a different mechanism of action and it is therefore likely that each will have differing side-effect profiles. The ALEX study evaluated the impact of the non-steroidal AIs, anastrozole and letrozole and the steroidal inactivator, exemestane on bone, lipid, coagulation and quality of life profiles in postmenopausal women with ER+ve breast cancer. The effects of tamoxifen after prior AI therapy was also assessed in the lipid and coagulation groups.

3.2 ALEX Material and methods

3.2.1 Study Design

ALEX (Anastrozole versus Letrozole versus Exemestane) compared the effects of AIs in a series of postmenopausal women with hormone sensitive breast cancer. This was a prospective, open-label, randomised pharmacodynamic study. Bone markers, lipid and coagulation parameters were measured in 120 postmenopausal women with invasive ER+ve breast cancer. QOL questionnaires were also collected. Patients were randomised as part of their adjuvant endocrine therapy to receive either 4 months of anastrozole or 4 months of letrozole or 4 months of exemestane. Patients were then switched to tamoxifen and further samples were collected at 12 months, as shown in figure 3.1.

All patients were treated in the Edinburgh Breast Unit, UK. Following informed consent, each patient was randomised to receive 4 months of anastrozole (1 mg) or letrozole (2.5 mg) or exemestane (25mg) orally once daily. Patients received adjuvant AI therapy within one month of surgery. Thereafter they were switched to tamoxifen and further samples collected at 12 months.

3.2.2 Ethical approval

The study was approved by the Lothian Research Ethics Committee and carried out in accordance with the Declaration of Helsinki and in keeping with Good Clinical Practice.

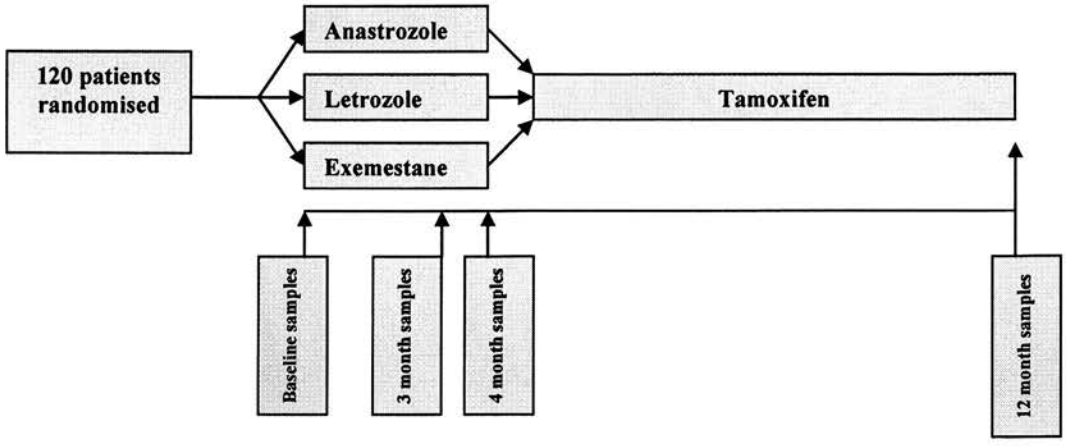


Figure 3.1 ALEX Study Design

3.2.3 Patient Selection

One hundred and twenty postmenopausal women with ER+ve breast cancer suitable for adjuvant therapy with an AI were enrolled. Patients were recruited within one month of surgery and were hormone treatment naïve. All patients were identified for trial entry at the multi-disciplinary team meeting using inclusion/exclusion criteria as shown in appendix D. Patient information sheets are shown in appendix E.

Women known to be hyperlipidaemic or taking lipid-lowering medications were excluded as were those taking HRT within one month prior to recruitment. All patients included had histologically confirmed invasive cancer that was ER+ve (ALLRED score ≥ 4). Postmenopausal status was defined as amenorrhea for 1 year and/or luteinising hormone with follicular stimulating hormone levels in the postmenopausal range. Postmenopausal women who had early invasive breast cancer (T1-3, N0-1, M0) and who were able to give informed consent were considered eligible. All premenopausal women; women receiving concurrent or previous chemotherapy; women taking concomitant hormonal therapy, including hormone replacement therapy; women thought to be at risk of carrying HIV, Hepatitis B or C, women with a history of jaundice, hypothyroidism, diabetes or with known or suspected alcohol abuse and patients unable to give informed consent were excluded from entry into the study.

Randomisation was 1:1:1, anastrozole:letrozole:exemestane for patients 1-77 and 1:1:2 for the patients 78–120. This was to increase the numbers on exemestane to allow a comparison between steroidal versus non-steroidal AIs. After 4 months of AI patients were commenced on 20mg tamoxifen daily.

3.2.4 Oestrogen receptor testing

All patients eligible for the study were ER+ve as per the ALIQUOT study.

3.2.5 Collection of clinical data

Data were collected using the same method as the ALIQUOT study. A CRF was filled out for every patient. All data were collected prospectively.

3.2.6 Collection and storage of blood and urine samples

Fasting blood and urine samples were obtained at the beginning of the study, after 3, 4 and 12 months. Samples were obtained at the same time of day and on the same day of the week. The timing of samples was scheduled to minimise diurnal and diet-related effects.

Plasma samples were separated by centrifugation and stored at -80°C until analysed. The second voided urine of the day was collected for measurement of urinary bone markers and stored at -80°C.

Patient details were encoded to ensure anonymity. A detailed chronological log book of all samples collected was recorded, ensuring easy and accurate identification of specimens.

3.2.7 Bone turnover marker analysis

Samples were analysed using the same methods as the ALIQUOT study as shown on page 136.

3.2.8 QOL analysis

Patient information was collected using the same methods as the ALIQUOT study as shown on page 138.

3.2.9 Lipid analysis

Samples were analysed using the same methods as the ALIQUOT study as shown on page 139.

3.2.10 Coagulation analysis

Coagulation and natural anticoagulants were analysed in citrated plasma at the Department of Haematology, Royal Infirmary of Edinburgh, UK using the ACL-TOP which is an automated, random access coagulation instrument utilising coagulometric, chromogenic and immunological measurements. Analysis was carried out under blind conditions to avoid bias. The analytes measured are shown in table 3.1 alongside their normal reference ranges.

Pro-Coagulants	**Action**	**Normal reference ranges / units**
Plasminogen activating inhibitor (PAI) antigen	Major inhibitor of fibrinolysis which prevents lysis of clot.	0.7 - 24.9 iu/ml
Von Willebrand's Factor (vWF) antigen	Binds to Factor VIII and enhances haemostasis. High levels increase the risk of thrombosis.	0.42 - 1.22 iu/ml
Factor VIII	Clotting factor. Factor VIII and vWF circulate together.	0.5 - 1.5 iu/ml
Fibrinogen	An acute phase protein which determines blood viscosity. ↑ levels of fibrinogen result in an ↑ plasma viscosity.	1.5 - 4.0 g/l
Activated protein C resistance (APCR)	Increases result in a hypercoagulable state.	3.0 - 5.0 ratio
Anti-Coagulants	**Action**	
Antithrombin (AT)	A major naturally occurring anticoagulant.	0.8 - 1.2 iu/ml
Protein C	A vitamin K-dependent glycoprotein which is anticoagulant. It requires the co-factor, protein S, for full activity.	0.67 - 1.38 iu/ml
Protein S total	A vitamin K-dependent glycoprotein. ~ 60% circulates bound and is inactive. The remaining 40% is the active uncomplexed moiety.	0.64 - 1.54 iu/ml
Protein S free	An active natural anticoagulant.	0.61 - 1.43 iu/ml

Table 3.1 Plasma coagulation factors measured[271]

Plasma levels of plasminogen activating inhibitor (PAI) antigen and vWF antigen are both markers of increased thrombogenesis. Elevated factor VIII and fibrinogen levels are also associated with increased thrombosis. Fibrinogen was measured using

coagulometric (turbidimetric) measurements whereby the amount of time required for a plasma specimen to clot is measured. This technique assesses coagulation endpoint by measuring change in optical density. Resistance to activated protein C was measured as a ratio and is a further marker of thrombogenesis. Activated protein C resistance (APCR) is an inherited cause of venous thrombosis and results from the factor V Leiden mutation[71].

Plasma antithrombin (AT), protein C, protein S total and protein S free are natural inhibitors of coagulation and were measured using chromogenic (absorbance) measurements which employs the colourimetric principle of measuring absorbance of light by a solution. Recurrent venous and occasionally arterial thrombosis are associated with an autosomal dominant deficiency of AT[71]. Protein C and S are inhibitors of coagulation cofactors V and VIII. Protein C is a vitamin K-dependent serine protease which is activated by the binding of thrombin to an endothelial cell surface receptor, thrombomedulin. It is able to destroy activated factors V and VIII, thus preventing thrombin generation and its action is enhanced by another vitamin K-dependent protein, S. Protein C also enhances fibrinololysis. Protein S is a co-factor for protein C[71].

The interassay CVs and the intra-assay CVs were <10% on all quality controls.

Coagulation factors measured at baseline and after 3 and 4 months of each AI. After 4 months of AI patients were commenced on tamoxifen therapy and further coagulation factors were measured at 12 months.

3.2.11 Adverse events monitoring

The incidence, type and grade of AEs and serious AEs were recorded during treatment in accordance with the National Cancer Institute's common terminology criteria for adverse events (version 3.0). Side effect profiles and adverse were recorded at each visit and also during telephone interviews with patients.

3.3 ALEX RESULTS AND DISCUSSION

3.3.1 Bone turnover markers

3.3.1.1 Patients

Fasting blood and urine samples were collected from 120 patients as shown in the CONSORT diagram in appendix H. An additional 42 patient sample sets were added to the population from the ALIQUOT study (hormone naïve group only). Overall bone turnover markers were measured in 162 postmenopausal women. Data from 155 patients were available for analysis. Samples were obtained from patients at the same time of day and the same day at entry, after 3 and 4 months of each drug.

3.3.1.2 Specimen analysis

Bone marker were analysed using the same methods as the ALIQUOT study as described on page 138.

3.3.1.3 Statistical analysis

Analysis of variables was performed using mixed models and repeated measures. Bone ALP and PTH were log transformed to achieve Normality prior to analysis. Percentage change was analysed by the method *analysis of covariance*, where the baseline values are included as a covariate. Some variables were log-transformed to achieve Normality. This is indicated in the tables where appropriate by (t). Where data were unavailable at three months, four month data have been substituted to create a 'post-treatment' variable. Tukey's correction has been used to adjust for multiple comparisons.

3.3.1.4 Differences between anastrozole, letrozole and exemestane

Results are presented as percentage change from baseline for each group.

Anastrozole, letrozole and exemestane significantly increased bone turnover. These effects are summarised in table 3.2.

Exemestane showed a greater rise by percentage change in bone turnover markers compared with anastrozole and letrozole however there were no significant differences between the drugs. These changes are shown in figures 3.2-3.6.

	Drug: Mean percentage change (95% CI)			p-value
	Anastrozole *n = 53*	Letrozole *n = 55*	Exemestane *n = 47*	
PINP ng/l	**7.65 (0.51, 14.79)**	**11.28 (4.34, 18.22)**	**17.82 (10.31, 25.32)**	0.15
ALP(t) µg/l	**2.59 (0.84, 4.33)**	**2.59 (0.90, 4.30)**	**3.04 (1.19, 4.89)**	0.92
sCTX ng/ml	**11.99 (5.35, 18.63)**	**15.72 (9.27, 22.17)**	**13.72 (6.73, 20.72)**	0.73
uNTX (t)[1] nmolBCE/mmolCr	-1.03 (-4.49, 2.42)	-1.44 (-4.93, 2.05)	3.53 (-0.36, 7.43)	0.14
PTH (t)[2] pg/ml	**-1.94 (-3.74, -0.13)**	-0.14 (-1.90, 1.61)	0.27 (-1.64, 2.19)	0.21

[1] n = 52, 52, 45 respectively
[2] n = 46, 48, 41 respectively
The bold results show where there is a significant change from baseline. A positive mean with all positive confidence limits indicates a significant percentage rise from baseline, while a negative mean with all negative confidence limits indicates a significant fall from baseline

Table 3.2 Percentage changes from baseline between each drug

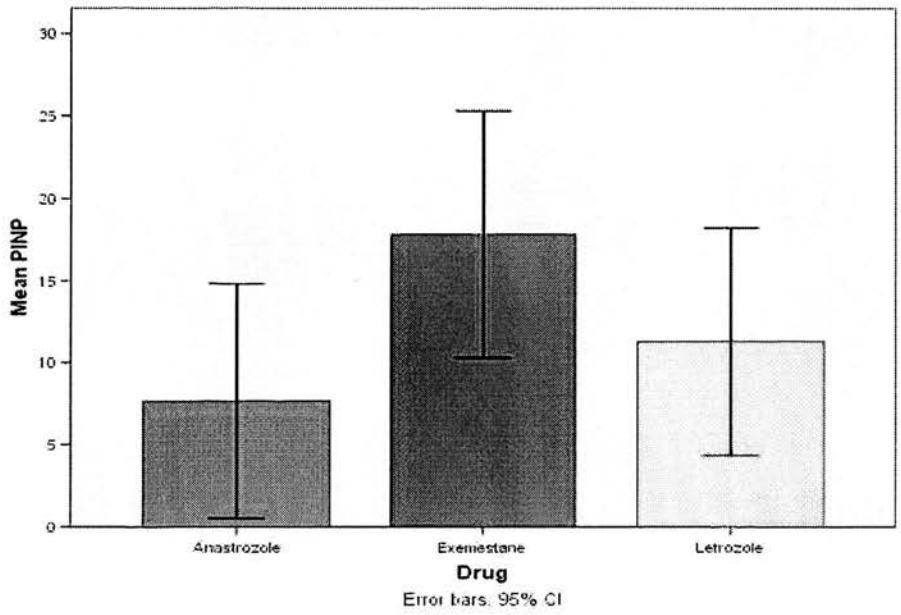


Figure 3.2 PINP mean percentage change per drug
No statistically significant difference detected between the drugs p=0.15

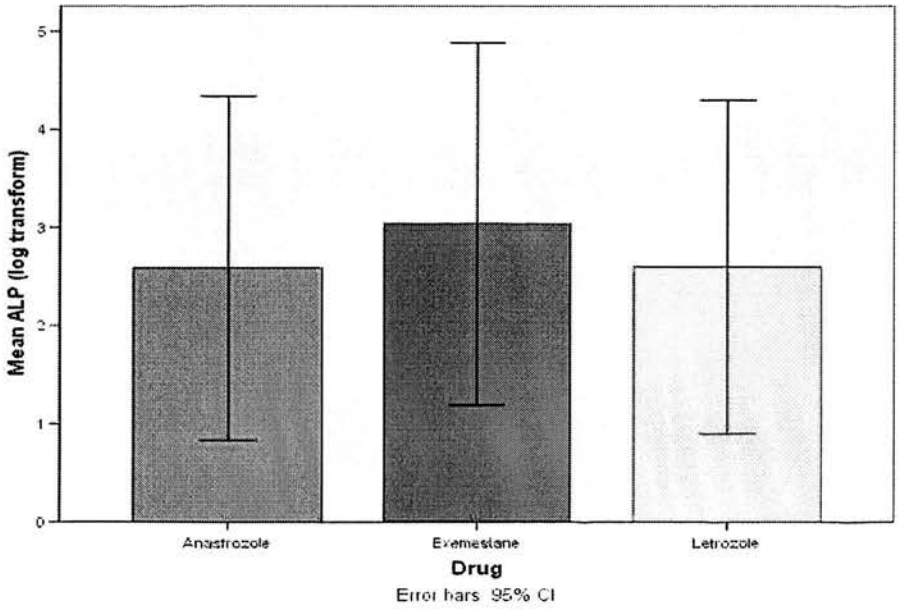


Figure 3.3 Bone ALP mean percentage change per drug
No statistically significant difference detected between the drugs p=0.92

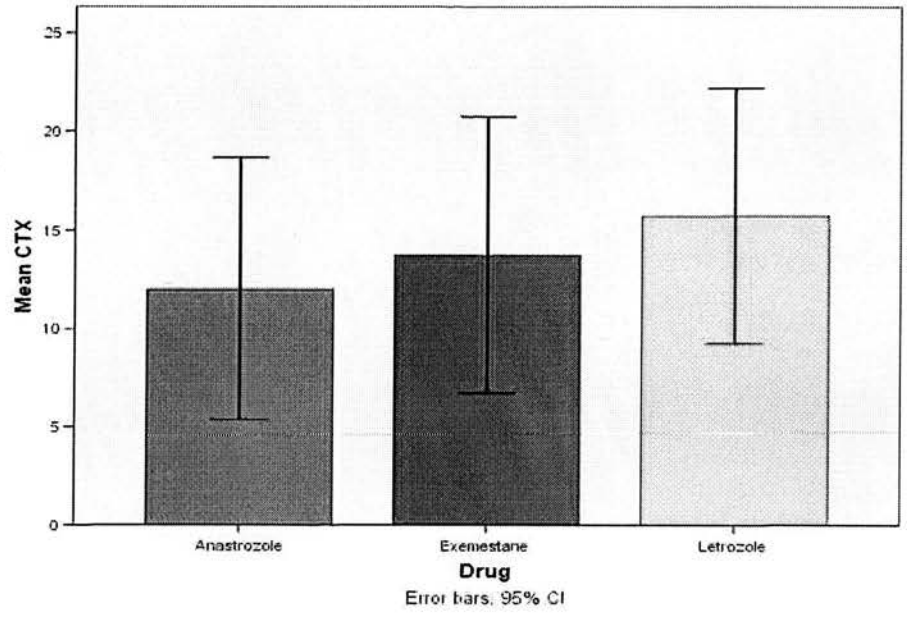


Figure 3.4 sCTX mean percentage change by drug
No statistically significant difference detected between the drugs p=0.73

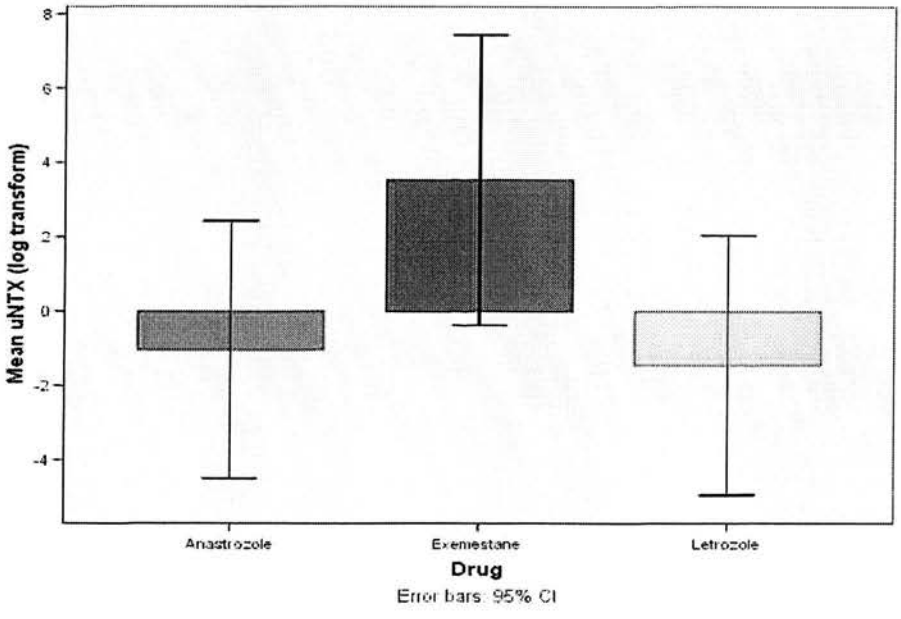


Figure 3.5 uNTX mean percentage change by drug
No statistically significant difference detected between the drugs p=0.14

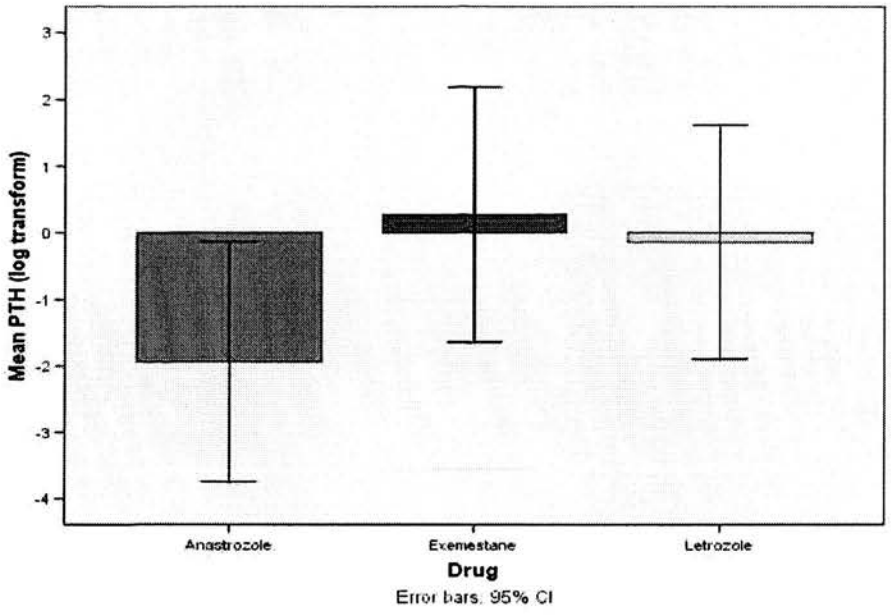


Figure 3.6 PTH mean percentage change per drug
No statistically significant difference detected between the drugs p=0.21

3.3.1.5 Differences between non-steroidal and steroidal AIs

Differences between non-steroidal and steroidal AIs are demonstrated in figures 3.7-3.11. Exemestane resulted in a greater increase in PINP compared to the non-steroidal AIs however this showed borderline significance (p=0.07). Exemestane resulted in a statistically greater increase in uNTX compared to non-steroidal AIs (p=0.05). PINP, bone ALP and CTX all had significantly higher than zero percentage changes, regardless of drug or drug type. These results are summarised in table 3.3.

	Drug type: Mean percentage change (95% CI)		p-value
	A+L *n = 108*	E *n = 47*	
PINP ng/l	**9.52 (4.55, 14.49)**	**17.81 (10.32, 25.31)**	0.07
ALP (t) µg/l	**2.59 (1.38, 3.81)**	**3.04 (1.20, 4.88)**	0.69
sCTX ng/ml	**13.91 (9.28, 18.53)**	**13.72 (6.74, 20.70)**	0.97
uNTX (t)[3] nmolBCE/mmolCr	-1.23 (-3.71, 1.24)	3.53 (-0.35, 7.41)	**0.05**
PTH (t)[4] pg/ml	-1.02 (-2.28, 0.25)	0.26 (-1.66, 2.18)	0.27

[3] n = 104, 45 respectively
[4] n = 94, 41 respectively

Table 3.3 Percentage changes from baseline between non-steroidal and steroidal AIs
(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

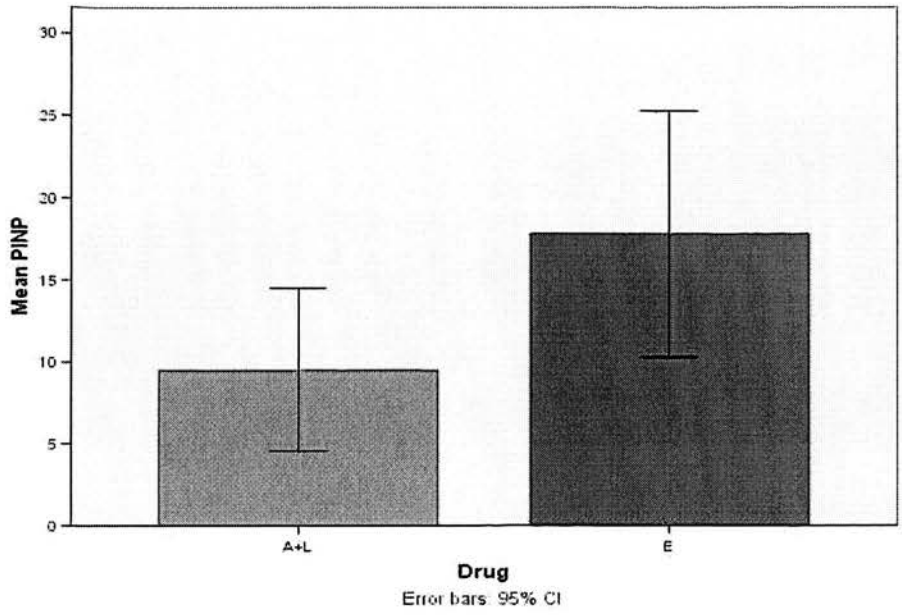


Figure 3.7 PINP mean percentage change from baseline
Non-steroidal versus steroidal AIs
No statistically significant difference detected between the classes of drug p=0.07

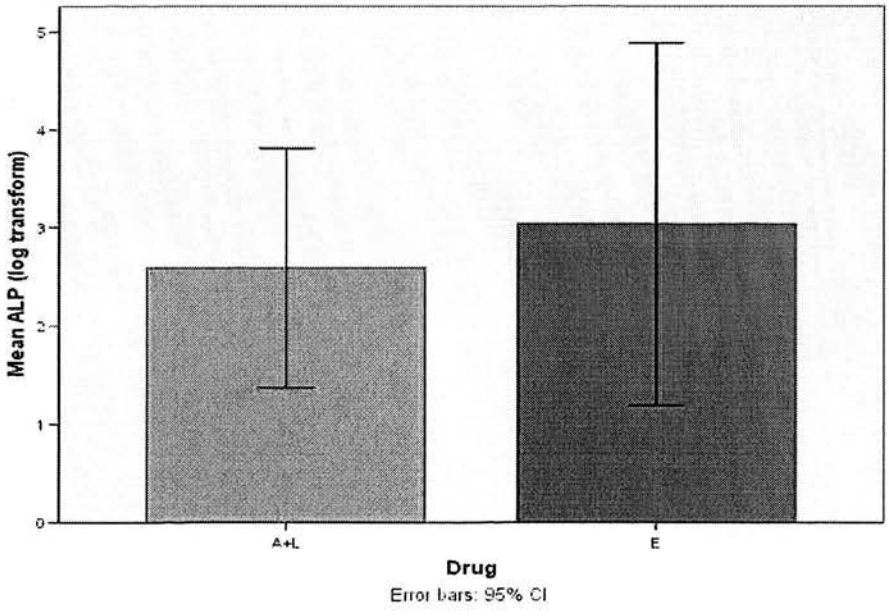


Figure 3.8 ALP mean percentage change from baseline
Non-steroidal versus steroidal AIs
No statistically significant difference detected between the classes of drug p=0.69

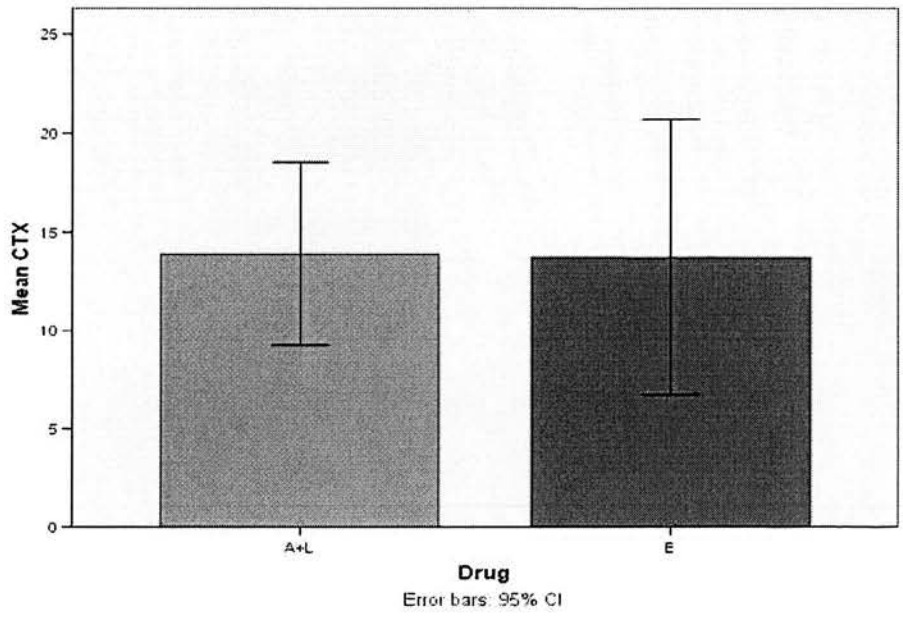


Figure 3.9 sCTX mean percentage change from baseline
Non-steroidal versus steroidal AIs
No statistically significant difference detected between the classes of drug p=0.97

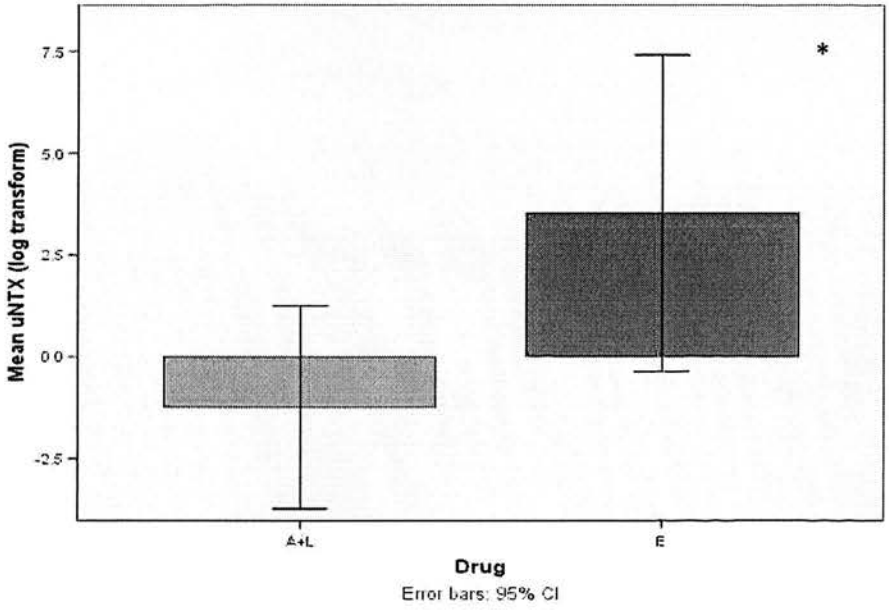


Figure 3.10 uNTX mean percentage change from baseline
Non-steroidal versus steroidal AIs
Statistically significant difference detected between the classes of drug p=0.05*

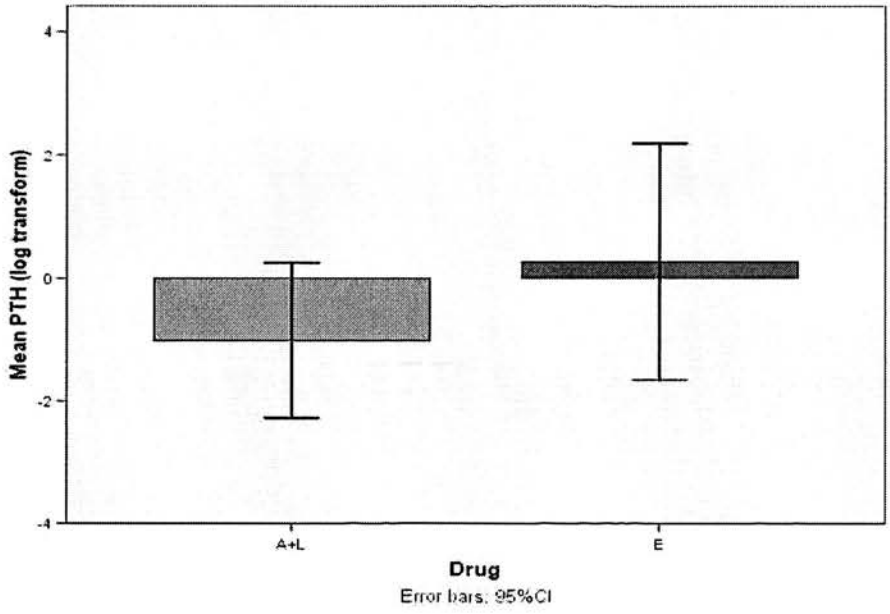


Figure 3.11 PTH mean percentage change from baseline Non-steroidal versus steroidal AIs

No statistically significant difference detected between the classes of drug p=0.27

3.3.1.6 Bone discussion and conclusions

This study demonstrated that each AI significantly increased bone turnover markers however the effects at these clinically used doses were very similar. The steroidal AI, exemestane had a greater effect on bone turnover compared with non-steroidal AIs, anastrozole and letrozole however this difference was not marked.

Due to their oestrogen depriving properties, AIs are associated with osteoporosis and an increased fracture risk. Oestrogen inhibits bone resorption by directly suppressing early osteoblastic and osteoclastic precursors in the bone marrow through the initiation of receptor activator of nuclear factor-kappa B ligand (RANKL) production by osteoblastic precursors. Previous studies have shown that oestrogens stimulate the production of osteoprotegerin, the potent anti-osteoclastogenic factor that blocks the binding of RANKL. Oestrogen also regulates the production of many bone-resorbing cytokines by osteoblasts and bone marrow stromal cells, progenitor cell differentiation, and inhibits apoptosis in both osteoblasts and osteocytes[272,273,274].

The two classes of AIs differ in both their structure and in their interaction with aromatase. The steroidal exemestane is structurally similar to the androgen substrate androstenedione rendering it more androgenic. In addition, the primary metabolite of exemestane is 17-hydroxyexemestane, which is also androgenic and binds tightly to the androgen receptor. Androgens positively influence bone density and strength. Their predominant effect appears to be on the osteoblast because androgens primarily increase bone formation rather than decreasing bone resorption[275]. Positive correlations between endogenous androgen levels and BMD in pre and postmenopausal women and also in men have been reported[276,277]. Studies have shown that low oestrogen concentrations have caused an increase in androgen receptor sensitivity in bone. In comparison, the non-steroidal AIs, anastrozole and

letrozole have no demonstrable androgenic effects[278]. This may partly expain the differences between the two classes of drug and their effects on bone.

One major limitation of this study is that the patients recruited had cancer and potentially other conditions rather than well patients with no other conditions such as those included in the LEAP study. Other limitations include the relatively small number of subjects investigated. In addition, there was no control/placebo group therefore we are unable to state that the changes detected were definitely a result of tamoxifen/AI use. The groups were not aged-matched however there was no significant difference in ages in each group. Compliance may also have been a limitation as there is no way of knowing whether the patients adhered strictly to the regimen. Both studies would have benefitted from having age-matched and cancer-free control groups.

All three AIs are associated with an increased fracture rate compared to tamoxifen or placebo in adjuvant trials. After a median follow-up of 68 months, the ATAC trial reported an increase in the numbers of fractures associated with anastrozole (11% fracture rate) compared with tamoxifen (7.7% fracture rate). The incidence of hip fractures was low in both groups however those taking anastrozole had a significantly higher rate of vertebral fractures compared with tamoxifen (1.5% versus 0.9% respectively)[189]. Similarly in the BIG-1-98 study, comparing letrozole with tamoxifen, letrozole was reported to have a higher (5.7%) fracture rate compared with tamoxifen (4.0%) after a median follow-up of 25.8 months[206]. The recent publication of the TEAM trial results has also confirmed a significantly higher incidence of fractures and osteoporosis in women treated with exemestane monotherapy (5% and 10% respectively) compared with those treated with tamoxifen (3% and 6% respectively) after a median follow-up of 60 months[196]. The TEAM sub-study evaluated the effects of exemestane and tamoxifen on markers of

bone turnover. Exemestane significantly increased bone turnover markers and tamoxifen significantly decreased them. PINP was increased by 36.7% after 3 months of exemestane compared with -18% in the tamoxifen group. These changes increased at 6 and 12 month time points[279]. The earlier IES study demonstrated a 3.1% fracture rate in women treated with exemestane compared with a 2.3% rate with tamoxifen[200].

The type of fracture associated with AI use is of interest. In the ATAC study of anastrozole, there were 45 vertebral fracture cases in the anastrozole group and 27 in the tamoxifen group. There were only small differences in the rate of hip and forearm fractures. It is likely that larger changes in bone turnover are required to reduce non-vertebral fractures. Tamoxifen is known to increase BMD of the spine, but not the forearm or total body BMD[280].

AI induced bone loss is preventable and treatable using bisphosphonates alongside dietary and lifestyle changes. Early detection using baseline bone mineral density tests are recommended prior to commencing an AI. Bisphosphonates can be administered to prevent the bone loss associated with AIs as demonstrated in the Zometa-Femara Adjuvant Synergy trials (Z-FAST, ZO-FAST and E-ZO-FAST)[281] and the Austrian Breast and Colorectal Cancer Study Group trial[180]. In addition, 80% of breast metastases develop in bone, which further increases the risk of fracture through osteoclast-mediated destruction of surrounding bone[282].

Current guidelines to minimise AI associated bone loss include healthy lifestyle changes e.g. weight-bearing exercises, smoking cessation and dietary supplements including calcium and vitamin D. Vitamin D supplementation is advisable in women with low vitamin D levels who are at higher risk of bone loss when receiving AIs[283].

In conclusion, treatment with exemestane resulted in a greater change in bone turnover markers compared with the non-steroidal AIs, although these differences

were not marked. This may be explained by the androgenic properties of exemestane. Increased changes in bone turnover markers are indicative of increased bone loss however at clinically used doses, there is unlikely to be any difference between the classes of AIs in the rates of osteoporosis or fractures.

3.3.2 ALEX Quality of life and bone turnover

3.3.2.1 Baseline data

The results shown in figures 3.12 – 3.16 demonstrate a comparison between the joint pain scores and bone markers at baseline, before any confounding effect of drug was possible. Joint pains (BRM1) were scored as follows (also shown in appendix C):

0 = I have pains in my joints – not at all
1 = I have pains in my joints – a little bit
2 = I have pains in my joints – somewhat
3 = I have pains in my joints – quite a bit
4 = I have pains in my joints – very much

Scores of 3 and 4 were combined, due to small numbers. ° = outliers

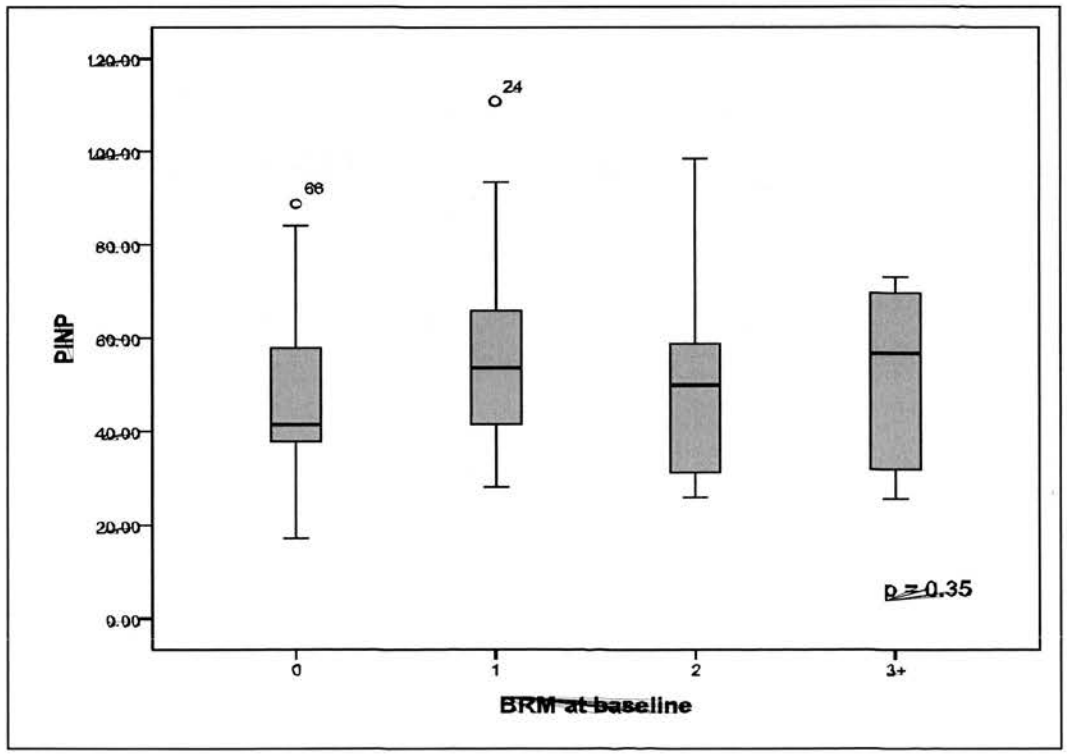


Figure 3.12 Baseline PINP and joint pain scores (BRM)

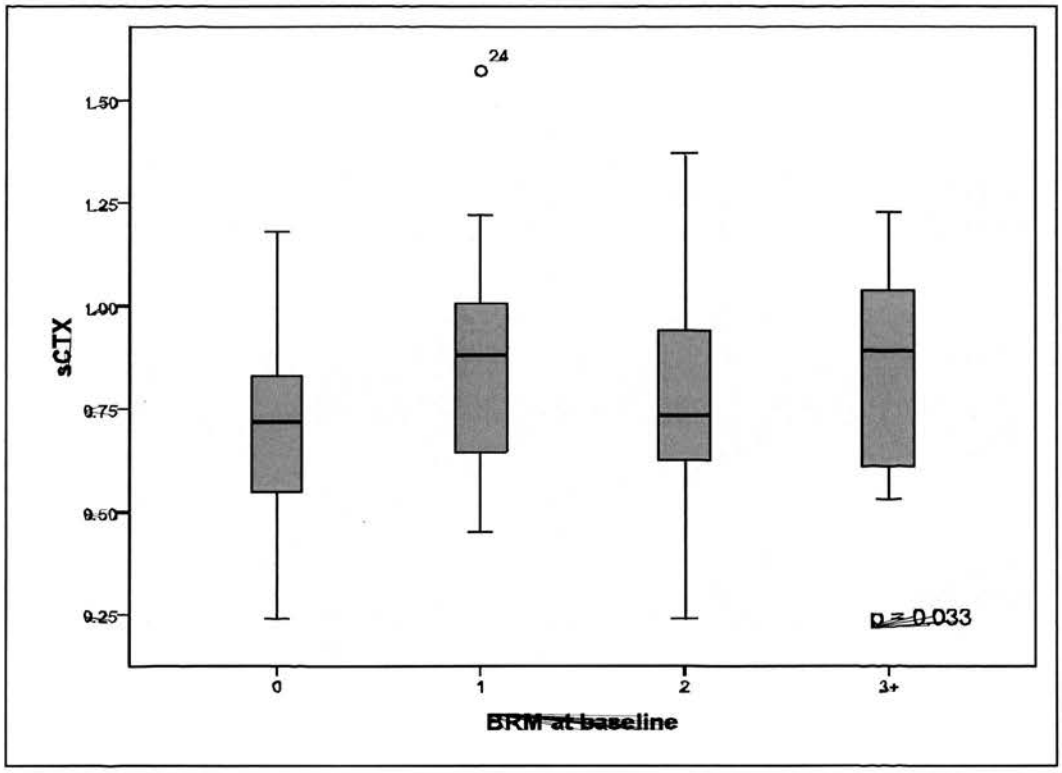


Figure 3.13 Baseline sCTX and joint pain scores (BRM)

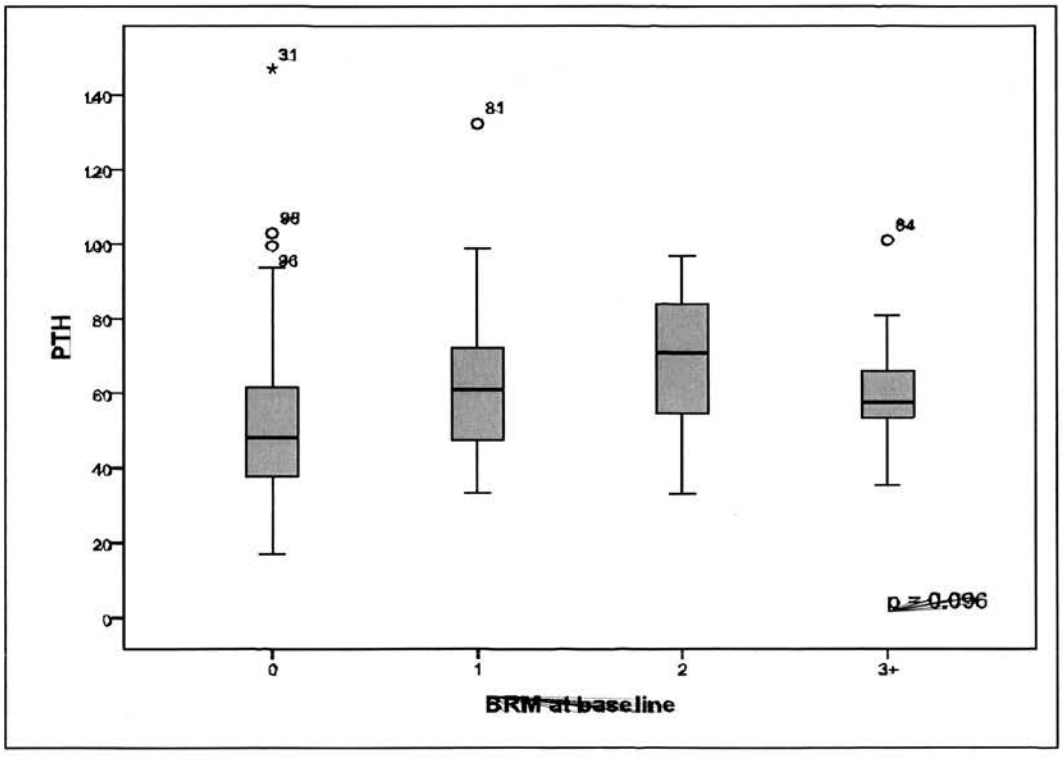


Figure 3.14 Baseline PTH and joint pain scores (BRM)

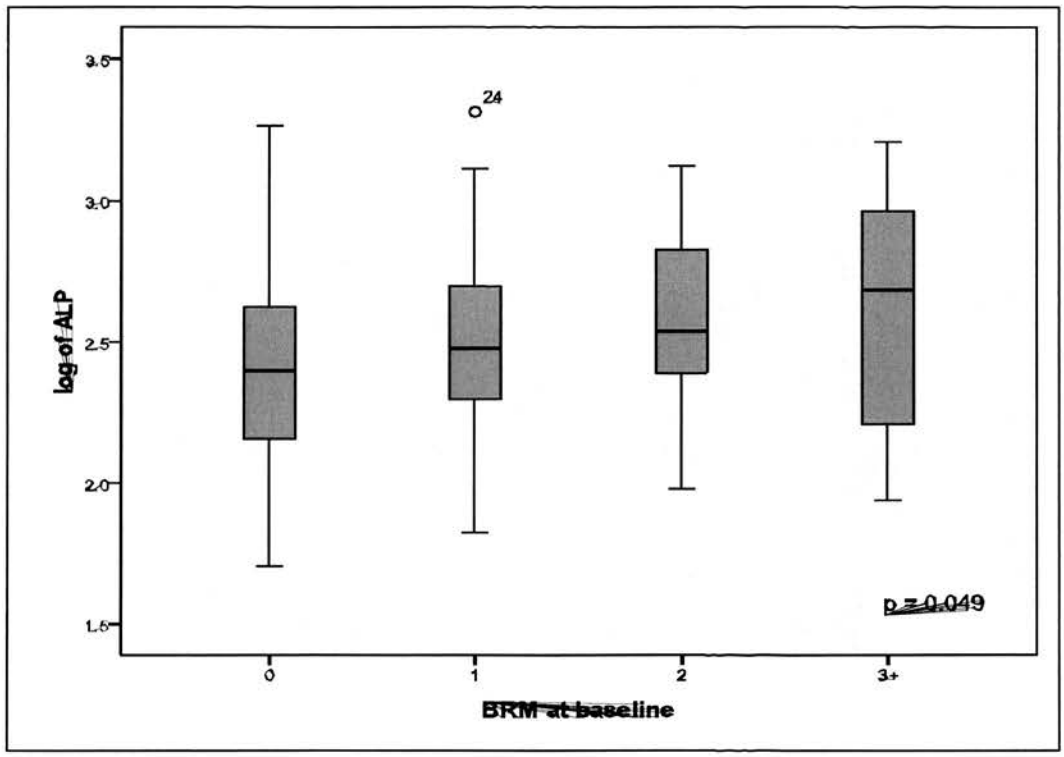


Figure 3.15 Baseline ALP and joint pain scores (BRM)

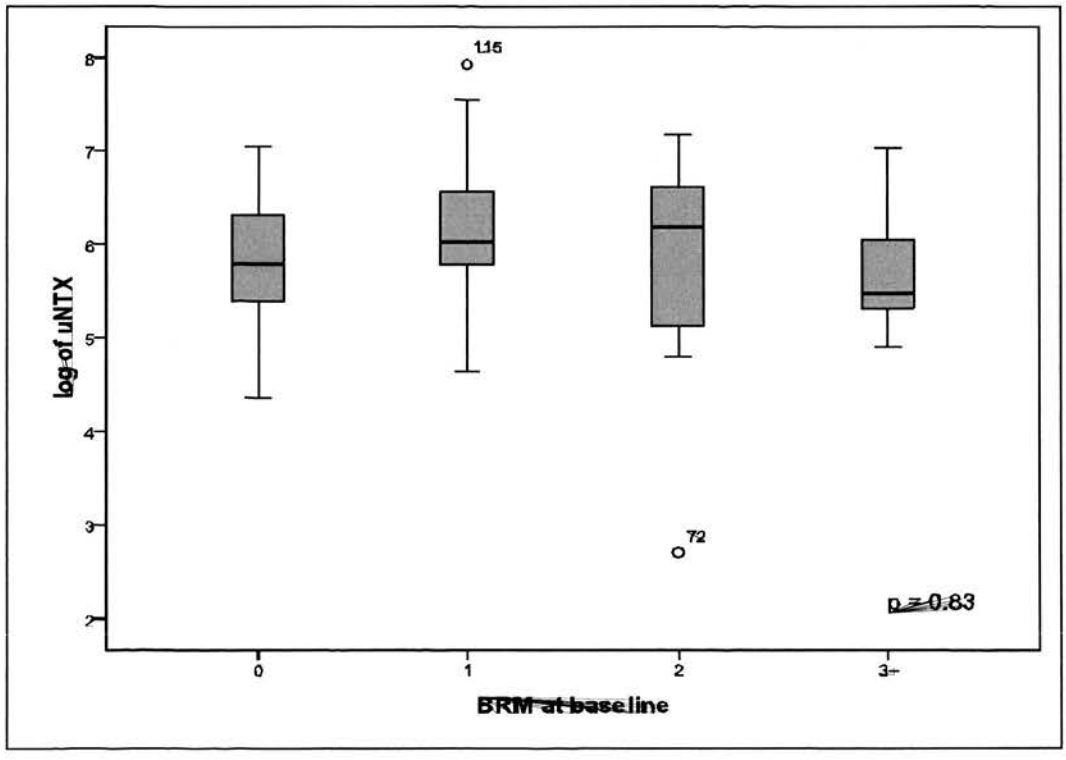


Figure 3.16 Baseline uNTX and joint pain scores (BRM) ° = outliers

3.3.2.2 Post-drug data

Sample sizes for drug versus joint pain scores (BRM) classes were very small as shown in table 3.4 and therefore confidence intervals were very large.

		BRM (joint pain scores)					Total no. of patients
		0	1	2	3	4	
Drug	A	7	7	6	6	4	30
	E	15	10	3	3	6	37
	L	7	8	4	5	2	26
	Nil	50	25	12	5	5	97
Total patients		79	50	25	19	17	190

Table 3.4 Joint pain scores (BRM) per drug
A = anastrozole, E = exemestane and L = letrozole

If 'no drug' is compared with 'any drug' (which is the same as using 'visit' as a variable), the following results are obtained as shown in table 3.5.

	BRM p-value i.e no drug	Visit p-value i.e any drug
PINP	0.44	**0.053**
log(ALP)	0.12	0.21
sCTX	0.60	**0.03**
log(uNTX)	0.47	0.92
PTH	0.21	0.82

Table 3.5 BRM according to no drug versus drug
Visit 1 – baseline Visit 2 – 4 months Visit 3 – 12 months
The bold results show where there is a significant difference

From this, it would appear that only 'visit' has an influence on the bone markers; that is, the bone markers PINP and sCTX are usually higher at the second visit than the first, regardless of BRM score.

3.3.2.3 Discussion and conclusions

Baseline data

There was some evidence to suggest that there is a linear relationship between joint pain scores and sCTX (p=0.033), and between joint pain scores and log ALP (p=0.049). These relationships were not strong.

All data

Sample size became problematic once drug was included in the models. There are fewer than 10 patients in each anastrozole/BRM and letrozole/BRM combination, and particularly few in the higher BRM scores. Using 'visit' as a surrogate for 'no drug' versus 'any drug' only the presence of a drug had any significant effect on bone markers (PINP p=0.053 and sCTX p=0.03).

Recent studies have detected a positive relationship between vasomotor menopausal symptoms and bone turnover markers. Several studies have found that mean BMD is lower among women with vasomotor symptoms compared with women without symptoms. Vasomotor symptoms including hot flushes and night sweats may be markers for risk of adverse bone health[284]. In addition data from the ATAC trial has demonstrated that the development of AI associated joint pain might predict improved breast cancer outcomes[285]. There is currently no available published data demonstrating a relationship between bone symptoms and bone turnover markers. Results from the ALIQUOT study suggested there might be a relationship between PINP and sCTX with musculoskeletal symptoms. From the ALEX results it would

appear that joint pain score is not predictive of bone markers in most cases, and where it is, the relationship is not strong. Further studies are required to establish if these findings can be demonstrated in larger populations of women taking AIs.

3.3.3 ALEX Lipids Results

3.3.3.1 Patients

Of the 120 patients randomised in this study, 117 patients had evaluable lipid samples after either 3 or 4 months of AI treatment. 96 patients were switched thereafter to tamoxifen and had evaluable lipids measurements at 12 months. Patient disposition is shown in the CONSORT diagram in appendix H. Overall, patient demographics were well balanced between the three patient groups as seen in table 3.6.

	Anastrozole n=34	**Letrozole** n=34	**Exemestane** n=49
Age yrs (median)	61.0	59.9	60.6
Height cm (median)	162.5	161.0	162.0
Weight kg (median)	66.0	67.5	66.5
BMI kg/m^2 (median)	25.3	26.4	25.7
Prior HRT *n (%)*	20 (59%)	13 (38%)	22 (45%)
Hysterectomy *n (%)*	4 (12%)	4 (12%)	10 (20%)
Bilateral oophorectomy *n (%)*	1 (3%)	1 (3%)	4 (8%)

Table 3.6 Patient baseline demographics

3.3.3.2 Statistical analysis

Percentage change was calculated as (post-treatment – baseline)/baseline, where a negative value indicated a fall from baseline. A repeated measures analysis was performed, since measurements were of the same patients over a series of time points. Estimates of effect size (and 95% CI) have been calculated from the least squares means, with baseline values entered into the model as covariates. Each

variable's mean and 95% CI have been adjusted for the presence of the other variables. Tukey's correction has been used to adjust for multiple comparisons.

3.3.3.3 Differences between anastrozole, letrozole and exemestane

Lipid measurements were similar at baseline in the three groups. The changes from baseline to 4 months for each lipid/lipoprotein measured in shown in table 3.7.

Exemestane caused a significant fall in triglycerides (-11.53% p=0.0015), cholesterol (-5.48% p=0.03) and HDL (-7.90% p=0.008) from baseline however only tryglycerides showed a significant difference between the drugs at any time point as shown in table 3.7. There were borderline significant changes between drug types for ApoA1 (p=0.08). No other drug on its own achieved a significant change from baseline. There was no significant change in LDL or Apo B in either of the three drugs. The period (3 or 4 months) did not have a significant effect on the overall results.

Exemestane caused a significant increase in the atherogenic risk ratios, chol:HDL (+4.70% p=0.004), LDL:HDL (+9.04% p<0.0001) and ApoB:ApoA-1 (+8.77% p=0.0005), from baseline compared to anastrozole and letrozole as shown in table 3.7. This was mediated by the fall in the levels of atheroprotective HDL and ApoA1.

Anastrozole resulted in moderate non-significant alterations in lipid profile. The atherogenic triglycerides (-3.91%), cholesterol (-5.32%), cLDL (-6.42%) and Apo B (-5.39%) decreased and there was a minor fall in the atheroprotective HDL (-1.67%) and ApoAl (-0.34). The atherogenic risk ratios chol:HDL and LDL:HDL fell by -2.85% and -3.97% respectively whereas there was a minor increase in the ApoB:ApoA1 ratio, +0.18%.

No significant changes were demonstrated for letrozole.

	Drug: mean percentage change (95% CI)			
	Anastrozole	Letrozole	Exemestane	p-value
Atherogenic lipids/lipoproteins				
Triglycerides mmol/l	-3.91 (-12.70,+4.88)	+2.29 (-6.12, +10.70)	**-11.53 (-18.57, -4.50) p=0.0015**	**0.04**
Cholesterol mmol/l	-5.32 (-11.52,+0.88)	+0.15 (-5.82, +6.13)	**-5.48 (-10.49, -0.46) p=0.03**	0.31
cLDL mmol/l	-6.42 (-14.14, +1.30)	-0.72 (-8.15, +6.72)	-1.30 (-7.53, +4.94)	0.50
Apo B g/l	-5.39 (-14.02,+3.25)	+3.07 (-5.22, +11.36)	+0.60 (-6.42, +7.61)	0.34
Atheroprotective lipids/lipoproteins				
HDL mmol/l	-1.67 (-8.81, +5.48)	-0.37 (-7.24, +6.50)	**-7.90 (-13.67. -2.14) p=0.008**	0.20
ApoA-1 g/l	-0.34 (-10.66, +9.98)	7.37 (-2.52, +17.27)	-7.70 (-16.07, +0.66)	0.08
Atherogenic risk ratios				
Chol:HDL	-2.85 (-6.85, +1.15)	+0.47 (-3.37, +4.30)	**+4.70 (+1.50, +7.91) p=0.004**	**0.01**
LDL:HDL	-3.97 (-9.45, +1.51)	-0.20 (-5.45, +5.05)	**+9.04 (+4.65, +13.44) p<0.0001**	**0.0008**
ApoB:ApoA-1	+0.18 (-5.89, +6.24)	+1.30 (-4.50, +7.11)	**+8.77 (+3.90, +13.63) p=0.0005**	**0.05**

Table 3.7 Percentage change from baseline to 4 months

(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

3.3.3.4 Differences between non-steroidal and steroidal AIs

The results showed greater statistical significance when the drugs were grouped into non-steroidal versus steroidal classes as demonstrated in table 3.8. Exemestane showed a significant fall from baseline in triglycerides (-11.53% p=0.0015), cholesterol (-5.45% p=0.03) and HDL (-7.90% p=0.007) as shown in figures 3.17 - 3.19. There was a significant difference between the groups for triglycerides (p=0.02) and a borderline significant difference for HDL (p=0.07). There were borderline significant differences between the drug types for ApoA-1 (p= 0.046). No effect of period was seen. There were significant differences between the lipid ratios and drug types as shown in figures 3.20-3.22. Exemestane caused a significant increase in chol:HDL (4.69% p=0.005) LDL:HDL (9.02% p<0.0001) and ApoB:ApoA-1 (8.76% p=0.0005) ratios which are associated with an increase risk of cardiovascular disease.

	Drug: mean percentage change (95% CI)		
	Non-steroidal AIs (anastrozole + letrozole)	Steroidal AI (exemestane)	p-value
Atherogenic lipids/lipoproteins			
Triglycerides mmol/l	-0.67 (-6.76, +5.42)	**-11.53 (-18.56, -4.50) p=0.0015**	**0.02**
Cholesterol mmol/l	-2.48 (-6.80, +1.83)	**-5.48 (-10.47, -0.42) p=0.03**	0.38
cLDL mmol/l	-3.45 (-8.82, +1.90)	-1.27 (-7.51, +4.97)	0.60
Apo B g/l	-0.96 (-7.07, +5.15)	+0.58 (-6.44, +7.61)	0.74
Atheroprotective lipids/lipoproteins			
HDL mmol/l	-0.99 (-5.93, +3.94)	**-7.90 -13.66. -2.16) p=0.007**	0.07
ApoA1 g/l	+3.67 (-3.51, +10.84)	-7.70 (-16.04, +0.69)	**0.046**
Atherogenic risk ratios			
Chol:HDL	-1.11 (-3.88, +1.66)	**+4.69 (+1.48, +7.90) p=0.005**	**0.008**
LDL:HDL	-1.98 (-5.79, +1.81)	**+9.02 (+4.63, +13.41) p<0.0001**	**0.0003**
ApoB:ApoA1	+0.76 (-3.41, +4.94)	**+8.76 (+3.92, +13.61) p=0.0005**	**0.01**

Table 3.8 Mean percentage change from baseline to 4 months (non-steroidal versus steroidal group)

(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

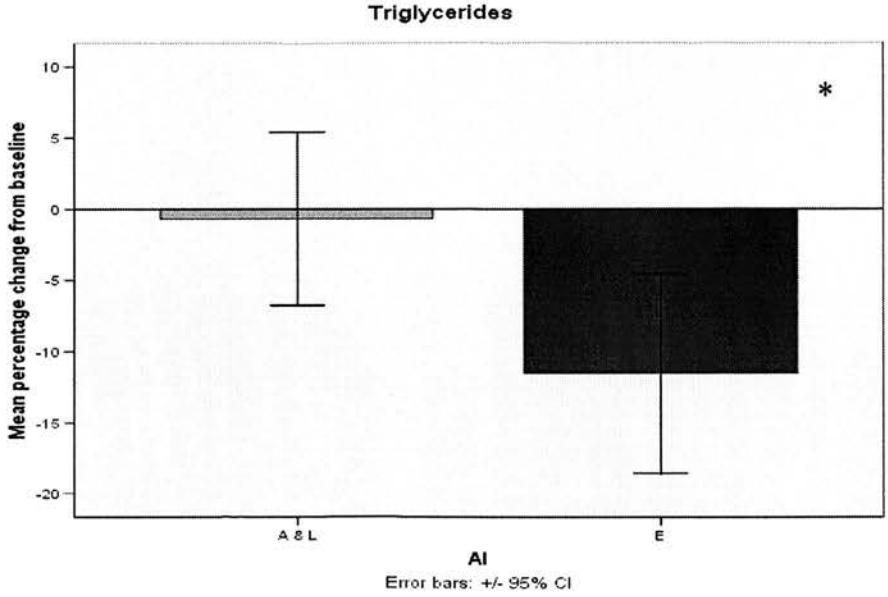


Figure 3.17 Triglyceride results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.02*

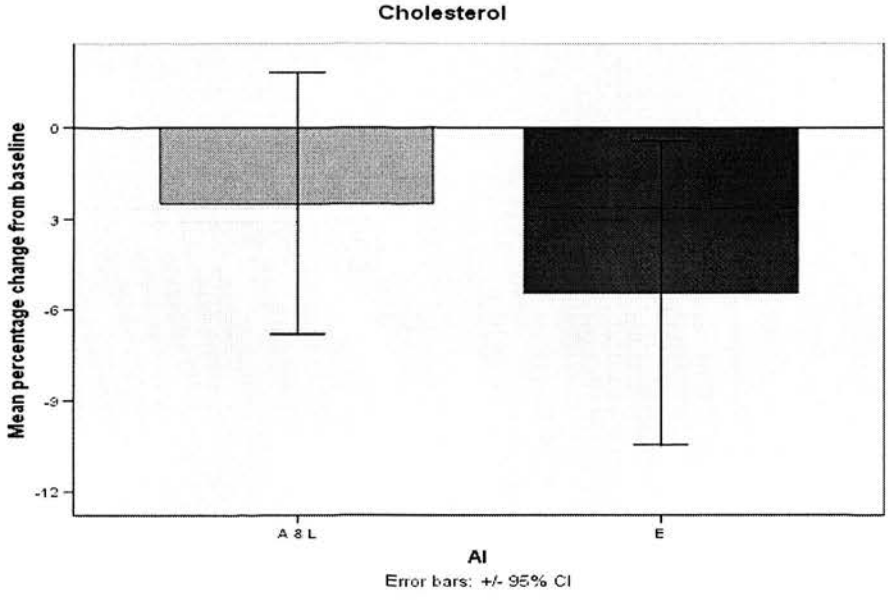


Figure 3.18 Cholesterol lipid results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.38

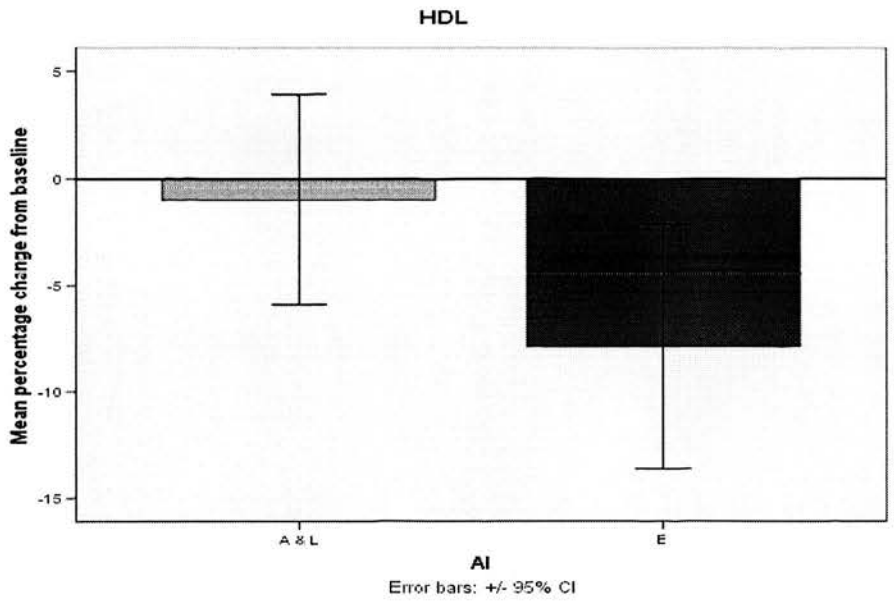


Figure 3.19 HDL lipid results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.07

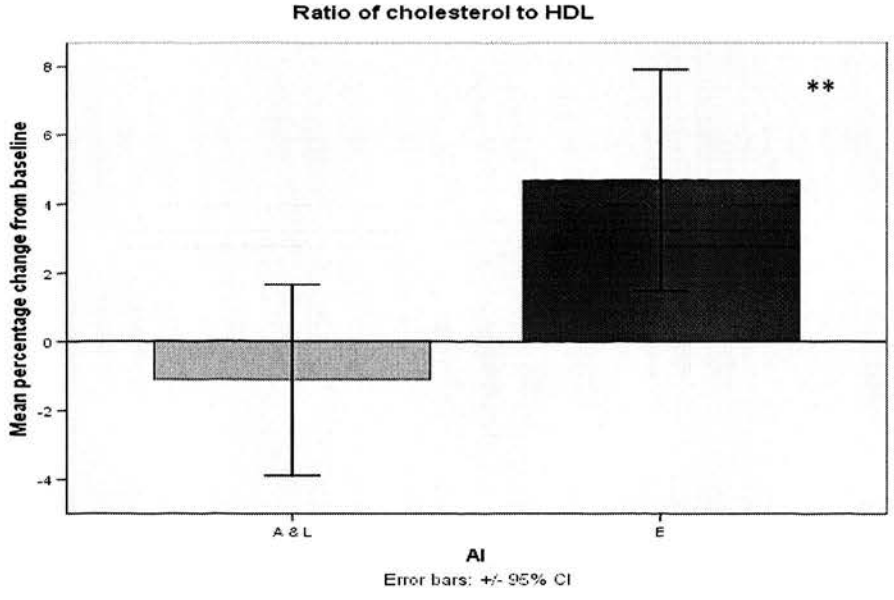


Figure 3.20 Cholesterol:HDL lipid results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.008**

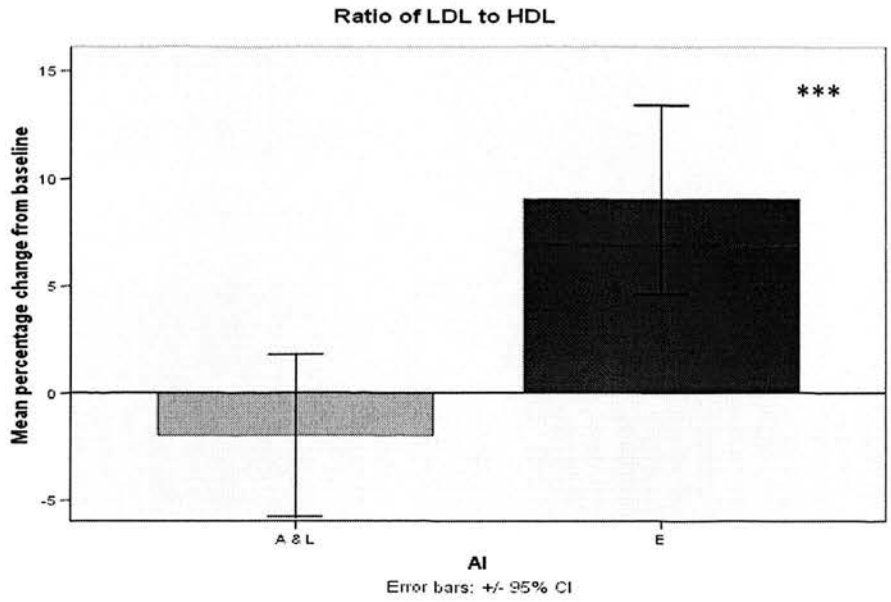


Figure 3.21 LDL:HDL lipid results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.0003***

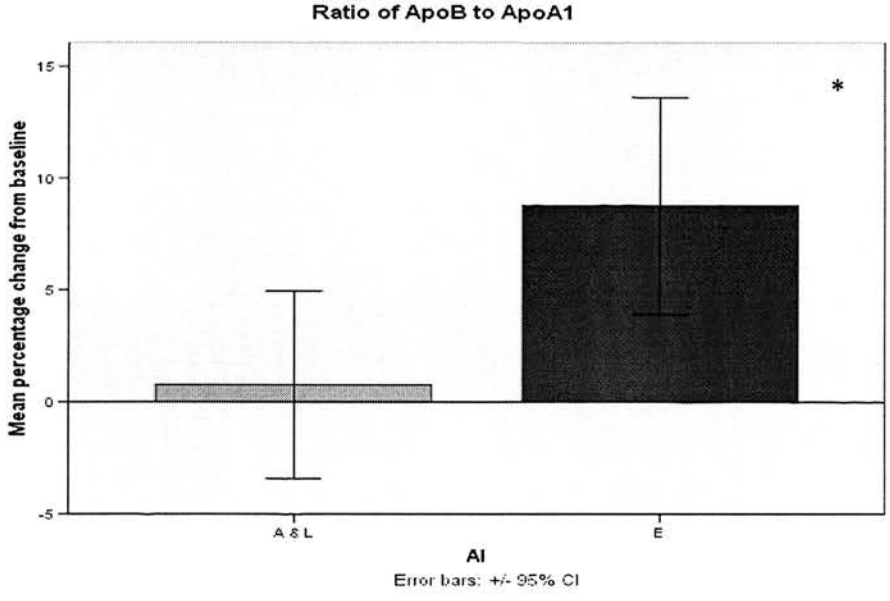


Figure 3.22 ApoB:ApoA-1 lipid results for non-steroidal versus steroidal drugs
Difference between the drug type p=0.01*

3.3.3.5 Effects of tamoxifen

Tamoxifen change from baseline following treatment with AIs

As expected, cholesterol, LDL and chol:HDL, LDL:HDL, ApoB:ApoA-1 ratios were significantly lower in each group following introduction of tamoxifen. There is no evidence suggesting that the change from baseline following treatment with tamoxifen is influenced by the type of AI that the patient was on prior to tamoxifen.

Lipid changes caused by tamoxifen use (after previous AI therapy)

Tamoxifen increased HDL by a statistically significant amount following exemestane +15.52% p=0.01 compared with anastrozole or letrozole (+0.59, +1.89%) p=0.009 as shown in table 3.9. This may reflect the significant decrease in HDL caused by the introduction of exemestane presumably because exemestane was producing greater changes from baseline. Some of the effect is therefore exemestane withdrawal.

	Drug: mean percentage change (95% CI)			
	Anastrozole	Letrozole	Exemestane	p-value
Atherogenic lipids /lipoproteins				
Triglycerides mmol/l	9.56 (-3.94, 23.06)	12.27 (-0.33, 24.87)	25.05 (14.01, 36.09)	0.16
Cholesterol mmol/l	-8.69 (-15.71, -1.66)	-2.28 (-8.08, 3.51)	-7.88 (-14.62, -1.14)	0.30
LDL mmol/l	-14.31 (-22.85, -5.77)	-12.58 (-20.68, -4.48)	-10.72 (-17.69, -3.75)	0.80
Apo B g/l	-8.40 (-17.69, 0.90)	-6.89 (-15.69, 1.90)	1.93 (-5.75, 9.60)	0.18
Atheroprotective lipids/lipoproteins				
HDL mmol/l	0.59 (-8.03, 9.21)	1.89 (-6.38, 10.16)	**15.52 (8.39, 22.66) p=0.01**	**0.009**
ApoA1 g/l	5.41 (-7.71, 18.54)	12.06 (-0.60, 24.72)	25.09 (14.05, 36.13)	0.07
Atherogenic risk ratios				
Chol:HDL	-7.57 (-13.09, -2.05)	-9.62 (-18.42, -9.39)	-13.90 (-14.82, -4.41)	0.19
LDL:HDL	-12.33 (-19.89, -4.77)	-14.46 (-27.64,15.27)	-21.45 (-21.58, -7.33)	0.14
ApoB:ApoA1	-7.68 (-15.70, 0.34)	-11.56 (-19.14, -3.98)	-17.93 (-24.49,-11.37)	0.13

Table 3.9 Lipid changes caused by tamoxifen use (after previous AI therapy)

(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

3.3.3.6 ALEX Lipid
Discussion and conclusions

Several large clinical AI trials have demonstrated a small but significant increase in cardiovascular events compared with tamoxifen[64,65,66]. Data from BIG 1-98 demonstrated an increased incidence of hypercholesterolaemia and cardiac events in postmenopausal women taking letrozole, compared with tamoxifen[286]. However these adverse events were rare and were outweighed by the superior control of recurrence afforded by letrozole compared with tamoxifen[286]. These results are shown in table 1.8 and 1.9. Exemestane has a minor androgenic effect due to significant suppression of the sex-hormone-binding globulin. This may have a protective effect on lipids, especially triglycerides. It causes an increase in gonadotrophins[287].

The impact of the third generation AIs on lipid metabolism and subsequent cardiovascular damage has been difficult to compare in previous phase 3 trials as most collaborators have chosen the alternative treatment, tamoxifen as the comparator. To date there are no published studies comparing the effects of adjuvant anastrozole, letrozole and exemestane on serum lipid profiles in postmenopausal women with early breast cancer.

Our results demonstrated significant differences between the steroidal and non-steroidal AIs. Exemestane caused a significant fall and hence beneficial effect on serum triglycerides and cholesterol. These changes were also observed in the EORTC trial which demonstrated that exemestane and tamoxifen had opposite effects on triglyceride levels: exemestane lowered while tamoxifen increased triglyceride levels over time[287]. The mechanism for this is unclear but oestrogen appears to have a negative effect on triglycerides which are a major risk factor for coronary artery disease and are a greater risk factor in women compared with men[288].

Of interest exemestane had a negative effect on the atheroprotective lipoproteins HDL and ApoA-1 which resulted in an unfavourable increase in each of the three risk ratios chol:HDL, LDL/HDL and ApoB:ApoA-1. Exemestane is an irreversible inhibitor of the aromatase enzyme. It is a steroidal compound which mimics the natural substrate of aromatase, androstenione and permanently inactivates aromatase, unlike letrozole and anastrozole. Its androgenic effects have been considered of possible positive value. Many tissues including the breast have androgen receptors. Its degree of androgenicity is unclear but it is likely that some of its effects and particularly the effects not seen with letrozole and anastrozole are in relation to its androgenicity. The different effects of exemestane may therefore be due to its irreversible nature and also to its androgenic effects which make it different from the non-steroidal AIs.

One major limitation of this study is that the patients recruited had cancer and potentially other conditions rather than well patients with no other conditions such as those included in the LEAP study. Other limitations include the relatively small number of subjects. In addition, there was no control/placebo group therefore we are unable to state that the changes detected were definitely a result of tamoxifen/AI use. The groups were not aged-matched however there was no significant difference in ages in each group. Compliance may also have been a limitation as there is no way of knowing whether the patients adhered strictly to the regimen. Both studies would have benefitted from having age-matched and cancer-free control groups.

Previous studies demonstrated that letrozole significantly increased the amount of plasma total cholesterol, cLDL, apolipoprotein B and the atherogenic risk ratios total cholesterol:HDL and LDL:HDL[259] and therefore had an unfavourable effect on serum lipid profiles. Other studies have suggested that AIs do not have adverse effects on lipid metabolism. The MA.17 lipid sub-study found that five years of

letrozole did not alter serum cholesterol, HDL, LDL, or triglycerides[266]. The steroidal AI exemestane has previously demonstrated stabilising effects on triglycerides and HDL[287]. It has been suggested that exemestane may have beneficial effects on lipid metabolism due to its slight androgen-agonistic effect of its major metabolite[289].

Results from other studies assessing lipid parameters have been variable. Large studies investigating the non-steroidal AIs have been discussed in depth on page 179 (ALIQUOT lipids discussion). The non-significant but overall beneficial effects of anastrozole on lipids have been previously demonstrated[290]. LEAP demonstrated no significant differences between anastrozole and letrozole and their effects on LDL:HDL ratios, triglycerides and non-HDL concentrations however exemestane significantly decreased total cholesterol at 3 months (-5.5%) in healthy postmenopausal women. Exemestane also significantly increased the LDL:HDL cholesterol ratio (+17.0% at 6 months) and ApoB:ApoA-1 ratio (+9.0% at 6 months)[247]. These results are consistent with the findings of our study. IES randomised patients to exemestane or tamoxifen after 2-3 years of tamoxifen. A higher rate of myocardial infarction was reported with exemestane compared with tamoxifen but these differences were not statistically significant (1.3 vs. 0.8%)[291]. Results from the TEAM study demonstrated that there was a higher incidence of hyperlipidaemia in the exemestane group (5%) compared with the sequential therapy group (tamoxifen followed by exemestane <1%). In addition, there was a higher incidence of cardiac failure in the exemestane group (1%) versus the sequential group (<1%). The TEAM Japan substudy demonstrated that anastrozole and exemestane had no clinically significant effects on the lipidaemic profile of postmenopausal women with breast cancer although women treated with tamoxifen resulted in relatively favourable changes[292].

The MA-17 study demonstrated different safety profile between anastrozole verus exemestane. There were fewer reports of dyslipidaemia in the group taking exemestane. Elevated triglyceride levels were demonstrated in only 2% of the exemestane group versus 3% of the anastrozole group. Hypercholesterolaemia was found in 15% of those taking exemestane versus 18% taking anastrozole[197].

Tamoxifen has oestrogen-like properties and is known to favourably affect lipid profiles. The changes in lipids from baseline were expected, tamoxifen caused a fall in the atherogenic ratios however the class of AI administered prior to tamoxifen did not affect the changes. Exemestane followed by tamoxifen therapy caused a significant increase in HDL when the change was measured from exemestane withdrawal. This can be explained by the withdrawal of exemestane.

In conclusion, our results are largely consistent with those of previous studies, suggesting that the two classes of AIs have different effects of lipid metabolism. Exemestane treatment appears to cause a beneficial fall in triglycerides and cholesterol however, perhaps more importantly, exemestane was associated with a detrimental fall in the atheroprotective lipoproteins and an increase in atherogenic ratios. The results also confirm the favourable although statistically insignificant effects of anastrozole on serum lipids. Further long-term follow-up is warranted to establish if these effects are of clinical significance in the management of women with breast cancer. Current studies investigating the effects of AIs on lipid profiles are underpowered and only collection of lots of data will discover any long-term harmful effects. If an AI is recommended then exemestane may be a better choice for postmenopausal women with lipid abnormalities. Otherwise, tamoxifen may better for patients at increased risk of cardiovascular events.

3.3.4 ALEX Coagulation

3.3.4.1 Patients

One hundred and twenty postmenopausal women with ER+ve early breast cancer were enrolled in this study. Of these, 116 patients had evaluable blood samples. Patient disposition is shown in the CONSORT diagram in appendix H. Overall, patient demographics were well balanced between the three patient groups as seen in table 3.10.

	Anastrozole n=34	**Letrozole** n=34	**Exemestane** n=49
Age yrs (median)	61.0	59.9	60.6
Height cm (median)	162.5	161.0	162.0
Weight kg (median)	66.0	67.5	66.5
BMI kg/m^2 (median)	25.3	26.4	25.7
History of previous HRT use *n (%)*	20 (59%)	13 (38%)	22 (45%)
Hysterectomy *n (%)*	4 (12%)	4 (12%)	10 (20%)
Bilateral oophorectomy *n (%)*	1 (3%)	1 (3%)	4 (8%)

Table 3.10 Patient baseline demographics

3.3.4.2 Statistical analysis

The statistical analysis was conducted by an independent statistician. Hormone therapy for each patient was coded to maintain the blind assessment and avoid bias. Data was analysed using SPSS version 12. Analysis of variables was performed using the method of analysis of covariance. Some variables were transformed to achieve Normality, either the log transformation or the square root was taken. Tukey's correction was used to adjust for multiple comparisons.

3.3.4.3 Differences between anastrozole, letrozole and exemestane

The effects of AI treatments on coagulation parameters are shown in table 3.11. For most parameters there was little evidence to suggest significant drug effects on coagulation variables. However exemestane significantly decreased AT from baseline (-8.55% (-13.33, -3.79)), p=0.0006 whereas anastrozole and letrozole had no effect. There was no significant difference between the drugs, p=0.4. Exemestane significantly decreased protein C levels compared to anastrozole and letrozole (15.75% (-21.48, -10.02) vs -3.80% (-10.77, 3.16) versus -3.63% (-10.50, 3.25)), p=0.008. Exemestane and anastrozole significantly increased protein S free levels from baseline (E 6.90% (1.98, 11.82), A 7.36% (1.37, 13.34), p=0.007. There was no significant difference between the drugs, p=0.69. These results are demonstrated in figures 3.23–3.35.

	Drug: Mean percentage change (95% CI)			
	Anastrozole n=33	Letrozole n=46	Exemestane n=31	p-value
Pro-coagulants				
PAI (log)	-0.66 (-5.60, 4.27)	3.46 (-1.42, 8.33)	1.43 (-2.63, 5.48)	0.50
vWF (sqrt) iu/ml	6.48 (-1.84, 14.80)	6.56 (-1.77, 14.88)	5.02 (-1.91, 11.95)	0.95
Factor VIII Iu/ml	-2.96 (-16.38, 10.45)	11.88 (-1.34, 25.11)	9.54 (-1.58, 20.66)	0.24
Fibrinogen g/l	3.91 (-7.46, 15.29)	3.19 (-7.99, 14.37)	1.62 (-7.72, 10.96)	0.95
APCR	6.41 (-2.19, 15.01)	0.38 (-7.10, 7.86)	-2.02 (-8.25, 4.19)	0.30
Anti-coagulant				
AT iu/ml	-3.48 (-9.31, 2.35)	-5.60 (-11.43, 0.23)	**-8.55 (-13.33, -3.79) p=0.0006**	0.40
Protein C iu/ml	-3.80 (-10.77, 3.16)	-3.63 (-10.50, 3.25)	**-15.75 (-21.48, -10.02) p<0.0001**	**0.008**
Protein S total iu/ml	-2.75 (-11.91, 6.40)	5.58 (-4.37, 15.52)	2.45 (-4.30, 9.20),	0.43
Protein S free^{sqrt} iu/ml	**7.36 (1.37, 13.34) p=0.02**	4.04 (-1.95, 10.03)	**6.90 (1.98, 11.82) p=0.007**	0.69

Table 3.11 Anastrozole versus letrozole versus exemestane
Mean percentage change from baseline to 4 months
(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

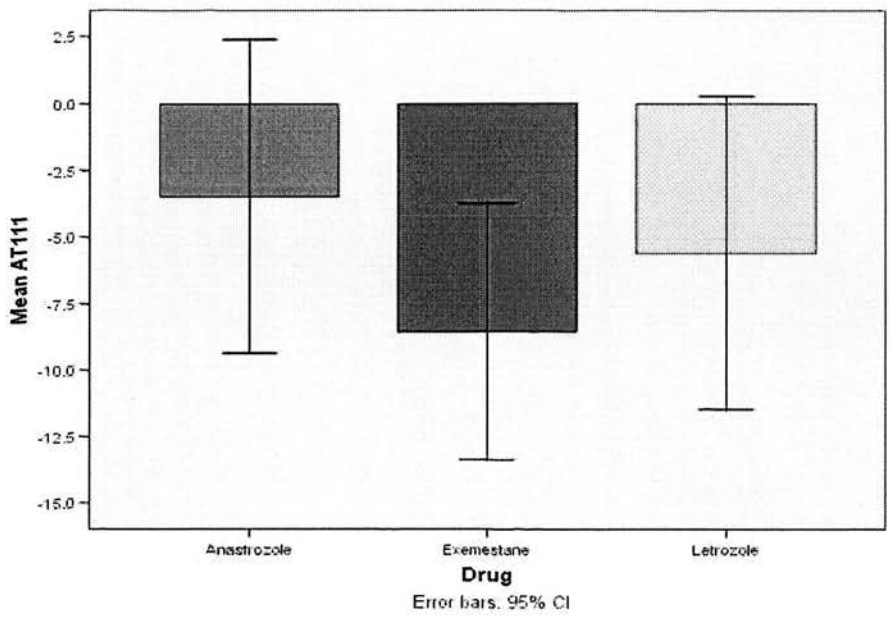


Figure 3.23 AT mean percentage change from baseline by drug
No statistically significant difference detected between each drug p=0.40

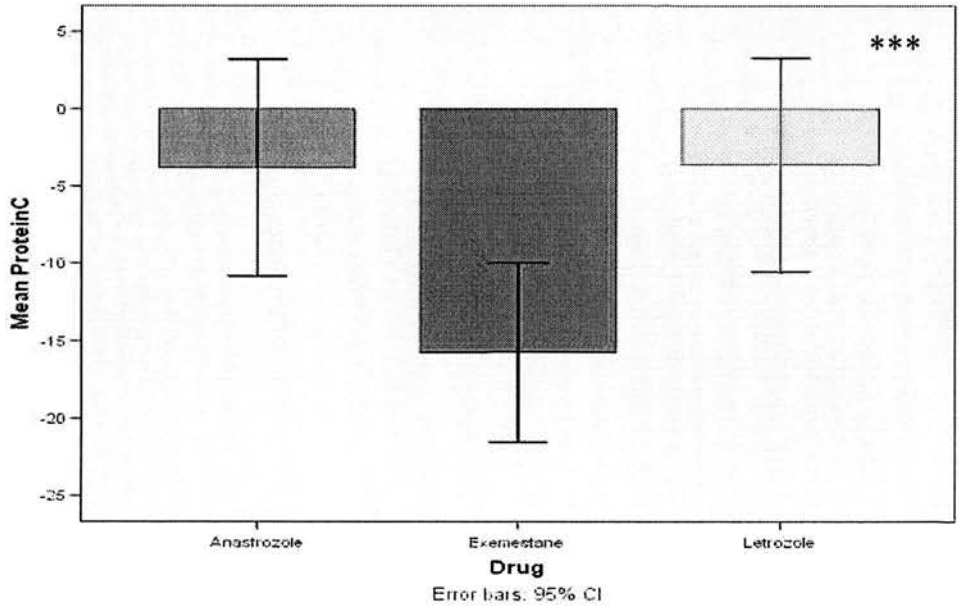


Figure 3.24 Protein C mean percentage change from baseline by drug
Statistically significant difference detected between exemestane and the other two AIs p=0.0008***

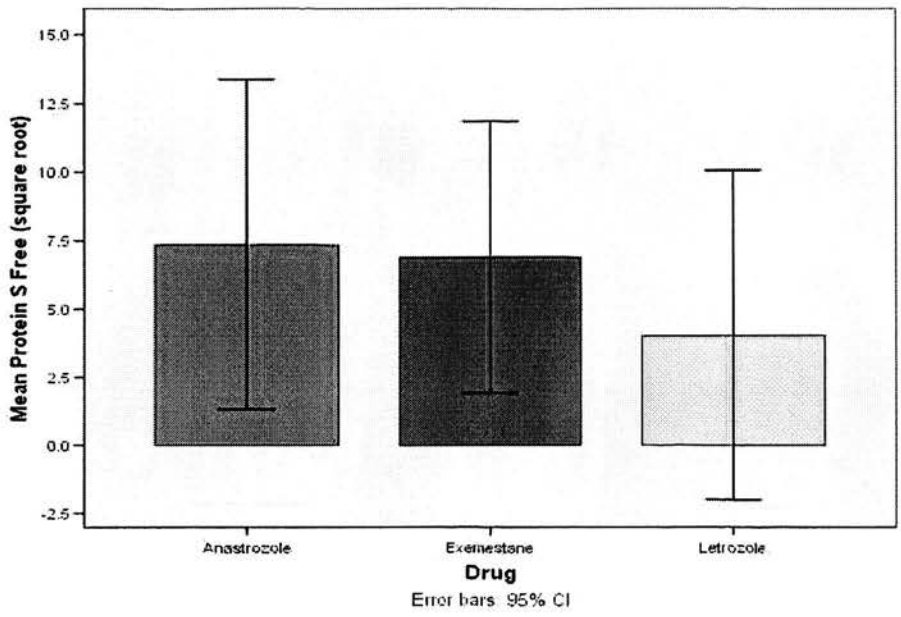


Figure 3.25 Protein S free mean percentage change from baseline by drug
No statistically significant difference detected between each drug p=0.69

3.3.4.4 Differences between non-steroidal and steroidal AIs

The effects of steroidal versus non-steroidal AIs on coagulation parameters are shown in table 3.12 and figures 3.26–3.29. Anastrozole and letrozole combined significantly increased vWF from baseline (6.52% (0.66, 12.38), however this increase in vWF was not statistically different from exemestane (5.02% (-1.88, 11.91)), p=0.74.

AT decreased significantly from baseline with anastrozole and letrozole combined (-4.54% (8.64, -0.43)), p=0.03 and also with exemestane (-8.55% (-13.31, -3.70), p=0.0006 however there was no significant difference between the groups of drugs, p=0.21.

Protein C levels again fell significantly with exemestane compared with anastrozole and letrozole combined (-15.75% (-21.45, -10.05) vs -3.71% (-8.58, 1.15)), p=0.002.

There was a significant increase in protein S free from baseline in both groups, anastrozole plus letrozole (5.70% (1.47, 9.92)), p=0.009 and with exemestane (6.90% (1.98, 11.81)), p=0.006 however the difference between the groups was not significant p=0.72.

	Drug: Mean percentage change (95% CI)		
	Non-steroidal AIs (anastrozole + letrozole)	Steroidal AI (exemestane)	p-value
Pro-coagulants	n=64	N=31	
PAI (log)	1.42 (-2.05, 4.89)	1.43 (-2.63, 5.48)	0.99
vWF (sqrt) iu/ml	**6.52 (0.66, 12.38) p=0.03**	5.02 (-1.91, 11.95)	0.74
Factor VIII iu/ml	4.57 (-4.96, 14.10)	9.54 (-1.58, 20.66)	0.51
Fibrinogen g/l	3.55 (-4.39, 11.47)	1.62 (-7.72, 10.96)	0.75
APCR	2.97 (-2.68, 8.63)	-2.02 (-8.25, 4.19)	0.25
Anti-coagulant			
AT iu/ml	**-4.54 (-8.64, -0.43) p=0.03**	**-8.55 (-13.33, -3.79) p=0.0006**	0.21
Protein C iu/ml	-3.71 (-8.58, 1.15)	**-15.75 (-21.48, -10.02) p<0.0001**	**0.002**
Protein S total iu/ml	1.07 (-5.76, 7.89)	2.45 (-4.30, 9.20),	0.77
Protein S free^{sqrt} iu/ml	**5.70 (1.47, 9.92) p=0.009**	**6.90 (1.98, 11.82) p=0.006**	0.72

Table 3.12 Non-steroidal versus steroidal AIs

Percentage change from baseline to 4 months

(results are presented as percentage change from baseline for each group, figures in bold reflect statistical significance)

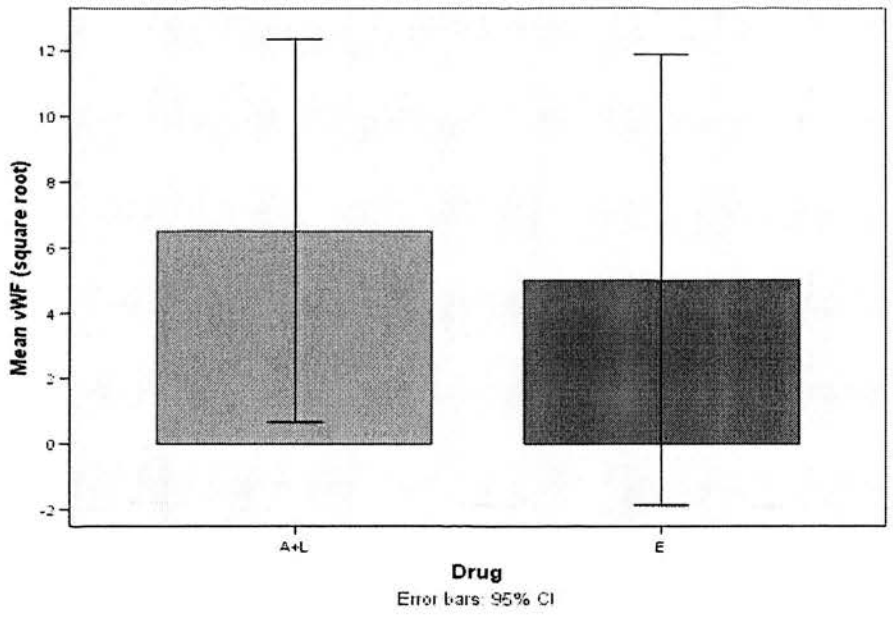


Figure 3.26 vWF mean percentage change from baseline
Non-steroidal versus steroidal drugs
No statistically significant difference detected between each group of AI p=0.74

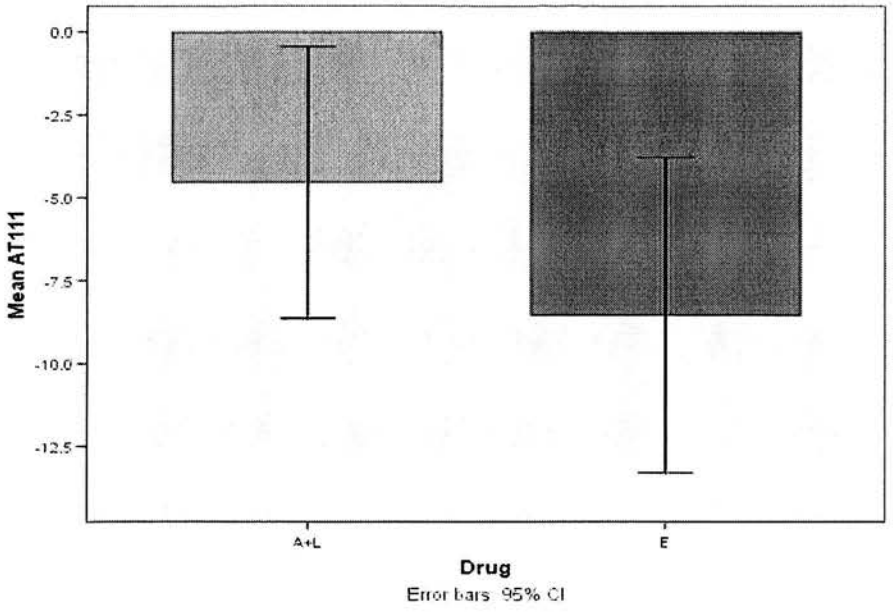


Figure 3.27 AT mean percentage change from baseline
Non-steroidal versus steroidal drugs
No statistically significant difference detected between each group of AI p=0.21

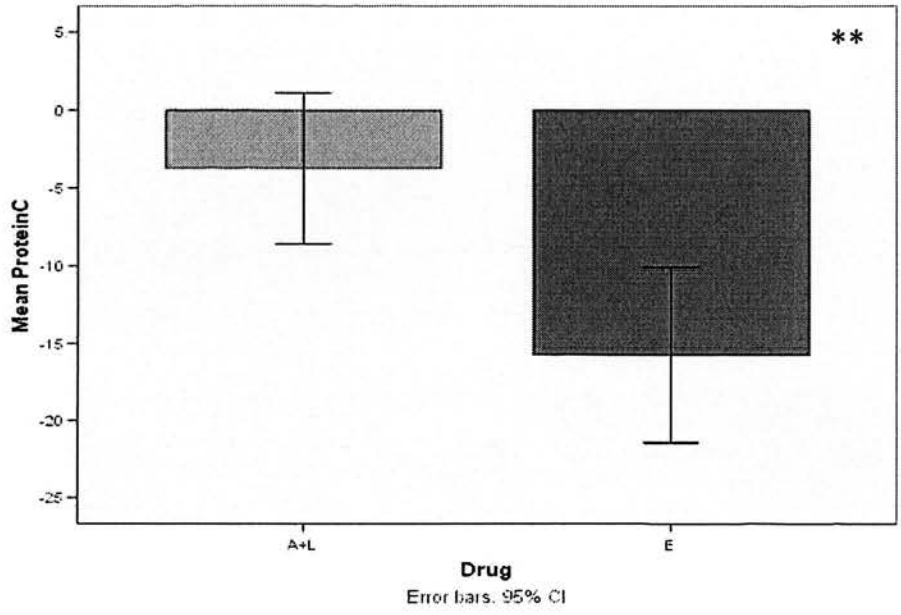


Figure 3.28 Protein C mean percentage change from baseline Non-steroidal versus steroidal drugs
Statistically significant difference detected between each group of AI p=0.002**

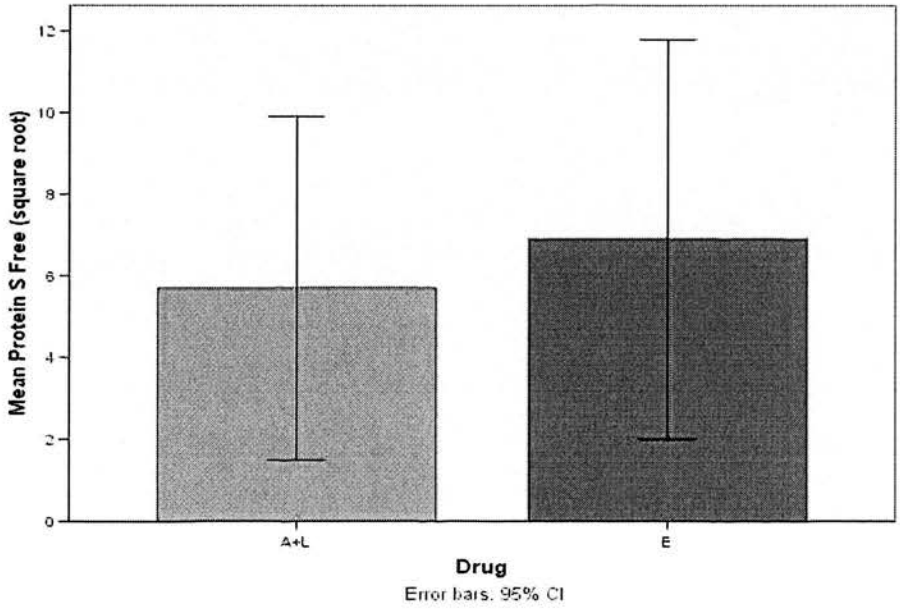


Figure 3.29 Protein S free mean percentage change from baseline Non-steroidal versus steroidal drugs
No statistically significant difference detected between each group of AI p=0.72

3.3.4.5 Effects of tamoxifen

Effects of 8 months of tamoxifen post AIs from baseline

The effects of tamoxifen on coagulation parameters after treatment with AIs are shown in table 3.13. Tamoxifen treatment given after anastrozole did cause a significant increase in vWF (10.05 (1.68, 18.41)) but this increase following anastrozole was not different following either of the other two drugs. Fibrinogen, AT, protein C and protein S free all showed significant changes from baseline regardless of which drug the patient was taking during the initial trial phase. Significant falls in fibrinogen (A-16.89, L-23.29, E-18.73) ($p<0.0001$) and AT levels were seen in each group (A-12.26, L-19.59, E-16.89) ($p=0.0001$). Tamoxifen also increased protein S free levels in each group (A 10.75, L 10.85, E 6.15) ($p= 0.0003$). There was no evidence to suggest that any particular AI had any influence on the post tamoxifen effects.

	Drug: mean percentage change (95% CI)			
	Anastrozole n=33	Letrozole n=46	Exemestane n=31	p-value
Pro-coagulants				
PAI (log)	-3.49 (-8.98, 2.01)	-4.47 (-9.47, 0.52)	-0.48 (-5.91, 4.95)	0.55
vWF (sqrt) iu/ml	**10.05** **(1.68, 18.41)** **p=0.02**	6.61 (-1.16, 14.37)	7.4 (-0.95, 15.81)	0.83
Factor V111 iu/ml	3.69 (-15.74, 23.13)	8.49 (-9.45, 26.43)	13.36 (-5.78, 32.52)	0.78
Fibrinogen g/l	**-16.89** **(-26.77, -7.02)** **p<0.001**	**-23.29** **(-32.25, -14.33)** **p<0.0001**	**-18.73** **(-28.43, -9.03)** **p<0.0002**	0.61
APCR	0.28 (-6.80, 7.35)	-0.29 (-6.48, 5.89)	-1.28 (-8.06, 5.49)	0.95
Anti-coagulants				
AT iu/ml	**-12.26** **(-18.63, -5.89)** **p=0.0001**	**-19.59** **(-25.37, -13.81)** **p=0.0001**	**-16.89** **(-23.26, 10.52)** **p=0.0001**	0.24
Protein C iu/ml	**-12.34** **(-20.73, -3.96)** **p=0.004**	**-20.77** **(-28.36, -13.17)** **p<0.0001**	**-10.81** **(-19.05, -2.56)** **p=0.01**	0.16
Protein S Total iu/ml	-11.48 (-25.49, 2.53)	-3.55 (-13.52, 6.40)	4.15 (-11.53, 19.82)	0.32
Protein S Free (sqrt) iu/ml	**10.75** **(5.51,15.99)** **p=0.0003**	**10.85** **(6.10, 15.61)** **p=0.0003**	**6.15** **(0.91, 11.39)** **p=0.0003**	0.35

Table 3.13 Effects of 8 months of tamoxifen post AIs from baseline
(results are presented as percentage change for each group, figures in bold reflect statistical significance)

Effects of 8 months of tamoxifen post AIs from 4 months

Tamoxifen significantly decreased fibrinogen levels after all three AIs. Tamoxifen also caused a significant reduction in AT following both exemestane and letrozole. There was no evidence to suggest that non-steroidal or steroidal AIs had any influence on results when patients were taking tamoxifen (p=0.53, p=0.27 respectively). Tamoxifen significantly increased protein S free levels following all three AIs, with no evidence to suggest the drug type had any influence. These results are demonstrated in figures 3.30 – 3.32.

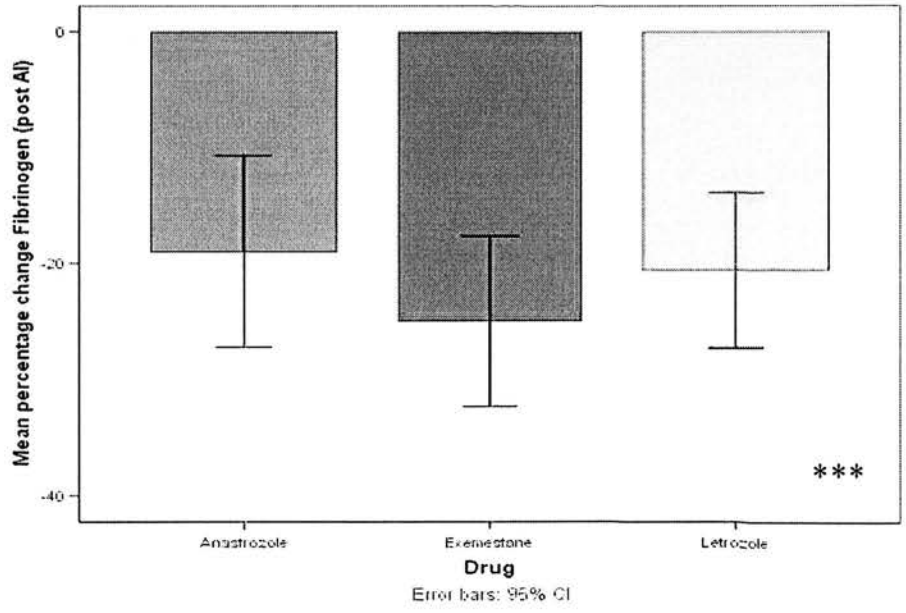


Figure 3.30 Fibrinogen mean percentage change post AI followed by tamoxifen
Statistically significant difference detected between the three AIs p<0.001***

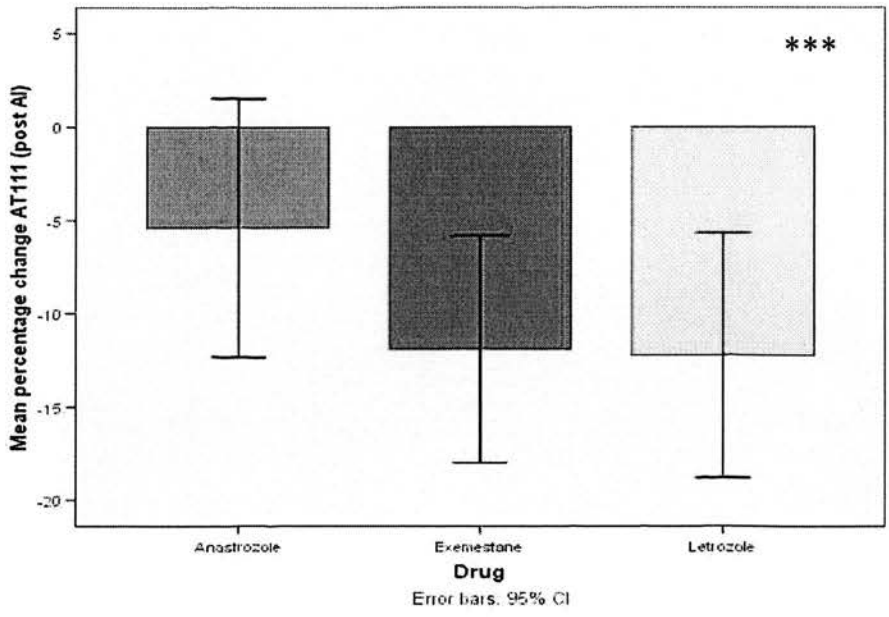


Figure 3.31 AT mean percentage change post AI treatment followed by tamoxifen

Statistically significant difference detected between the three AIs $p<0.001$***

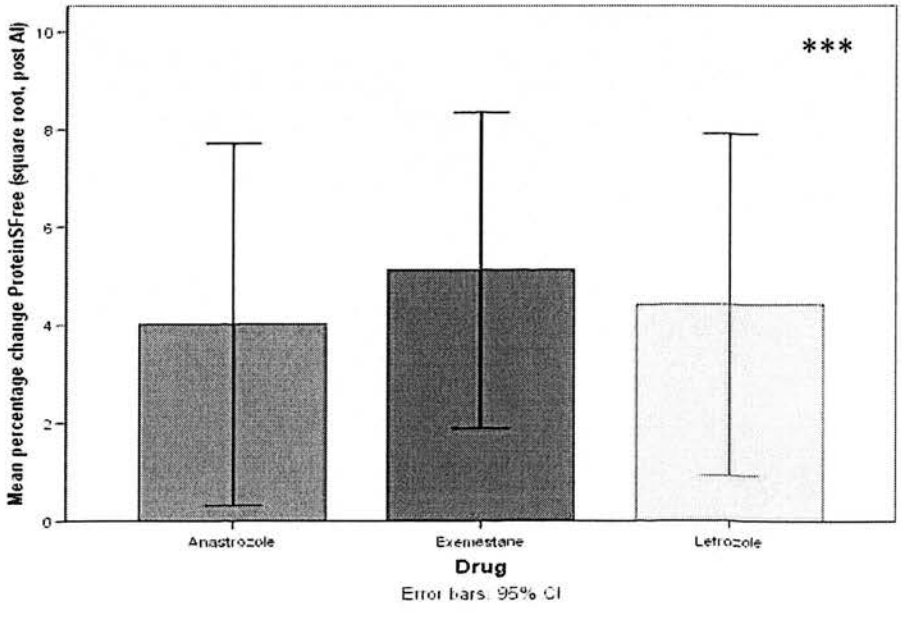


Figure 3.32 Protein S free mean percentage change post AI followed by tamoxifen

Statistically significant difference detected between the three AIs $p<0.001$***

3.3.4.6 ALEX Coagulation Discussion and Conclusion

Oestrogen has detrimental effects on coagulation and tamoxifen is associated with an increased risk of thromboembolism. AIs significantly reduce the amount of circulating oestrogen and in theory should have either no significant effects on coagulation or should inhibit coagulation pathways. The impact of third generation AIs on coagulation have not been directly compared in previous trials which generally compared each AI with tamoxifen.

This study demonstrated a significant fall in the anticoagulants AT and protein C in patients given the steroidal inactivator exemestane compared to anastrozole and letrozole. Exemestane also caused a significantly greater increase in the anticoagulant protein S free level. There was no significant change in coagulation factors detected with letrozole and anastrozole and no difference between these two drugs. Exemestane is an irreversible inhibitor of the aromatase enzyme. It is a steroidal compound which mimics the natural substrate of aromatase, androstenione and permanently inactivates aromatase, unlike letrozole and anastrozole. Its androgenic effects have been considered of possible positive value. Many tissues including the breast and bone have androgen receptors. Its degree of androgenicity is unclear but it is likely that some of its effects and particularly the effects not seen with letrozole and anastrozole are in relation to its androgenicity. The different effects of exemestane may therefore be due to its irreversible nature and also to its androgenic effects which make it different from the non-steroidal AIs.

One major limitation of this study is that the patients recruited had cancer and potentially other conditions rather than well patients with no other conditions such as those included in the LEAP study. Other limitations include the relatively small number of subjects. In addition, there was no control/placebo group therefore we are

unable to state that the changes detected were definitely a result of tamoxifen/AI use. The groups were not aged-matched however there was no significant difference in ages in each group. Compliance may also have been a limitation as there is no way of knowing whether the patients adhered strictly to the regimen. Both studies would have benefitted from having age-matched and cancer-free control groups.

Overall AIs caused a minor increase in the procoagulants and a fall in the most clinically relevant anticoagulants predisposing to potential thrombosis. These effects may be more concerning in those with advanced breast cancer where there is increased tumour burden and a higher secretion of procoagulants including tissue factors which activates coagulation[293].

Anastrozole trials

The 68 month follow-up data from the ATAC study demonstrated that adjuvant treatment with anastrozole monotherapy reduced the incidence of VTEs by 39% when compared with those taking tamoxifen ($p<0.0001$)[294]. Results demonstrated a 2.8% incidence of VTEs and 1.6% incidence of deep venous thromboembolic events in women taking anastrozole which is greater that the estimated risk in healthy women[176,295]. The combined analysis of ABCSG 8 (Austrian Breast and Colorectal Cancer Study Group) and German ARNO9 5 (Arimidex-Nolvadex) trials (total n=3224) showed that women taking anastrozole were significantly less likely to develop thromboses compared with those treated with tamoxifen. Only three patients taking anastrozole developed thromboses compared with 12 patients in the tamoxifen arm after 28 months of follow-up, $p=0.034$. In addition there was a trend suggesting fewer emboli; only two patients affected versus nine in the tamoxifen group ($p=0.064$)[199].

The ITA trial (n=448) reported a 6.0% incidence of venous disorders in the tamoxifen group versus a 2.5% incidence in the anastrozole group[198].

Of interest, anastrozole was found to have no effect on coagulation factors after eleven days of treatment in a small population of healthy males (n=16). This may be due to the short duration of treatment and follow-up[296]. Our results are consistent with this finding, suggesting no major coagulation effects resulting from anastrozole.

Letrozole trials

The BIG 1-98 trial (n=8028) demonstrated a decreased rate of thromboembolic events in the letrozole versus tamoxifen group. Those taking letrozole had a 1.5% incidence of adverse thromboembolic events compared with 3.5% in those taking tamoxifen ($p<0.001$)[195]. An updated safety analysis concluded that the risk of grade 1-5 thromboembolic adverse events in those taking letrozole was 0.9% compared with 2.3% in the tamoxifen arm ($p<0.01$) and the risk of overall cardiovascular adverse events (grade 1-5) was 1.7% versus 3.9% in the respective arms ($p<0.001$)[297].

The MA-17 trial demonstrated that after 30 months of follow-up there was no significant difference between letrozole and placebo in terms of thromboembolic events. The rate of thromboembolism was 0.4% in the letrozole group versus 0.2% in the placebo group[202]. These results are in keeping with our data which suggests that letrozole has little effect on coagulation factors.

Exemestane trials

The IES study (n=4724) randomised women to exemestane alone or following tamoxifen. The incidence of venous thrombembolic events was only 1.2% in patients receiving exemestane compared with 2.4% in the tamoxifen group ($p=0.004$)[298].

Side-effects from exemestane were rare and some may have been due to tamoxifen withdrawal[207].

The TEAM trial (n=9779) randomised women to exemestane alone or following tamoxifen for five years. The sequential treatment arm was associated with a higher incidence of venous thrombosis (2%) when compared with the exemestane alone (1%) (p=<0.0001)[196]. Both IES and TEAM demonstrated that exemestane was associated with a significantly lower incidence of thromboembolic events compared with tamoxifen.

The EORTC 10951 trial (n=382) randomised women with metastatic breast cancer to either exemestane or tamoxifen. The incidence of deep vein thromboses was similar in each group[299]. A smaller study (n=147) demonstrated that exemestane had no effect on plasma coagulation factors in patients with early breast cancer compared with placebo[300].

Our study has demonstrated significant differences between non-steroidal and steroidal AIs. Exemestane caused a significant fall in the anticoagulants protein C and AT, predisposing to a potential increased risk of thromboembolic disease. Non-steroidal AIs caused a significant increase in vWF which is associated with a potential increased risk of thrombosis. Previous studies have suggested that exemestane does not appear to have long-term effects on coagulation parameters[172] and therefore AI induced coagulation changes are unlikely to persist after the drug is discontinued. The effects of tamoxifen on thromboembolism are mediated via a reduction in known anticoagulants. Exemestane reduces significantly these same anticoagulants and may also have greater pro-thrombotic properties compared with the non-steroidal AIs. Non-steroidal AIs can therefore be expected to cause less thromboembolic adverse events.

Tamoxifen

The oestrogenic effects of tamoxifen are associated with an increased risk of thromboembolic events including DVT, stroke and PE[301]. It has complex and significant effects on coagulation factors.

The IBIS-1(International Breast Cancer Intervention Study-1) found that five years of tamoxifen in women at higher risk of developing breast cancer resulted in an increased risk of thromboembolism compared with placebo[302].

Our results demonstrated significant changes in coagulation factors consistent with increased risk of DVT and PE with tamoxifen. Tamoxifen therapy administered after an AI caused a significant fall in fibrinogen, AT and protein C. There was no evidence to suggest that the AIs had any effect on fibrinogen levels pre-tamoxifen. Tamoxifen caused an increase in protein S free however this showed statistical significance in the anastrozole group only. Overall, the type of AI therapy administered prior to tamoxifen had little effect.

This study is the first to directly compare the effects of adjuvant anastrozole, letrozole and exemestane on serum coagulation parameters in postmenopausal women with early breast cancer. There were significant differences demonstrated between non-steroidal and steroidal AIs and their effects on important coagulation parameters. Exemestane caused a reduction in important anticoagulants, potentially predisposing to an increased risk of thromboembolism. These differences were not reflected in the results of the MA.27 trial (n= 7,576) which is the first large study to directly compare anastrozole with exemestane. Cardiovascular effects were similar in both groups and were infrequent[197]. Our study showed no differences between anastrozole and letrozole, and generally these drugs have little thrombotic activity.

AIs are associated with a reduced incidence of thromboembolic events compared with tamoxifen and are therefore better suited for use in high risk groups who have risk factors for thromboembolism. In conclusion, tamoxifen and AIs have significant effects on coagulation which might help explain their clinical effects on thrombotic and thromboembolic disease.

SECTION 4: SUMMARY

Incorporation of an AI improves disease-free survival in postmenopausal women with hormone receptor positive breast cancer compared with tamoxifen however, for some women, the risks and side effects outweigh the potential benefits. AIs cause a profound decrease in circulating oestrogen levels causing a variety of systemic effects. These small studies revealed interesting and in some areas, unexpected results. The most interesting finding was the effects prior tamoxifen use had on bone turnover and the effects of adding a subsequent AI. Previous studies suggested that prior tamoxifen use may have beneficial bone preserving effects which may reduce increased bone turnover caused by AIs. Our findings demonstrated the opposite, showing that there appears to be a rebound increase in turnover which is detrimental to overall bone health from prior tamoxifen use. An attempt was made to identify a relationship between bone markers and musculoskeletal symptoms but unfortunately the studies were inadequately powered to demonstrate statistically significant results. Of further interest was the different effects exemestane had on lipid metabolism when compared with the non-steroidal AIs. These studies have demonstrated a decrease in atherogenic ratios and exemestane use suggesting that the androgenic, steroidal AI may be safer for use in women with dyslipidaemias. Finally exemstane also showed a difference in coagulation parameters when compared with the non-steroidal AIs. Our results suggest that it is slightly more thrombogenic compared with letrozole and anastrozole which again may be explained by its androgenic properties. The long-term tolerability and toxicity profiles of AIs are important considerations when trying to encourage compliance and optimise outcomes. An enormous amount of research has already been undertaken on the effects of AI use in breast cancer. Despite this, there are few trials which directly compare the different

types of AI. Future research may demonstrate further information on the effects of increased bone turnover and musculoskeletal symptoms. The value of the current work is that it provides important information on the effects of the different drugs and assists considerably in choosing the most appropriate drug for the patient.

SECTION 5: APPENDICES

Appendix A ALIQUOT Study Inclusion / exclusion criteria

Inclusion criteria:

Operable T1, T2 and T3, N0-N1, M0 primary breast cancer.

Invasive, ER +ve tumour confirmed on core biopsy / surgical excision.

Postmenopausal women defined as a woman who has not menstruated over the preceding 12 months or who have FSH/LH levels within the post-menopausal range.

HRT stopped ≤ 4 weeks ago.

Patient able to give written informed consent.

Women taking drugs likely to affect bone metabolism, including steroids and bisphosphonates were randomised to participate in the quality of life study only. Blood and urine samples were not therefore obtained from this group.

Exclusion criteria:

Risk (in the investigator's opinion) of transmitting Human Immunodeficiency Virus (HIV) or Hepatitis B or C, through blood or other bodily fluids.

Any evidence of severe or uncontrolled systemic disease e.g. severe renal or hepatic impairment.

Women receiving post-operative chemotherapy.

If a patient is suitable then please contact a member of the research team.

Appendix B ALIQUOT Study Patient Information Leaflet

Invitation to Take Part in a Research Project
Anastrozole versus Letrozole:
An Investigation into Quality of Life and Tolerability

Background Information

The purpose of this leaflet is to explain to you as openly and clearly as possible all the procedures involved in this study before you decide to take part. The study will be conducted at the Edinburgh Breast Unit and has been funded by an educational grant by Novartis Pharma. 185 women will be invited to take part in this study.

Please read this leaflet carefully and feel free to ask your medical team any questions you have however simple they may seem. Their names and telephone numbers are found at the end of this leaflet. You may also wish to discuss the study with family and friends or your general practitioner.

Current drugs used in the treatment of breast cancer

There are many different treatments for breast cancer, including hormone drugs that remove or reduce the effect of the female hormones produced by your body. They appear to work because most breast cancers require female hormones to grow. The most common of these hormones are called oestrogens. Currently the drug tamoxifen, which is known as an anti-oestrogen, is the most commonly used and is a very effective drug in the treatment of hormone sensitive breast cancer. This drug works by reducing the amount of oestrogen available to cancer cells and by doing this it deprives the cancer cells of the oestrogen they need and so it can stop breast cancer cells from growing. It also increases the number of cells dying in a cancer and when given alone to women with breast cancer it can shrink the cancer. It is usually given for 5 years.

A new class of drugs called aromatase inhibitors have been developed and these drugs have been shown to be effective against hormone sensitive breast cancers. These drugs stop you from making oestrogen and so deprive the cancer of the oestrogen it needs to grow. The most commonly used of these new aromatase inhibitors are anastrozole and letrozole.

Studies of these new aromatase inhibitors have demonstrated that they are very active treatments in postmenopausal woman with hormone sensitive breast cancers but their long-term effects when given for 5 years have not yet been established.

Purpose of the Study

It may be that in future these new aromatase inhibitors will be prescribed instead of tamoxifen or after tamoxifen for the treatment of breast cancer. All drugs have side effects. Studies comparing tamoxifen suggests that the aromatase inhibitors have different side effects to tamoxifen. What we want to know is whether there are differences in side effects between the two commonly used aromatase inhibitors, anastrozole and letrozole. You will receive each of these drugs for three months so that in total you will receive six months of these two new aromatase inhibitors. It is important before these drugs are introduced for long term use over 5 years in some women that we know which of the two drugs patients prefer and which is best tolerated by patients.

To date there have been no studies directly comparing these two drugs when given for long periods after surgery for breast cancer. The aim is to compare how each of these drugs affects common symptoms by using a questionnaire and to determine which of these two drugs patients prefer.

By reducing the levels of oestrogen your body produces the aromatase inhibitors also affect bones and might increase the rate of osteoporosis and fracture when given over many years. It may also affect the level of cholesterol in your blood. You will not be at risk of these problems because you are only taking these drugs for 6 months but what we aim to do by collecting samples of blood and urine is to test what effects

these two drugs have on hormone production and the effects they might have on bones.

Why have I been chosen to take part?

You have been invited to take part in this study because we know at the time of your original surgery when the tumour was removed, it was oestrogen sensitive.

You are about to complete 5 years of hormone treatment after surgery for your breast cancer. If you agree to take part in the study you will receive aromatase inhibitors for six months after finishing your tamoxifen. You will receive three months of each of the two drugs. You will start treatment after you have signed the consent form. After six months of aromatase inhibitors – three months of letrozole and three months of anastrozole – you will then stop treatment. If you do not want to take part in the study you will receive no further treatment after your five years of tamoxifen.

What are the benefits of taking part?

There is some evidence that the aromatase inhibitors add to the effects tamoxifen in hormone sensitive tumours but there have been no studies of their long-term use. You will be given six months of drug treatment after stopping tamoxifen with these newer and potentially more effective drugs. There may however be no advantages to taking the aromatase inhibitors for only six months but equally it is highly unlikely there will be any disadvantages.

What extra tests will I have to have by being involved in the study?

If you do decide to take part you will be asked to take either:-
3 months of letrozole tablets followed by three months of anastrozole or
3 months of anastrozole followed by three months of letrozole.

There will be 3 extra visits to hospital at baseline, three months after starting the tablets.

When you enter the study and at three and six months we will take 20ml (four teaspoons) of blood and a small sample of urine to measure how these aromatase inhibitors affect your hormones, lipids and bones.

You will also be asked to complete a series of questionnaires asking about how you feel physically and emotionally throughout the study period. These questionnaires are simple to complete and take approximately 10 minutes to complete. The first questionnaire is completed when you enter the study. We then ask you to complete questionnaires after one and three months of each of the two aromatase inhibitors. We will also ask you specifically about any side effects you experience with either of the two drugs and will record these. At the six month visit, you will also be asked to state which drug of the two drugs, anastrozole or letrozole, you preferred and why you preferred that drug.

How will my treatment be decided?

If you enter the study, you will not be able to choose which of the two drugs you receive first. All patients in the study however will have three months of each of the two drugs, anastrozole and letrozole. You will be aware of which drug you are taking and you will be fully informed of any potential side effects. You will take one tablet of either anastrozole or letrozole every day. It is best to take this tablet at the same time each day and the recommendation is that you take it in the morning. For the remainder of the 5 years of hormonal treatment you will receive tamoxifen.

Side Effects

The side effects of aromatase inhibitors are similar to those described for tamoxifen. The majority of people who have taken aromatase inhibitors in the past have reported mild or moderate side effects. In studies looking at these drugs individually, only a few women have had to stop treatment because of these side effects. The side effects which have been reported by patients taking aromatase inhibitors include:

Tiredness, hot flushes or increased sweating, changes in weight, dizziness, itching and skin rash, muscular pains, headache, changes in appetite, water retention, nausea and sickness, constipation or diarrhoea, stomach upset or pain, vaginal dryness or

bleeding. Less common side effects are chest pain, viral infections, pain in bones and joints, shortness of breath, coughing and blood clots.

Please do not be alarmed by this list of possible side effects, you may not get any of them.

In this study you will be carefully monitored. You can contact your GP or any member of the study team at any time if you are worried or concerned (contact details of who and how to contact them are provided at the end of this information leaflet). If you decide to take part in the study we will inform your GP both of your entry into the study and the treatment that you are taking. If any side effects are too troublesome then you may stop the treatment straight away. Your own GP or a member of the study team may also stop your treatment at any stage if they feel that this is in your best interest.

Is there anything else I should know?

After the study period your follow up will be as normal.

In the unlikely event of any injury arising from your participation in the trial, information about procedures for obtaining compensation may be obtained from your doctor.

You may read in the newspapers, see on the television and hear on the radio alot about these new drugs. It is important to try and not let these news items influence how you rate the two drugs and which one gives you the least problems. If we knew which of the drugs was better and which was better tolerated there would be no point doing this study. The results of the study you are involved in will greatly assist doctors and patients decide which drug to use. Your participation is greatly appreciated.

Outline of Study

Due to finish 5 years of tamoxifen therapy
Details of study outlined
Information sheet given to patient
↓
One Week Later
Review in Clinic
Blood and urine samples collected
Randomised to receive 3 months of anastrozole or letrozole
↓
One Month Later
Complete further FACT-ES quality of life questionnaire
Return in stamped addressed envelope provided
Telephone interview to check for any side effects
↓
Three Month Follow Up Visit
Complete further FACT-ES quality of life questionnaire
Blood and urine samples collected
Patients receiving anastrozole change to letrozole
Patients receiving letrozole change to anastrozole
↓
One Month after starting second drug
Complete further FACT-ES quality of life questionnaire
Return in stamped addressed envelope provided
↓
Two months
Telephone interview to check if any side effects
↓
Six Month Follow Up Visit
Further FACT-ES quality of life questionnaire completed
Blood and urine samples collected
Complete a preference questionnaire – letrozole or anastrozole with explanation as to why

End of Study

What now?

If you decide you would like to take part in this study after you have read this information leaflet and discussed it with friends and relatives, then please sign the consent form in the presence of your doctor. It is important that you have time to think about and discuss this study. If you have any questions at all about the study, then please ask your doctor, the research nurse or the breast care nurse.

If any new information on the safety or effectiveness of the drugs used in this trial comes to light, then we will inform you immediately.

If you require any assistance with travelling expenses for any extra visits, please let your doctor know.

Further information

Your participation is voluntary. If you decide you do not want to take part or you wish to withdraw from the study at any time, you are completely at liberty to do so and your future care will not be affected. Your legal rights are not affected by you giving consent to participate. All your medical records will be confidential although information from the study will be analysed to assess the results in an anonymous fashion.

Please note that all your doctors and nurses are specialised in this form of treatment, and all of us are here to help. If you do have any questions or queries then please do not hesitate to contact us.

Appendix C FACT-B+ES (Version 4) questionnaire Endocrine symptoms

By circling one (1) number per line please indicate how true each statement has been for you during the past 7 days.

	ENDOCRINE SYMPTOMS	not at all	a little bit	some -what	quite a bit	very much
ES1	I have hot flushes	0	1	2	3	4
ES2	I have cold sweats	0	1	2	3	4
ES3	I have night sweats	0	1	2	3	4
ES4	I have vaginal discharge	0	1	2	3	4
ES5	I have vaginal itching/irritation	0	1	2	3	4
ES6	I have vaginal bleeding or spotting	0	1	2	3	4
ES7	I have vaginal dryness	0	1	2	3	4
ES8	I have pain or discomfort with intercourse	0	1	2	3	4
ES9	I have lost interest in sex	0	1	2	3	4
ES10	I have gained weight	0	1	2	3	4
An9	I feel lightheaded (dizzy)	0	1	2	3	4
O2	I have been vomiting	0	1	2	3	4
C5	I have diarrhoea	0	1	2	3	4
An10	I get headaches	0	1	2	3	4
Tax1	I feel bloated	0	1	2	3	4
ES11	I have breast sensitivity/tenderness	0	1	2	3	4
ES12	I have mood swings	0	1	2	3	4
ES13	I am irritable	0	1	2	3	4
BRM 1	I have pain in my joints	0	1	2	3	4

Appendix D ALEX Study –Inclusion / exclusion criteria

Inclusion criteria:

Operable T1, T2 and T3, N0-N1, M0 primary breast cancer.

Invasive, ER +ve tumour confirmed on core biopsy / surgical excision.

Postmenopausal women defined as a woman who has not menstruated over the preceding 12 months or who have FSH/LH levels within the post-menopausal range.

HRT stopped $\leq$ 4 weeks ago.

Patient able to give written informed consent.

Exclusion criteria:

Risk (in the investigator's opinion) of transmitting Human Immunodeficiency Virus (HIV) or Hepatitis B or C, through blood or other bodily fluids.

Any evidence of severe or uncontrolled systemic disease e.g. severe renal or hepatic impairment.

Women with a history of hypothyroidism or diabetes.

Women with a history of jaundice.

Women with abnormal lipid profiles, prior to randomisation.

Women taking drugs likely to affect lipid or bone profiles including:
Lipid regulating drugs; fibrates, nicotinic acid, fish oil, anion-exchange resins, neomycin, probucol, statins.
Other drugs including beta-blockers with ISA, cyclosporin, corticosteroids, phenytoin, retinoids, thiazide diuretics, bisphosphonates, glucosamine and St John's wort.

Women receiving post-operative chemotherapy.

Women with known alcohol abuse or in whom you suspect alcohol abuse.

If a patient is suitable then please contact a member of the research team.

Appendix E ALEX Study Patient Information Sheet

A study to compare the effects of anastrozole, letrozole and exemestane in postmenopausal women with hormone sensitive breast cancer

Patient information sheet
Invitation to take part in a research project

Background Information

The purpose of this leaflet is to explain to you as openly and clearly as possible all the procedures involved in this study before you decide to take part. The study will be conducted at the Edinburgh Breast Unit.

120 women will be invited to take part in this study.

Please read this leaflet carefully and feel free to ask your medical team any questions you have, however simple they may seem. Their names and telephone numbers are found at the end of this leaflet. You may also like to discuss the trial with family and friends.

Current drugs used in the treatment of Breast cancer

There are many different treatments for breast cancer, including drugs which remove or reduce the effect of the female hormones in your body. They appear to work because most breast cancers require female hormones to grow. The most common of these hormones are called oestrogens.

Currently the drug tamoxifen, which is known as an anti-oestrogen, is the most commonly used drug in the treatment of breast cancer. This drug works by reducing the amount of oestrogen available to cancer cells so it can stop a breast cancer from growing. It has also been seen to shrink breast cancers when given alone. A new class of drugs called aromatase inhibitors have been developed and these drugs have been shown to be effective against hormone sensitive breast cancers. These drugs stop you from making oestrogen and so deprive the cancer of the oestrogen it needs to grow. These new aromatase inhibitors are anastrozole, letrozole and exemestane.

Studies looking at the effects of these new aromatase inhibitors have demonstrated that they are active treatments in postmenopausal woman with hormone sensitive tumours.

Purpose of the Study

It is likely that in future these new aromatase inhibitors will be prescribed instead of tamoxifen for the treatment of breast cancer. We know that by reducing the amount of oestrogen you have in your body over a prolonged period could cause potential problems. These include changes in the levels of lipids such as cholesterol in the blood, changes in clotting, and they also are likely to affect bones because oestrogen reduces the chances of the bones becoming thin or osteoporotic. As you will receive the drug for only 4 months, the effects on lipids and bones will be very short lived and you should have no long term adverse effects. It is important however before we introduce these drugs for use over many years that we understand the effects these drugs have on blood lipids, clotting and on bone by measuring blood lipids and markers of bone activity before, during and after 4 months treatment with different aromatase inhibitors.

To date there has been no study that has directly compared these 3 different aromatase inhibitors. We hope to compare how each drug affects clotting, lipids and bones. This will not only improve our scientific knowledge, but should also help surgeons and oncologists decide which of the 3 drugs causes the least long term problems.

Why have I been chosen to take part?

You have been invited to take part in this study because your tumour is oestrogen sensitive.

Your doctor has decided that the best treatment for you will be surgery followed by drug treatment. If you agree to take part in the study you will receive one of the 3 aromatase inhibitors for 4 months after surgery. You will start treatment after you have signed the consent form. After the study period you will switch to taking tamoxifen for 5 years. If you do not want to take part you will simply take tamoxifen for 5 years after surgery.

What are the benefits of taking part?

There is some evidence that the aromatase inhibitors may be superior to tamoxifen in their action on hormone sensitive tumours. You will be being treated with both an aromatase inhibitor and the standard 5 years of tamoxifen.

What extra tests will I have to have by being involved in the study?

If you do decide to take part you will take one tablet per day for 4 months and it will mean 4 extra blood tests. You will also be asked to supply 3 samples of urine during the course of the 4 months of treatment and one sample once you are on tamoxifen one year after your operation. It will involve 2 additional trips to hospital out with routine appointments over the 4 month study period. You will also have samples taken at your routine one year follow up appointment. . However, you will be

providing important information to help doctors assess how these 3 drugs work on breast cancer cells and what effects they have blood lipids and bones.

You will also have to complete a quality of life questionnaire at the start of the study after 4 months on the drug and at 1 year.
At the end of the 4 month period you will stop taking the aromatase inhibitor and you will then receive tamoxifen for 5 years. The anti-oestrogen drug tamoxifen remains the current standard treatment after surgery for the majority of women with breast cancer. If taken for 5 years it has been shown to significantly reduce the chances of breast cancer returning.

How will my treatment be decided?

If you enter the study, you will not be able to choose which of the 3 drugs you receive. All patients in the study, however, will receive active treatment and you will be randomly assigned to receive either:

anastrozole 1mg daily or
letrozole 2.5mg daily or
exemestane 25mg daily

You will be aware of which drug you are taking and you will be fully informed of any potential side effects. The treatment schedule for each drug is the same regardless of which drug you receive. Each patient in this study will take 1 tablet of anastrozole or letrozole or exemestane every day. It is best to take the tablet at the same time each day and the recommendation is that you take it in the morning.

Side effects

The side effects with the 3 aromatase inhibitors are similar. The majority of people who have taken aromatase inhibitors in the past have reported no side effects. In studies performed looking at the three drugs individually, only a few women have had to stop treatment because of serious side effects. The majority of side effects reported with the aromatase inhibitors are similar to the side effects which patients report when taking tamoxifen. The side effects which have been reported by patients taking aromatase inhibitors include:

Tiredness, hot flushes or increased sweating, changes in weight, dizziness, itching and skin rash, headache, changes in appetite, water retention, nausea and sickness, constipation or diarrhoea, stomach upset or pain, vaginal dryness or bleeding. Less common side effects are chest pain, viral infections, pains in the muscles, bones and joints, shortness of breath, coughing and blood clots.

Please do not be alarmed by this list of possible side effects. You will probably not have any of them.

In this study you will be carefully monitored. You can contact your GP or any member of the study team at any time if you are worried or concerned (contact details of who and how to contact them are provided at the end of this information leaflet). If

you decide to take part in the study we will inform your GP both of your entry into the study and the treatment that you are taking. If any side effects are too troublesome then you may stop the treatment straight away. Your own GP or a member of the study team may also stop your treatment at any stage if they feel that this is in your best interest.

What will happen over next 4 months?

Outline of Study

Post Surgical Follow Up Clinic
Results of surgery discussed.
Study discussed with you and you meet trial personnel
Given information sheet and you have time to decide whether you wish to enter study

If you decide to enter the study,

Visit One
Informed consent obtained
Fasting blood sample taken
Give a sample of urine
Start treatment with either anastrozole, letrozole or exemestane
Complete Quality of Life Questionnaire

Visit 2
Three months after starting treatment
Fasting blood sample taken
Give a sample of urine
Continue on aromatase inhibitor

Visit 3
Four months after starting treatment
Fasting blood sample taken
Give a sample of urine
Stop aromatase inhibitor
Commence tamoxifen
Complete Quality of Life Questionnaire

Visit 4
One year after initial surgery
Fasting blood sample taken
Give a sample of urine
Complete Quality of Life Questionnaire
Continue tamoxifen

Is there anything else I should know?

After the study period your follow up will be as normal with annual clinical examination and mammograms for 5 years.

In the unlikely event of any injury arising from your participation in the trial, information about procedures for obtaining compensation may be obtained from your doctor.

What now?

If you decide you would like to take part in this study after you have read this information leaflet and discussed it with friends and relatives, then please sign the consent form in the presence of your doctor. It is important that you have time to think about and discuss this study. If you have any questions at all about the study, then please ask your doctor, the research nurse or the breast care nurse.

If any new information on the safety or effectiveness of the drugs used in this trial comes to light, then we will inform you immediately.

If you require any assistance with travelling expenses for any extra visits, please let your doctor know.

Further information

Your participation is voluntary. If you decide you do not want to take part or you wish to withdraw from the study at any time, you are completely at liberty to do so and your future care will not be affected. Your legal rights are not affected by you giving consent to participate. All your medical records will be confidential although information from the study will be analysed to assess the results in an anonymous fashion.

Please note that all your doctors and nurses are specialised in this form of treatment, and all of us are here to help. If you do have any questions or queries then please do not hesitate to contact us.

Appendix F Tables summarising trials discussed in Section 1

Summary of trials / studies included in section 1 – part (i)							
Trial / study	**Start date**	**Design**	**Sample size**	**Duration of follow-up**	**Goals**	**Arms**	**Results**
Womens' Health Initiative study[37]	1993	Randomised, controlled, primary prevention trial	16, 608 healthy post-menopausal women	5.2 years (planned duration 8 years)	To determine the risks and benefits of oestrogen plus progestin on overall health	(i) oestrogen plus medroxyprogesterone (ii) placebo	Stopped early due to adverse events. HRT doubled risk of death from breast cancer. Estimated hazard ratio1.26 [95% CI 1.00-1.59]
Million Women study[32]	1996	Cohort	1, 084 110 women between 50-64 years	4.1 years	To investigate the effects of specific types of HRT on incident and fatal breast cancer	Study questionnaire sent to women attending for routine mammography. Information about use of HRT collected	Current users of HRT at increased risk of breast cancer, adjusted RR 1.66 [95% CI 1.58-1.75. Oestrogen only compounds RR 1.30 [1.21-1.40], oestrogen-progestagen compounds RR 2.00 [1.88-2.12], tibolone RR 1.45 [1.25-1.68]
Danish Nurse Cohort study[36]	1993	Cohort	23, 178 female nurses aged 45 years and above	6 years	To determine the risks and benefits associated with different types of HRT via questionnaires	(i) oestrogen alone (ii) combined HRT (iii) tibolone	Current users of HRT at increased risk of breast ca. Oestrogen alone RR 1.96 [95% CI 1.16-3.35]. Combined HRT RR 2.70 [1.96-3.73. Tibolone RR 4.27 [1.74-10.51] compared with never used HRT
UK General Practice Research Database study [38]	1998	Population-based case-control	37, 863	6 years	To determine the effect of different types of HRT on the risk of breast cancer in postmenopausal women	6, 347 incident cases of breast cancer were matched with 31, 516 controls	Opposed oestrogens associated with increased risk of breast cancer RR1.38 [95% CI 0.81-1.43]. Unopposed oestrogens RR 0.97 [0.8601.09]. Tibolone RR 0.86 [CI 0.65-1.13]

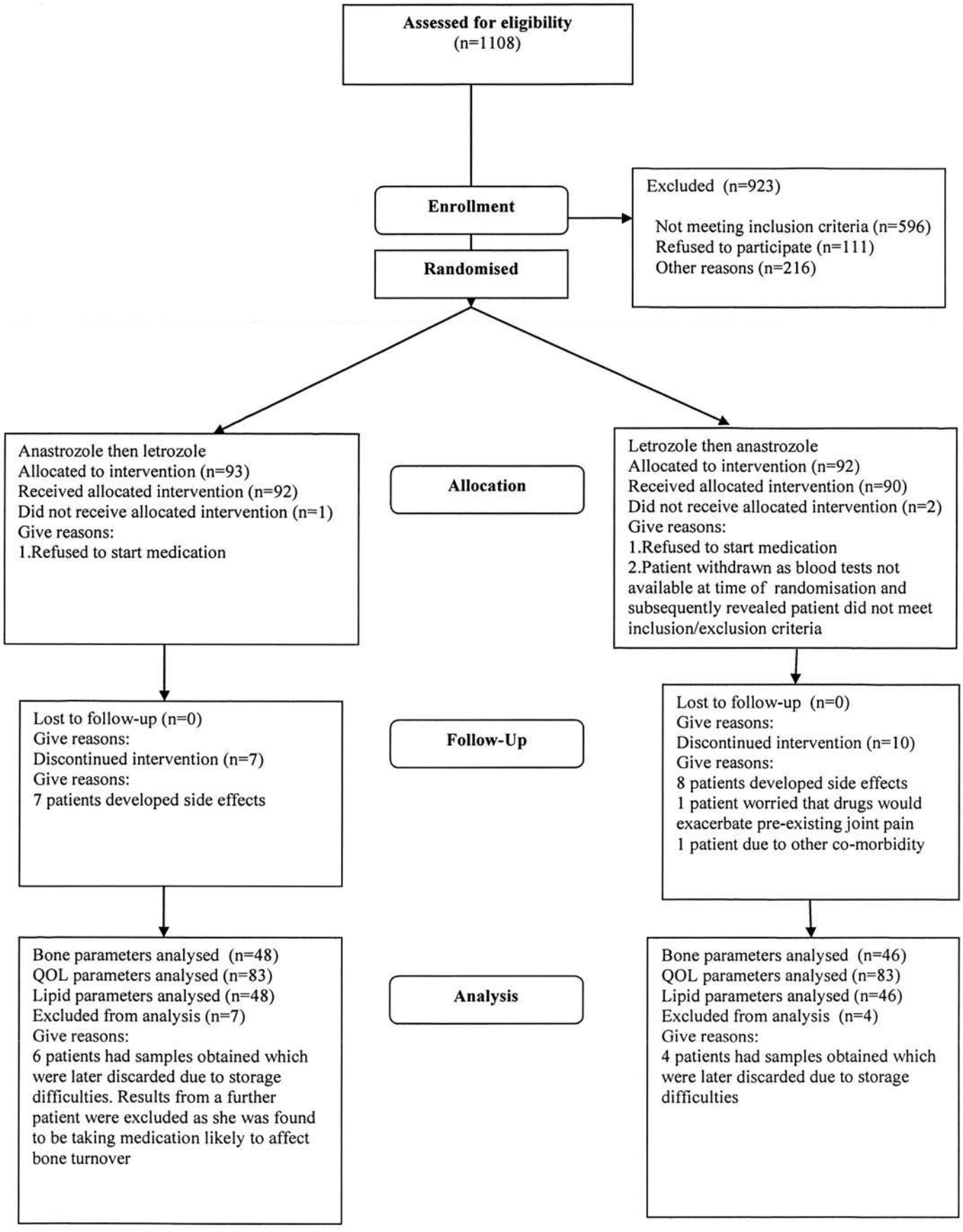
Summary of trials / studies included in section 1 – part (ii)							
Trial / study	**Start date**	**Design**	**Sample size**	**Duration of follow-up**	**Goals**	**Arms**	**Results**
LIFT study [39]	2001	Randomised, double-blind, placebo-controlled	4538 post-menopausal women	34 months	To test if tibolone reduces risk of vertebral fractures +/- modifies risks of breast cancer, DVT, CVD	(i) tibolone 1.25mg (ii) placebo	Decreased risk of invasive breast cancer with tibolone. Relative hazard 0.32 [95% CI 0.13-0.80]
LIBERATE study[40]	2002	Randomised, double-blind, non-inferiority trial	3148 post-menopausal women with BMD T-score ≤2.5	3.1 years	To establish breast cancer recurrence/vasomotor symptoms after tibolone use versus placebo in women with vasomotor symptoms	(i) tibolone 2.5mg (ii) placebo	Increased risk of breast cancer with tibolone HR 1.40 [95% CI 1.14-1.70]
P-1 Breast Cancer Prevention trial[82]	1992	Randomised controlled trial	13, 388 women	69 months	To assess the value of using tamoxifen for breast cancer prevention in women at increased risk	(i) tamoxifen 20mg (ii) placebo	Tamoxifen reduced the risk of breast cancer. Risk ratio 0.51 [95% CI 0.39-0.66]
ABCSG-12 trial[180]	1999	Randomised controlled study	1, 803 pre-menopausal women	47.8 month	To examine the effects of zoledronic acid to a combination of either goserelin + tamoxifen or goserelin + anastrozole in women with early hormone responsive breast cancer	(i) goserelin 3.6mg + tamoxifen 20mg (ii) goserelin 3.6mg + anastrozole 1mg +/- zoledronic acid 4mg intravenously	94% DFS in group that received endocrine therapy + zoledronic acid versus 90.8% DFS in group that received endocrine therapy alone. Zoledronic acid addition resulted in a relative reduction of 36% in the risk of disease progression. Hazard ratio 0.64% [95% CI 0.46-0.91]. No significant different detected between tamoxifen and anastrozole

Summary of trials / studies included in section 1 – part (iii)							
Trial / study	**Start date**	**Design**	**Sample size**	**Duration of follow-up**	**Goals**	**Arms**	**Results**
IBCSG 25-02 / BIG 3-02 (TEXT)[303]	2003	Randomised phase III trial	Target = 2, 639 pre-menopausal women	5 years	A phase III trial evaluating the role of exemestane plus GnRH analogue as adjuvant therapy for premenopausal women with endocrine responsive breast cancer	(i) triptorelin + tamoxifen 20mg (ii) triptorelin + exemestane 25mg	Study closed to accrual Result awaited
P024[182]	1998	Randomised, double-blind, multicenter study	337 post-menopausal women	4 months	To compare the anti-tumour activity of letrozole vs. tamoxifen in postmenopausal women with ER and/or PgR+ve primary untreated breast cancer	(i) letrozole 2.5mg (ii) tamoxifen 20mg	Overall objective response rate (clinical palpation) was statistically significantly superior in the letrozole group, 55% compared to tamoxifen, 36% ($p < 0\ 001$) Secondary endpoints of ultrasound response, 35% vs 25% ($p = 0.042$). Mammographic response, 34% vs 16% ($P < 0.001$), and BCS. 45% vs. 35% ($p = 0.022$) between the letrozole and tamoxifen groups, respectively, showed letrozole to be significantly superior
Neoadjuvant AI Therapy: Results from a Multicenter Phase II Trial[184]	2002	Open-label multicenter phase II trial	115 post-menopausal women	4 months	To investigate effects on surgical outcomes of neoadjuvant letrozole in postmenopausal women with early hormone receptor positive breast cancer	letrozole 2.5mg	Neoadjuvant letrozole improved operability and facilitated breast conserving surgery. Clinical response rate 64%

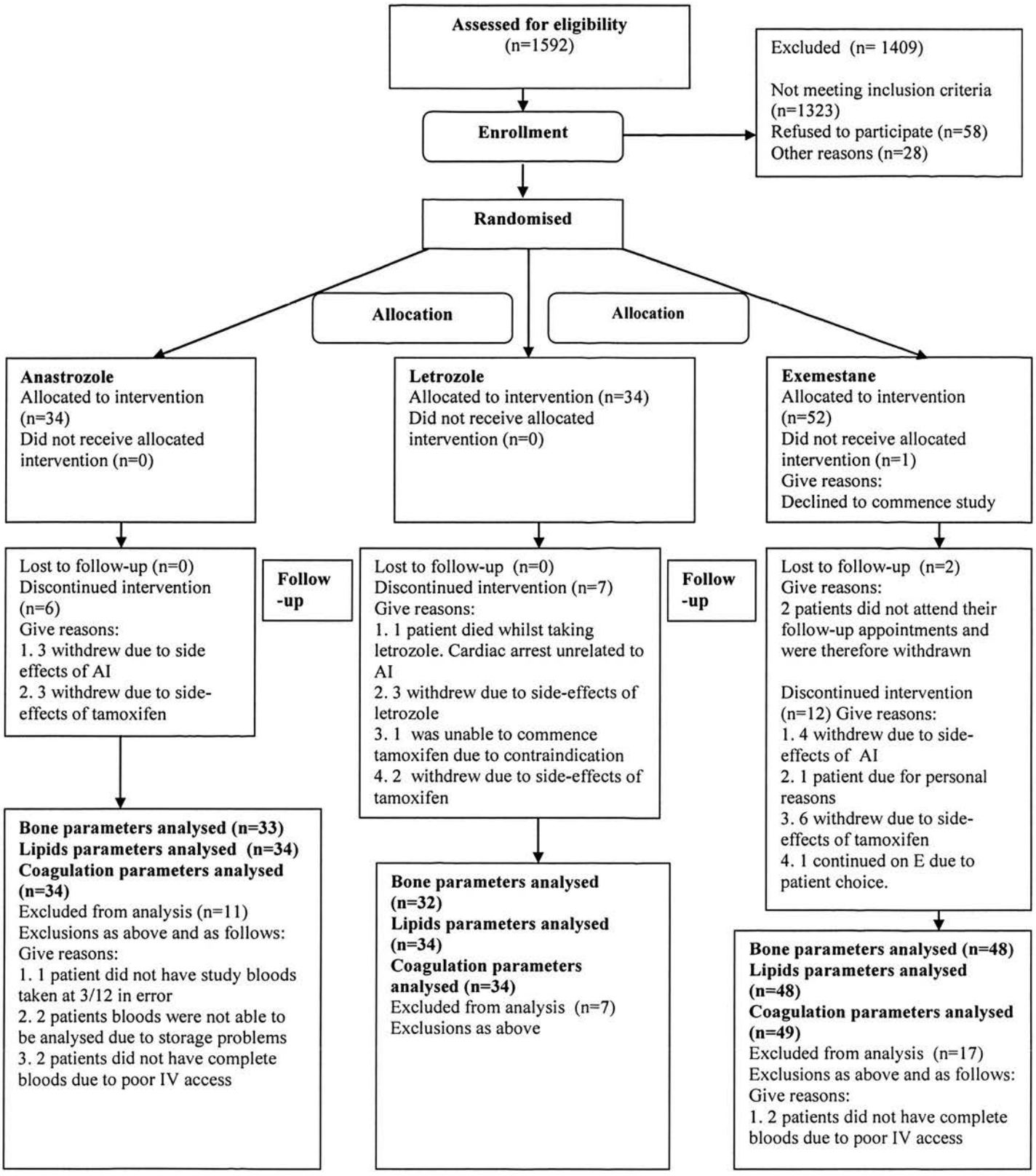
Summary of trials / studies included in section 1 – part (iii)							
Trial / study	**Start date**	**Design**	**Sample size**	**Duration of follow-up**	**Goals**	**Arms**	**Results**
IMPACT[185]	1997	Phase III randomised, double-blind, multicenter trial	330 post-menopausal women	13 weeks	To test the hypothesis that the clinical and/or biologic effects of neoadjuvant tamoxifen compared with anastrozole and with the combination of tamoxifen and anastrozole before surgery in postmenopausal women with ER+ve, invasive, nonmetastatic breast cancer might predict for outcome in the ATAC trial	(i) tamoxifen 20mg (ii) anastrozole 1mg (iii) tamoxifen + anastrozole	There were no significant differences in OR in the intent-to-treat population between patients receiving tamoxifen, anastrozole, or the combination. In patients who were assessed as requiring mastectomy at baseline, 44% of patients received BCS after anastrozole compared with 31% of patients after tamoxifen (p = 0.23). Neoadjuvant anastrozole was as effective and well tolerated as tamoxifen, but the hypothesis that clinical outcome might predict for long-term outcome in adjuvant therapy was not fulfilled
PROACT[186]	2000	Randomised, double-blind, multicenter study	451 post-menopausal women	5 years	To compare anastrozole with tamoxifen as a preoperative treatment of postmenopausal women with large, operable, or potentially operable breast cancer.	(i) anastrozole 1mg + tamoxifen placebo +/- chemotherapy (ii) tamoxifen 20mg + anastrozole placebo +/- chemotherapy	OR for anastrozole and tamoxifen occurred in 39.5% and 35.4% respectively on USS, and 50.0% and 46.2% respectively using calipers. Feasible surgery at baseline improved after 3 months in 43.0% of anastrozole patients and 30.8% receiving tamoxifen ($p = 0.04$).

Summary of trials / studies included in section 1 – part (iv)							
Trial / study	**Start date**	**Design**	**Sample size**	**Duration of follow-up**	**Goals**	**Arms**	**Results**
ACOSOG Z1031 [188]	2006	Phase II randomised neoadjuvant screening trial	377 post-menopausal women with stage II or III ER+ve breast cancer	16 – 18 weeks	Preoperative AIs promote BCS for ER+ve breast cancer. To study this treatment option, responses to three AIs were compared in a trial designed to select agents for phase III investigations.	(i) anastrozole 1mg (ii) letrozole 2.5mg (iii) exemestane 25mg	Letrozole and anastrozole were selected for further investigation due to superior clinical response. No differences in surgical outcome including preoperative endocrine prognostic index (PEPI) score or Ki67 suppression were detected between the drugs. The BCS rate for mastectomy-only patients at presentation was 51%. Neoadjuvant AIs markedly improved surgical outcomes. Ki67 and PEPI data demonstrated that the three agents tested are biologically equivalent and therefore likely to have similar adjuvant activities.

Appendix G CONSORT diagram - disposition of patients throughout the ALIQUOT study



Appendix H CONSORT diagram - disposition of patients throughout the ALEX study



SECTION 6: PUBLICATIONS

Publications and presentations arising from the work in this thesis

Publications

McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Hannon R, Dixon JM
A study of the effects of the aromatase inhibitors anastrozole and letrozole on bone metabolism in healthy postmenopausal women with estrogen receptor-positive breast cancer
Journal of Breast Cancer Research and Treatment 2010;119(3):643-51

Dixon JM, Renshaw L, Langridge C, Young OE, McHugh M, Williams L, Murray J, Macaskill EJ, **McCaig F M**, Dixon OM, Fallowfield LJ
Anastrozole and letrozole: an investigation and comparison of quality of life and tolerability in women with post-menopausal breast cancer
Journal of Breast Cancer Research and Treatment 2011;135(3):741-9

Dixon JM, Renshaw L, Langridge C, Young OE, McHugh M, Williams L, Murray J, Macaskill EJ, **McCaig F M**, Dixon OM, Fallowfield LJ
Anastrozole and Letrozole – an investigation and comparison of quality of life, tolerability and morbidity
The Breast 16 (2007) Supplement ppS57

Oral Presentations

A randomised study of the impact of endocrine therapy for breast cancer on bone turnover and quality of life
6th European Breast Cancer Conference, Berlin
April 2008

A randomised study of the effects of letrozole and anastrozole on bone turnover
Association of Surgeons of Great Britain and Ireland Meeting, Bournemouth
June 2008

A randomised study of the effects of letrozole and anastrozole on bone turnover
Edinburgh School of Surgery Day. *Clinical Prize awarded*
December 2008

Poster presentations

McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Riermersma R, Evans DB, Dixon JM
A randomized study of the effects of the aromatase inhibitors anastrozole, letrozole and exemestane on lipid metabolism in healthy postmenopausal women with oestrogen receptor-positive breast cancer
32st Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2009

Dixon JM, Renshaw L, Langridge C, Young OE, McHugh M, Williams L, Murray J, Macaskill EJ, **McCaig F M**, Dixon OM, Fallowfield LJ
A randomized study of the effects of the aromatase inhibitors anastrozole, letrozole and exemestane on quality of life in healthy postmenopausal women with oestrogen receptor-positive breast cancer
32st Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2009

McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Dawson P, Dixon JM
A randomised study of the effects of anastrozole, letrozole, exemestane and tamoxifen on coagulation in health post-menopausal women with estrogen receptor positive breast cancer
31st Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2008

McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Hannon R, Dixon JM

A randomised study of the effects of anastrozole, letrozole and exemestane on bone turnover in women with post-menopausal estrogen positive breast cancer
31st Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2008
McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Riermersma R, Evans DB, Dixon JM
A randomised study of the effects of anastrozole and letrozole on lipid metabolism in women with post-menopausal estrogen positive breast cancer
31st Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2008

McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Hannon R, Dixon JM
A randomised study of the effects of letrozole and anastrozole on bone turnover in women with post-menopausal estrogen positive breast cancer
30th Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2007

Dixon JM, Renshaw L, Langridge C, Young OE, McHugh M, Williams L, Murray J, Macaskill EJ, **McCaig F M**, Dixon OM, Fallowfield LJ
Comparison of joint problems as reported by patients in a randomised adjuvant trial of anastrozole and letrozole
30th Annual San Antonio Breast Cancer Symposium, Texas, USA
December 2007

Dixon JM, Renshaw L, Langridge C, Young OE, McHugh M, Williams L, Murray J, Macaskill EJ, **McCaig F M**, Dixon OM, Fallowfield LJ
Anastrozole and Letrozole – An Investigation and Comparison of Quality of Life, Tolerability and Morbidity
10th International Conference on Primary Therapy of Early Breast Cancer, St Gallen, Switzerland
March 2007

SECTION 7: REFERENCES

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