A COMPARISON OF CLINICO-BIOLOGICAL AND GENETIC MARKERS IN SPORADIC BREAST CANCER IN DIFFERENT POPULATIONS

Thesis submitted for the degree of Doctor of Philosophy

at

the University of Leicester

by

Wafa Makky Jonathan Ronald Nichols MSc (U.E.A)

Department of Pathology

University of Leicester

September 2000

UMI Number: U138619

UMI

Dissertation Publishing

ProQuest

ABSTRACT

A Comparison Of Clinico-Biological And Genetic Markers In Sporadic Breast Cancer In Different Populations

Wafa Makky Jonathan Ronald Nichols MSc (U.E.A)

The aim of this thesis is to examine the hypothesis that breast cancer exhibits ethnic differences in incidence, age of presentation and aggressiveness.

Two approaches were used:
Firstly, International data was examined to find if differences in the age of presentation of breast cancer existed between low and high breast cancer incidence populations.

Secondly, selected pathobiological parameters and genetic markers, chosen to act as surrogate markers of tumour behaviour, were examined in breast cancers from Western Region of Saudi Arabia, Leicester Asians and Leicester Europeans.

Analysis of International data showed countries with the lowest incidence of breast cancer to have a significantly lower mean age of onset than countries with the highest incidence. The mean age of onset in the Western Region was less than Leicester Asians and Leicester Europeans. Statistically significant differences in ASIR were found between the study groups that varied with age.

Breast cancers from Western Region and Leicester Asian women, when compared to the Leicester Europeans, were found to have a more aggressive profile of clinicopathological markers, but a less variable and possibly less aggressive nuclear morphometry.

Molecular alterations at the markers studied appeared to be related to age, 16q exhibiting more Loss of Heterozygosity in older groups and p53 in the younger groups. 6q also showed differences in Loss of Heterozygosity with respect to age, but these differences varied between the populations studied.

The results are consistent with a "two disease" model of breast cancer. It is proposed that the low age of onset seen in the Western Region is primarily due to a low incidence of "older type" of breast cancer rather than an absolute excess of "younger type".

ACKNOWLEDGEMENTS

CONTENTS

Section   **Subject**   **Page**

Section	Subject	Page

TABLES

Number   **Title**   **Page**

Number   **Title**   **Page**

Number   **Title**   **Page**

FIGURES

ABBREVIATIONS

+	Additional/present
-	Loss/less/subtracted
AA	Saudi study group
ADH	Atypical ductal hyperplasia
AI	Allelic imbalance
AL	Leicester Asian study group
AmA	American Asians
AmB	American Blacks
AmH	American Hispanics
AmW	American Whites
APC	Adenomatous polyposis coli gene
ASR	Age standardised rate
ASIR	Age specific incidence rate
AT	Ataxia telangiectasia
ATM	Ataxia telangiectasia gene
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CAMs	Cell adhesion molecules
CDK	Cyclin dependant kinase
CGH	Comparative genomic hybridisation
CKI	Cyclin dependant kinase inhibitor protein
CMI	Cellularity mean index
CML	Chronic myeloid leukaemia
Coeff var	Coefficient of variation
DCC	Deleted in colon cancer gene
DCIS	Ductal carcinoma in situ
del	Deletion (cytogenetics)
df	Degrees of freedom
DFI	Disease free interval
Dmins	Double minutes (cytogenetics)
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate

dup	Duplication (cytogenetics)
ECM	Extra cellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EL	Leicester European study group
ER	Oestrogen receptor
FAP	Familial adenomatous polyposis
GADD45	Growth arrest and DNA damage inducible gene
GS	Gardener syndrome
HNPCC	Hereditary non polyposis colon cancer
HRT	Hormone replacement therapy
HSR s	Homogeneously staining regions (cytogenetics)
IARC	International Agency for Research on Cancer
IDC	Infiltrating ductal carcinoma
ILC	Infiltrating lobular carcinoma
inf	Informativity
inv	Inversion (cytogenetics)
IUD	Inter uterine contraceptive device
KFSH&RC	Fahad Specialist Hospital and research centre
KSA	Kingdom of Saudi Arabia
LCIS	Lobular carcinoma in situ
LN	Lymph node
LOH	Loss of heterozygosity
MSI	Microsatellite instability
NA	Nuclear area
NA10	Mean area of ten largest nuclei
NCR	National Cancer Registry (Saudi)
ND1:ND2	Ratio of maximum and minimum nuclear diameter
Ndmax	Maximum nuclear diameter
NHSBSP	National Health Breast Screening Programme
NIH	National Institute of Health (USA)
NOS	Not otherwise specified
NPI	Nottingham Prognostic Index
OC	Oral contraceptive

-p	Short chromosome arm
PAGE	Poly acrylamide gel electrophoresis
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PgR	Progesterone receptor
PI	Prognostic index
-q	Long chromosome arm
relSIR	Relative standardised incidence rate
RFLP	Restriction fragment length polymorphism
S.Asian	South Asian
SD	Standard deviation
SDNA	Standard deviation of nuclear area
SDNP	Standard deviation of nuclear perimeter
SEER	Socio Economic End Results
Sh	Shape coefficient
SIR	Standardised incidence rate
STR	Short terminal repeats
t-	Translocation (cytogenetics)
TDLU	Terminal duct lobular unit
TGFβ	Transforming growth factor β
TSG	Tumour suppressor gene
UICC-AJC	Union Internationale Centre Cancer-American Joint Committee
UV	Ultra violet light
Var	Variance
VMI	Volume corrected mitotic index
VNA	Variation in nuclear area
VNTR	Variable number terminal repeats
Vv	Volume fraction
W.Region, WReg, WR	Western region of Saudi Arabia
YBCG	Yorkshire Breast Cancer Group
YBCGPI	Yorkshire Breast Cancer Group Prognostic Index

CHAPTER ONE

INTRODUCTION

Breast cancer is an important clinical problem world-wide, not only in countries like the UK, which have a high incidence of breast cancer, but also in countries with a much lower overall incidence such as Saudi Arabia. Breast cancer is attributable for 20% of all female malignancies in the UK (McPherson et al, 1994) and 18% of female malignancies in Saudi Arabia, (NCR 1996). The disease is very heterogeneous in terms of its epidemiology, incidence varying up to seven fold in different countries (Parkin et al, 1992), and also with regards to its biological and clinical course (Devilee et al, 1994). Publications from Saudi Arabia have assessed breast carcinoma as being aggressive and affecting a young age group (Al-Idrissi et al, 1992; Amr et al, 1995; Tandon et al, 1995; Ezzat et al, 1999) when compared with the west. Asian populations residing in the UK have also been found to have significantly lower rate of breast carcinoma, which affects a younger age group (Winter et al, 1999), and presents with more advanced disease (Potter et al, 1983). My study will comprise age matched populations of breast carcinoma patients from Saudi Arabia (Jeddah) and Asian and European breast carcinoma patients from the UK (Leicester).

In order to study the mechanisms by which a breast carcinoma achieves excessive, uncoordinated growth it is necessary to outline the basic structure, cell organisation, and control of cell division in normal breast tissue.

1.1 THE NORMAL BREAST

1.1.1 BASIC STRUCTURE

The normal breast of a female of reproductive age is formed of segments each comprising 6-10 lobules containing several acini, linked by terminal ducts; this forms the terminal duct lobular unit (TDLU) (Fig1.1). The TDLUs are connected via a heirachy of ducts to the lactiferous ducts and sinuses, which open to the outside.

Components of the breast

Acini: The acini, the milk producing glands of the breast, are lined with cuboidal epithelial cells attached to a basal layer of myoepithelial cells which adjoin the basement membrane.

Ducts: The areolar, nipple and openings of the lactiferous ducts are covered with stratified squamous epithelium; further along the major ducts this changes to first pseudostratified colomnular epithelium, and then to double layer of cuboidal epithelium. Parallel to the ducts is a layer of myoepithelium (Stirling and Chandler, 1976).

Lobules: The individual lobules are surrounded by dense collagenous inter lobular stroma, and all are further enclosed by a looser stroma, the intralobular connective tissue.

1.1.2 NORMAL BREAST CHANGES

Changes occur in the female breast associated with puberty, the menstrual cycle, pregnancy, lactation, and menopause.

Puberty

Prior to puberty there are a few ducts present in the breast, but no acini. After the onset of menarche, for the first 1-2 years, oestrogen predominates over progesterone synthesis, and causes the ducts to elongate, branch, and to develop terminal buds (which will later form the acini). The amount of fat and connective tissue in the breast also increases. Growth of the breast continues until the mid 20's unless pushed to maturity earlier by the occurrence of a pregnancy. An international study for the World Health Organisation (Morabia and Costanza, 1998) found menarche to have median values varying between 13-16 years.

Menstrual Cycle

The breast undergoes cyclic changes, in synchrony with ovulation and menstruation (Longacre and Bartow, 1986) from menarche until menopause, unless interrupted by a pregnancy. Normal breast tissue therefore is not static in nature, but undergoes a cycle of proliferation and apoptosis, under hormonal regulation. (Table 1.1)

Fig 1.1 Normal breast showing duct lobular unit, TDLU
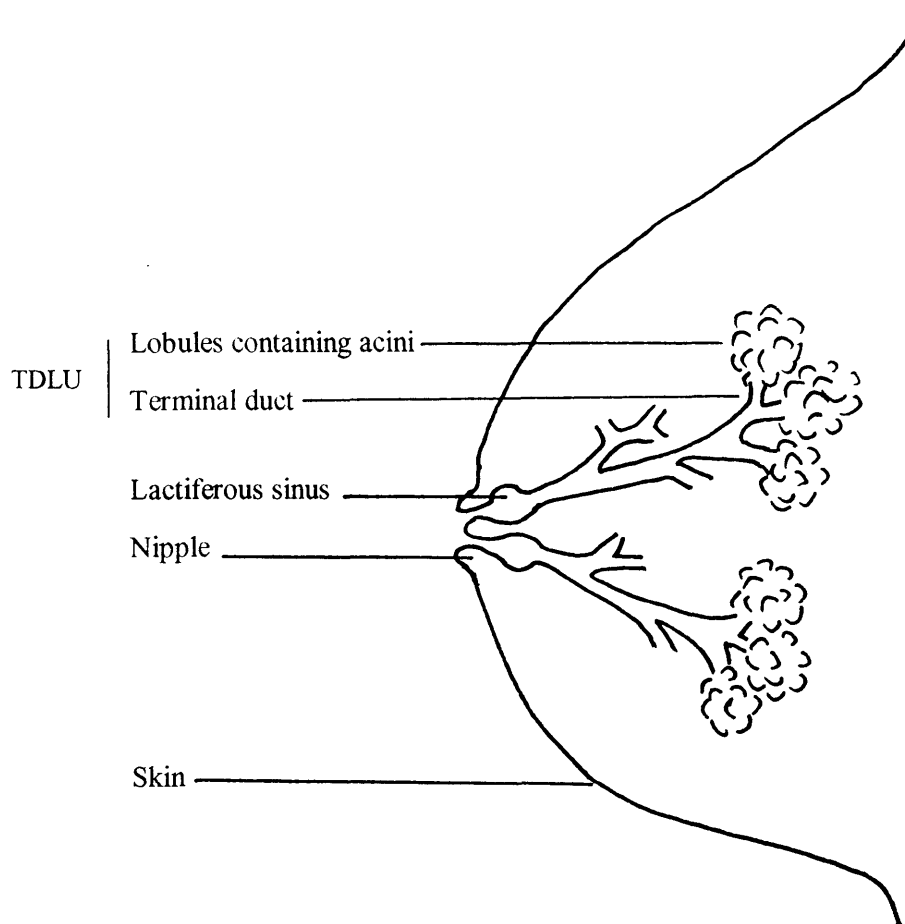
Lobules containing acini

TDLU

Terminal duct

Lactiferous sinus

Nipple

Skin

Cycles in which no ovulation occurs, anovulatory cycles, are not associated with the changes of hormone levels described. Many of the irregular cycles experienced by postmenarcheal girls are anovulatory (Vihko and Apter, 1984), strenuous physical exercise in this group may also lead to a decrease in the number of ovulatory cycles (Bernstein et al, 1987). In some women post partum cycles may either be absent or anovulatory during the months spent breast feeding.

Table 1.1 **Breast changes during the menstrual cycle** (Vogel et al, 1981)

Day of cycle	Phase and hormone levels	Events in Breast
14	Ovulation progressive rise in oestrogen	Cells of ducts and acini start to proliferate
22 - 24	Secretory phase influence of progesterone	Terminal ducts: increase in proliferation and structure. Basal epithelial cells: increase in mitotic activity, and vacuolisation Stromal cells : proliferation and stromal oedema
28	Menstruation fall in oestrogen and progesterone levels	Epithelial cells: apoptosis Intralobular connective tissue: atrophy Stromal cells : reduction of oedema Ducts and glands : shrinkage

Pregnancy

Proliferative changes occur in the breast through out pregnancy in preparation for nursing, under hormonal control. (Table 1.2)

Table 1.2 **Breast changes during pregnancy**

Hormone	Stage of Pregnancy	Function
Oestrogen	3-4 weeks 5-8 weeks	Increase branching of ducts Breast enlargement
Progesterone	3-6 months	Increase lobular formation. Acini produce colostrum causing an increase in the volume of the breast. Cells are in a pre secretory state.
Prolactin	increases slowly throughout pregnancy	Initiates protein synthesis in mammary epithelium.

Lactation

Birth causes an abrupt withdrawal of the hormones oestrogen and progesterone which previously dampened the effect of prolactin on the mammary epithelium. Prolactin, insulin, and cortisol, cause the mammary epithelial cells to progress to the secretory state. The continued production of prolactin is stimulated by suckling, and when breast feeding ceases the prolactin levels drop and there is rapid involution of the glandular tissue and the breast returns to its resting state.

Menopause

Reduced ovarian function results in involution of the epithelial structures and stroma. In post menopausal women the breast becomes increasingly composed of adipose tissue as the other components shrink. Morabia and Costanza (1998) found little international variation in age at natural menopause, the mean age world wide being 50 years.

Normal breast tissue can therefore be seen to be in a state of dynamic change throughout the life of the female, controlled by the interaction of ovarian hormones, until the post menopausal phase.

1.1.3 ORGANISATION OF EPITHELIAL TISSUE IN THE BREAST

Epithelial tissue is an ordered, polarised, compact tissue (Fig 1.2).The overall organisation of epithelial tissue is maintained by:

a. Cell - cell interaction: Cells "recognise" each other and adhere to one another via molecules and receptors on their surface, and by linking junctions.

b. Cell - matrix interactions: Cells have an intimate contact with the molecules of the extracellular matrix (ECM) .

c.Extra-cellular differentiation and growth factors: Tissues require growth factors, differentiation factors and hormones to maintain them in a particular state.

The surface membrane of epithelial cells

The cells of epithelial tissues are highly polarised, the cell membrane of the top (apical) surface of the cell having a different composition of components than the membranes at the side (lateral membranes) or those at the bottom (basal membrane).

The apical membrane

Due to its molecular composition the apical surface has a negative charge, one of the effects of which is to repel other cells so that the apical surface is always the free surface of the tissue. The apical surface may also perform the specialised function of secretion (as occurs in the breast during lactation).

Fig 1.2 **Organisation of epithelial cells in acinus**
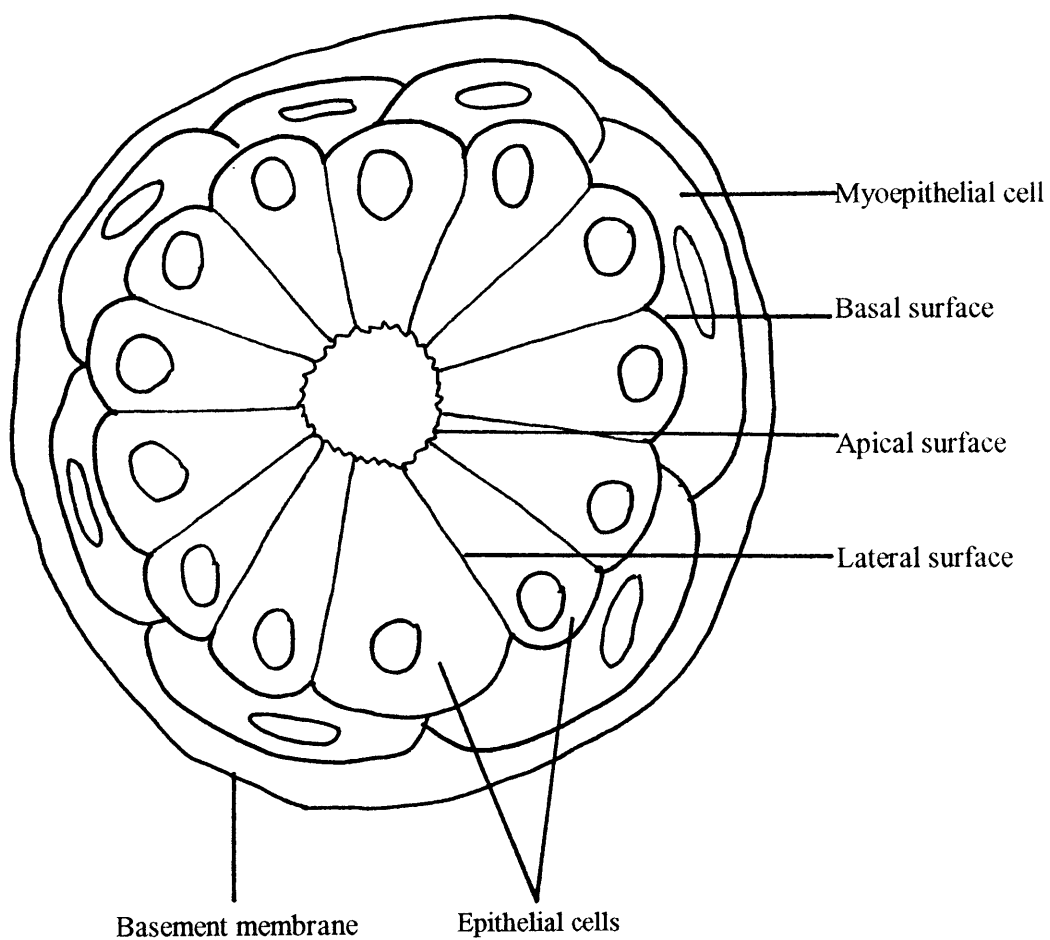
Myoepithelial cell

Basal surface

Apical surface

Lateral surface

Basement membrane   Epithelial cells

The basal membrane

The basal membrane is attached to the basement membrane via cell matrix junctions e.g. focal contacts and integrin receptors (Birchmeier and Birchmeier, 1993).

The lateral membrane

The lateral membrane has many of the compounds found on the basal membrane and also some unique features e.g. gap junctions, adherence junctions. In addition there are cell adhesion molecules (CAMs) which hold the cells tightly and are important for cell-cell recognition.

Cell adhesion molecules

There are three main types of CAM:

1.Cadherins:

Calcium dependant adhesion molecules are both receptor and ligand (Takeichi, 1990). All the cadherins are transmembrane proteins which tend to be at a greater density at cell junctions. There are at least three classes of cadherins in normal breast tissue:

E-Cadherin, located in adult epithelial tissue E cadherin is both involved in the formation of adherence junctions and in signal transduction pathways (Ben-Ze'ev, 1997).

H -cadherin, expressed predominantly in the heart, is also found in the epithelial tissue of the breast (Lee, 1996).

P-Cadherin, is found in the myoepithelial cells of the breast (Soler et al, 1999).

2.Integrins:

Integrins are transmembrane cell adhesion molecules; each sub family of integrin receptors (Hynes, 1992) has a common βsubunit interacting with a specific αsubunit. Binding to the ligand occurs by recognition of specific amino acid sequences found in several ECM proteins including laminin, and fibronectin (Ruoslahti and Pierschbacher, 1987). Integrins also interact with the cytoskeleton, and pass signals across the cell membrane and therefore have a role in the regulation of cell function.

3.Immunoglobin super family adhesion molecules:

Are also transmembrane molecules which includes VLA-4 which binds to VCAM-1 a ligand expressed by epithelial cells (Hemler et al, 1990).

In addition to its role in maintaining normal tissue structure, studies by Gadbois et al (1997) and Nigro et al (1997) demonstrate that loss of adhesion may result in the reduction of cell cycle check point controls.

The extracellular matrix

There are two types of extracellular matrix (ECM):

1. The basement membrane, a thin matrix which provides the foundation for epithelial cells, separates the tissue from the surrounding stroma and acts as a barrier controlling the passage of macromolecules and penetration by cells. The components of the basement membrane are in contact with the cells via receptors and cell adhesion molecules and are involved in the determination of the structure, polarity, and migration of the cells (Martinez-Hernandez and Amenta, 1983; Timpl and Dziadek, 1986; Birchmeier, 1993).

2. The interstitial connective tissue, found separating structures of the lobules of the breast.

The basement and interstitial connective tissue vary in organisation but are composed of similar components; collagens, glycoproteins, and proteoglycans. Adhesion to ECM molecules, via secondary signals, helps regulate cell division (Gadbois et al, 1997) and cell cycle checkpoint control via p53 (Nigro et al, 1997).

1.2 THE NORMAL CELL CYCLE

The normal cell cycle is a continuous process composed of four stages:

M phase : Mitosis : active cell division

G_1: gap phase one : cell prepares for DNA replication

S phase : synthesis phase : DNA is duplicated to form bivalent chromosomes

G_2: gap phase two : cell prepares for cell division

Those cells which are not actively dividing may either be terminally differentiated and unable to re-enter the cell cycle, or may be in a state of rest (G_0), not actively dividing but still capable of re-entering the cell cycle if subjected to the suitable stimulus.

1.2.1 GENERAL PATHWAY OF INITIATION

Normal breast tissue is regulated by an interaction of many hormones and growth factors. Growth factors bind to receptors on the surface of the cell, for example epidermal growth factor (EGF) binds to EGF receptors (EGFR) and activates them. After binding, some receptors have intrinsic protein tyrosine kinase activity such as EGFR. Receptors which do not have intrinsic protein tyrosine kinase activity activate intracellular protein kinases, which are proteins on or near the inner membrane, and pass the stimuli along to the secondary messengers which are phosphorylated proteins.

A series of secondary messengers pass the signal across the cytoplasm, it then crosses the nuclear membrane where transcription factors are activated and DNA synthesis occurs allowing the S phase of cell division. The steroid hormone receptors e.g. oestrogen, and progesterone receptors, are located within the nucleus, and are activated by binding of the respective hormone.

1.2.2 CONTROL OF THE CELL CYCLE

Growth factors stimulate the cell to progress from G_0 to G_1 and once stimulated the events of cell division are controlled by cyclins, proteins which then form complexes with the protein kinases CDKs. The cyclin/CDK complexes are then able to regulate cell division by phosphorylating the substrate proteins required during the cell cycle (Table 1.3). Once the cell has entered the cell cycle there are check point controls, including those between G_1 - S, and G_2 - M which function by ensuring that key events in the cycle have occurred correctly before mitosis can proceed. Cyclin dependant kinase inhibitor proteins (CKIs) bind to cyclin/CDK complexes and inhibit function thus stopping the cell cycle (Hartwell and Weinert, 1989) (Table 1.3).

Table 1.3 Cyclin / CDK complexes and interaction with CKI's

stage of cell cycle	cyclin	associated CDK	Function	associated CKI
Stage G_1	D	CDK4 CDK6	Control entry into S phase. Initiate DNA replication. The START checkpoint after which cells are committed to replication[a]	p16[e] p15[f] p27[g]
late G_1 S Phase	E	CDK2 CDK2	Drives the cell through the G_1 /S checkpoint[b] Starts S phase and ensures progression through to M[c]	p27[g] p21[h]
Stage G_2	B	CDK1	Controls transition from G_2 to M[d]	

a:(Scheer, 1993) b:(Reznitsky et al, 1995) c:(Girad et al, 1991, Schulman et al, 1998) d:(Cardoso et al,1993) e:(Serrano et al, 1993) f:(Hannon and Beach, 1994) g:(Koff et al, 1993) h:(Dulic et al, 1994)

Rb, and P53 are proteins which interact with cyclin/CDK complexes and CKI's in many of the pathways controlling cell division and apoptosis (Figs 1.3 and 1.4). Apoptosis is genetically controlled single cell death (Kerr et al, 1972). It can occur as part of normal development, e.g. the regression of normal glandular breast tissue after

Fig 1.3 Rb in G_1 check point control pathway

ACTIVE
Rb
(non phosphorylated)

INACTIVE
Rb
(phosphorylated)

Release of
transcription
factor
E2F
enables cell to
enter S phase

S
Phase

feedback

Cyclin D / cdk 4, 6   and   Cyclin E / cdk 2

p16 CKI
p15 CKI

p21 CKI
p27 CKI
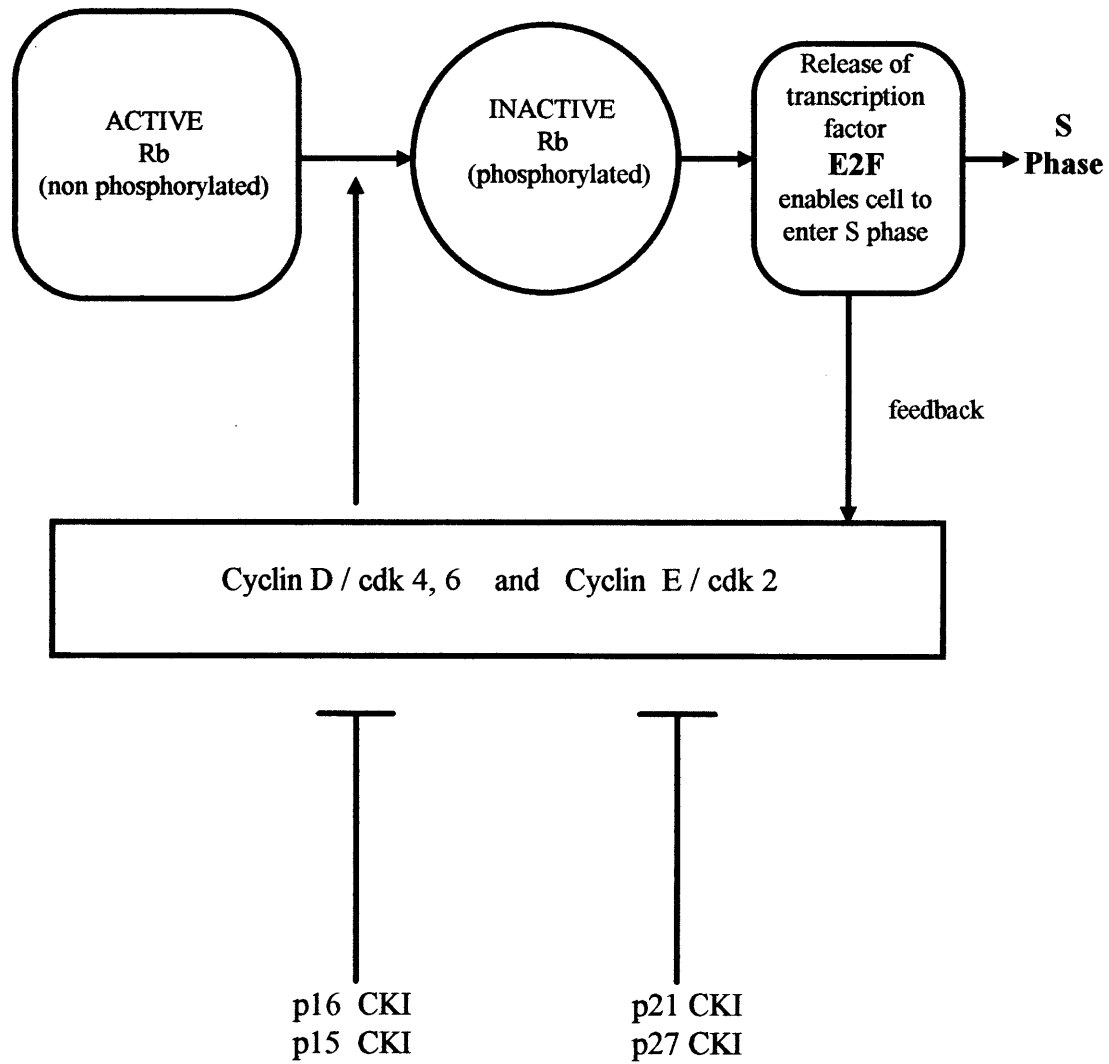
Adapted from Marshall (1999)

Fig 1.4   p53 in apoptosis and cell cycle arrest

Apoptosis

Growth arrest to allow DNA repair

Normal p53

DNA damage

Mutant p53

Normal Cell

Cell with mutations and chromosomal aberrations

Clone of cells with mutations and chromosomal aberrations and possible malignant potential
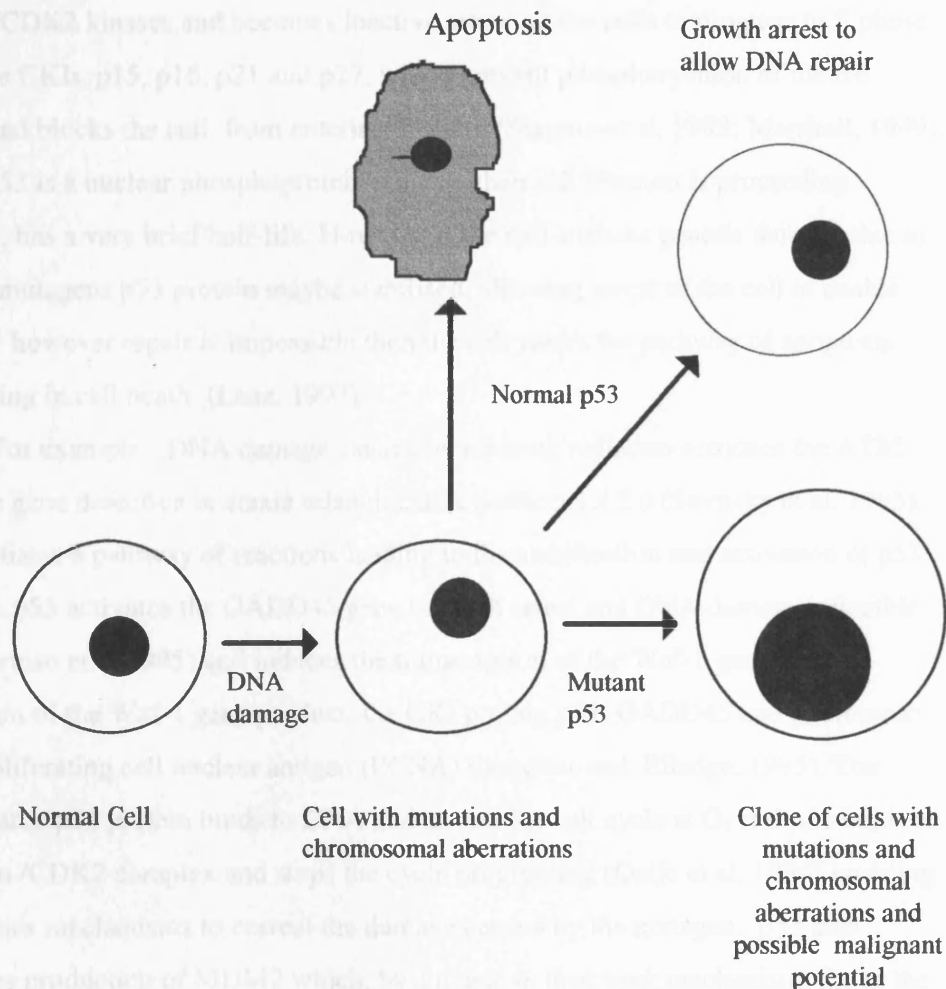
Adapted from Carson and Lois 1995

Rb when activated (underphosphorylated) stops the cell from making the transition from G_o or G_1 to the S phase by binding transcription factors in the pocket region of the molecule. When the cells are subjected to stimulation (e.g. by a growth factor) the Rb protein is phosphorylated by cyclin D/CDK4, 6 and subsequently by cyclin E/CDK2 kinases and becomes inactive allowing the cells to progress to S phase. Therefore CKIs, p15, p16, p21 and p27, which prevent phosphorylation of the Rb protein and blocks the cell from entering S phase (Serrano et al, 1993; Marshall, 1999).

p53 is a nuclear phosphoprotein which, when cell division is proceeding normally, has a very brief half-life. However if the cell sustains genetic damage due to external mutagens p53 protein maybe stabilised, allowing arrest of the cell to enable repair, if however repair is impossible then the cell enters the pathway of apoptosis culminating in cell death (Lane, 1993).

For example: DNA damage caused by ionising radiation activates the ATM gene (the gene defective in ataxia telangiectasia (section 1.4.3)) (Savitsky et al, 1995). ATM initiates a pathway of reactions leading to the stabilisation and activation of p53 (Fig 1.5). p53 activates the GADD45 gene (growth arrest and DNA damage inducible gene) (Artuso et al,1995) and induces the transcription of the Waf-1 gene and the production of the Waf-1 gene product, the CKI protein p21. GADD45 and p21 interact with proliferating cell nuclear antigen (PCNA) (Sanchez and Elledge, 1995). The accumulated p53 protein binds to DNA and arrests the cell cycle at G_1 and p21 binds to the cyclin /CDK2 complex and stops the cycle progressing (Dulic et al, 1994), enabling DNA repair mechanisms to correct the damage caused by the mutagen. p53 also stimulates production of MDM2 which, by a negative feed back mechanism, limits the function of p53 by blocking its transcriptional activation domain (Oliner et al, 1993).

There is also p53 dependant regulation of the G_2/M checkpoint (Fig 1.6), two proposed mechanisms are:

By decreasing intracellular levels of cyclin B1 and inhibiting cyclin B1 promoter activity (Innocente et al, 1999) since cyclin B1 is required for the G_2/M transition this mechanism blocks the cycle at G_2.

p53 dependant over expression of 14-3-3σ, a protein related to the Rad protein mitotic regulators in yeast, also effects an G_2 block (Hermeking et al, 1997). 14-3-3σ binds to the activator of cyclin B/CDK1 and blocks progression to M.

Fig 1.5 p53 regulation of growth arrest and apoptosis
at the G_1 checkpoint control

DNA DAMAGE

increase

P53

-ve Feed-back

increase

MDM2

P21

increase   increase

GADD45

Cyclin
CDK

PCNA

PCNA

P21   Cyclin
CDK

APOPTOSIS
PATHWAY

CELL ARREST AT G_1
AND DNA REPAIR
PATHWAY

Adapted from Carson and Lois 1995
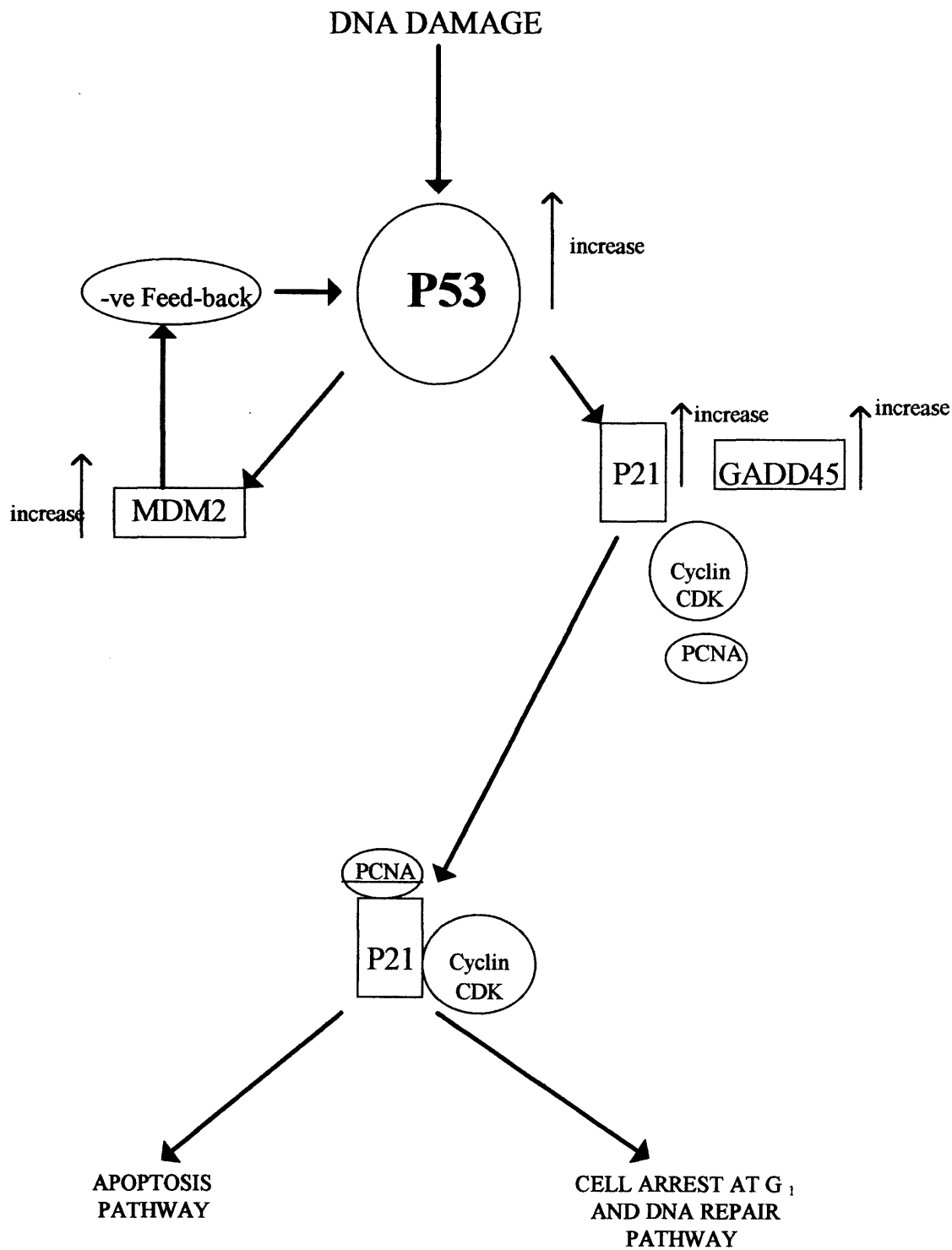
Fig 1.6 p53 in G_2 checkpoint control

DNA DAMAGE

Chan et al 1999, Innocente et al 1999, Piwnica-Worms 1999
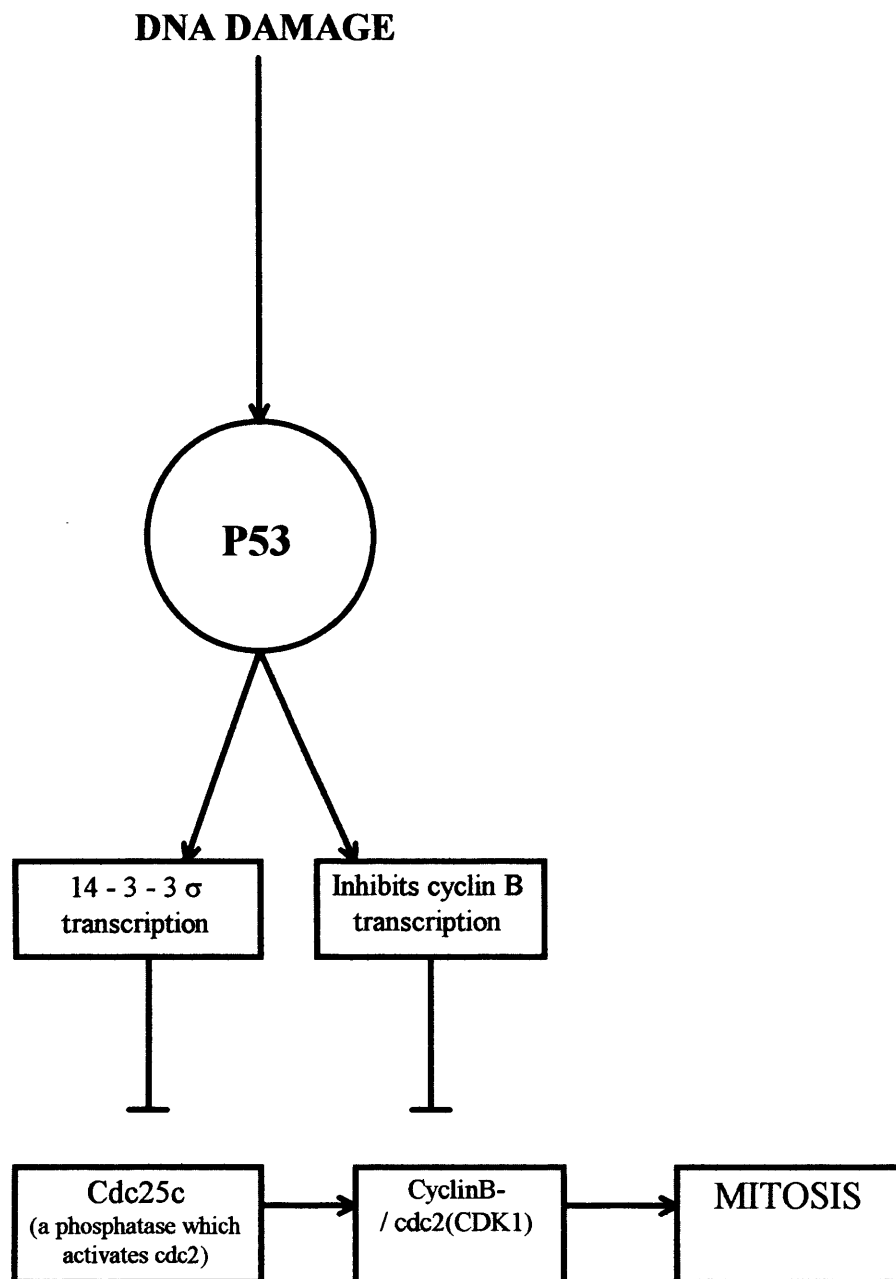
The above research on the p53 dependent check point was completed on ovarian and colorectal tumour cell lines and the relevance of either to breast has yet to be established.

P53 has also been implicated in the regulation of a mitotic spindle checkpoint (Cross et al, 1995), and may also induce growth suppression in neighbouring cells (Komarova et al, 1998). Due to its monitoring role and its involvement in many inhibitory pathways p53 has been named 'the guardian of the genome' (Lane, 1993). P53 can therefore be seen to have a central position in both the DNA repair and cell apoptosis pathways (Fig 1.4).

1.3 NEOPLASIA

The process of neoplasia can be divided into two main stages: initiation and promotion.

1.3.1 INITIATION AND PROMOTION

The cells of the human body are subjected to an almost continuous assault by potential initiating factors in particular carcinogens and UV radiation; and it is probable therefore that there will always be some initiated cells present. A few viruses have also been associated with the initiation of carcinogenesis in specific human tumours. E.g. Papillomavirus with carcinoma of the cervix, Epstein-Barr virus with Burkitts lymphoma, and Hepatitis B virus with hepatocellular carcinoma. An inherited genetic lesion may give cells of an individual a predisposition to develop tumours (Table 1.7) and act as an initiating step. Most of the genetic lesions in initiated cells are repaired by the cells repair mechanisms or where repair is impossible subjected to apoptosis as part of the normal cell cycle control (section 1.2.2).

Initiation is however is not sufficient on its own to produce tumours, a promotion factor is required to facilitate cell division of the initiated cell. Promotion factors could include growth factors and hormones, or mutation or loss of the genes responsible for the DNA repair mechanisms and induction of apoptosis would also facilitate the progression of the tumour. The theory of clonal expansion postulates that after exposure to the initiation factor and induction of cell proliferation by a promoting factor many different mutations occur and those cells having mutations which gives a proliferative advantage are selected for. This then becomes the dominant cell line (Nowell, 1976). Experimental evidence has demonstrated the clonal nature of many tumours (Fialkow, 1976; Nowell, 1976; Noguchi et al, 1992) which gives added credence to the theory of clonal expansion.

1.3.2 GENETIC CHANGES

Karyotypic changes in neoplasia were described by Boveri (1914), and types of chromosomal aberrations found in neoplasms are outlined in Table 1.4. If a section of chromosome is deleted or duplicated the genes encoded by that section are lost or increased accordingly, and similarly if a translocation occurs the genes are also subjected to a new location possibly coming under new promoters or becoming non functional due to disruption of the coding sequences.

Table 1.4 **Types of chromosomal aberrations found in neoplasia**

Class	Aberration	Net change in genetic material	Example
Numeric	monosomy	loss	monosomy chromosome 9 bladder carcinoma[a]
Numeric	polysomy	gain	trisomy chromosome 7 colon cancer[b]
Structural	deletion	loss	retinoblastoma del(13q14)[c]
Structural	amplification	gain	neuroblastoma dup(1 p31-32)[d]
Structural	translocation	no net loss or gain	chronic myeloid leukaemia t(9;22)[e]
Structural	Inversion	no net loss or gain	inv (16) Acute Myelo monocytic leukaemia[f]

Refs: a:(Gibas et al, 1984) b:(Ochi et al, 1983) c:(Knudson, 1985) d:(Schwab et al, 1984) e:(de Klein et al, 1992) f:(Larson et al, 1986)

Two particular classes of gene are associated with neoplasia, oncogenes and tumour suppressor genes.

Oncogenes

Originally identified in transforming viruses, v-oncs, these genes are now realised to be genes found in the normal genome. Proto-oncogenes are genes required for the signal transduction cascade and cell division, but which when modified become oncogenic, c-oncs (Verma, 1986). The normal function of oncogenes is to respond to growth initiation signals and overexpression or deregulation can result in unregulated growth (Table 1.5).

Table   1.5   **Mechanisms of oncogene activation:**

Type	Mechanism	Example
Structural change	Point mutation, resulting in a change in gene product.	Mode of activation frequently seen in Ras[a]
Structural change	Chromosomal translocation within coding regions giving rise to a fusion gene product.	Ph[1]chromosome in CML[b] Abl:Bcr fusion gene product[c]
Deregulation	Chromosomal translocation: gene under the influence of a strong or constitutive promoter.	Burkitts Lymphoma $t(8;14)^d$
Deregulation	Amplification of gene:formation of homogeneously staining regions (HSRs) and double minutes (Dmins)	Neuroblastoma amplification of Nmyc[e,f]

Refs: a:(McGrath et al, 1984) b:(Nowell and Hungerford, 1960) c:(Shtivelman et al, 1985) d:(Croce and Nowell, 1985) e:(Seeger et al, 1985) f:(Schwab, 1984)

Oncogenes can be classified into several "families" according to their position in the signal transduction cascade (Weinberg, 1989) see Table 1.6.

Table 1.6 **Oncogene families**

Class	Example	Action
Growth factor	c-sis	βchain of platelet derived growth factor (PDGF)[a]
Receptor	c-erb-B2	overexpression of cerbB2 associated with aggressive breast cancer[b]
Signal transducing	GTP binding proteins e.g. Ras non receptor kinase c-abl	Ras is mutated and is constituatively active [c] translocation t(9;22) and the formation of the abl:bcr fusion protein with increased kinase activity[d]
Nuclear proteins	Myc	activated by amplification[e] and deregulation [f]
Apoptosis	Bcl-2	over expression blocks apoptosis[g]

a: (Waterfield et al, 1983) b: (Slamon et al, 1987) c: (McCormick, 1993) d: (Sawyers et al, 1991) e:(Koskinen and Alitalo, 1993) f:(Jain et al, 1993) g:(Hockenbery, 1992)

Tumour Suppressor Genes

Another group of genes whose normal function is to respond to growth inhibitory signals causing the stoppage of the cell cycle or apoptosis (section 1.2.2) are the tumour suppressor genes (TSG).

Early cell fusion experiments (Harris et al, 1969) observed that the fusion of a normal cell with a tumour cell tended to result in the suppression of the malignant phenotype. From these observations it was apparent that some mutated genes causing the tumour were recessive to the normal wild type genes and that transformation and expression of the malignant phenotype appeared to be under separate genetic control (Stanbridge and Wilkinson, 1978).
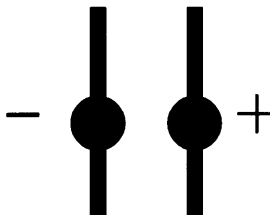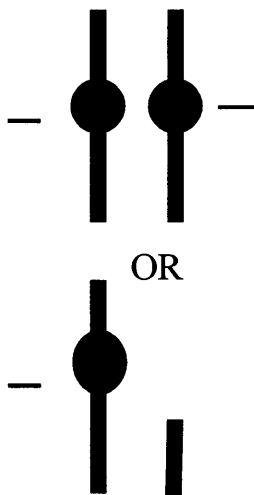
Knudson (1971), developed a two hit hypothesis for tumour development in retinoblastoma. Knudson's hypothesis postulated that in the familial form of the disease the child inherits a mutation present in the germline of one of the parents and only when a second somatic mutation occurs is the disease manifest, whereas in the sporadic form no mutation is inherited, and for the disease to occur two somatic mutations must arise in the developing retinal cells. This is generally known as the two-step or two-hit hypothesis (Fig1.7). The germline mutation anticipated in Knudson's early papers was later identified a deletion of q14 band on chromosome 13. Knudson used the term anti-oncogenes to describe this class of gene (Knudson, 1985), these are now called tumour suppressor genes (TSG). Some TSGs in inherited syndromes are listed in Table 1.7.

Table 1.7 **Tumour Suppressors with known germline mutation**

Gene	Syndrome	Effect of mutated gene
p53	Li-Fraumeni (section 1.4.3)	loss of G_1 / S checkpoint loss or reduction of p53 apoptosis pathway patients with Li-Fraumeni have an increased risk of developing neoplasms including breast carcinoma [a]
Rb	Familial Retinoblastoma	loss of G_o - G_1 / S checkpoint[b]
WT	Familial Wilm's Tumour	loss of normal cell growth repression function[c]
APC	Familial adenomatous polyposis FAP	mutated gene product no longer has the ability to bind β catenins and form adherens juctions[d]

a:(Frebourg and Friend, 1992) b:(Dowdy, 1993) c:(Guan et al, 1998) d:(Bonneton et al, 1997)

Fig 1.7 **Two hit hypothesis**

Mechanism in Inherited Cancers		Mechanism in Sporadic Cancers
STAGE ONE (FIRST HIT TO EITHER CHROMOSOME) • Inherited mutation		**STAGE ONE (FIRST HIT TO EITHER CHROMOSOME)** • Chromosomal rearrangement with gene disruption • New gene deletion • or point mutation
STAGE TWO (SECOND HIT TO NORMAL CHROMOSOME) • Loss of normal chromosome and duplication of abnormal chromosome. • Deletion of normal gene. • New mutation in normal gene • Recombination between chromosomes at mitosis leading to disruption of normal gene		**STAGE TWO (SECOND HIT TO NORMAL CHROMOSOME)** • Loss of normal chromosome and duplication of abnormal chromosome. • Deletion of normal gene. • New mutation in normal gene • Recombination between chromosomes at mitosis leading to disruption of normal gene
	+ Normal (Wild Type) gene – Mutant Allele	

Adapted from Kingston 1989

Some mutant tumour suppressor genes form a protein which binds to the normal gene product, as occurs with some mutant forms of p53, thus resulting in loss of function even though a wild type allele is present. This is known as dominant negative (Lane, 1994). The tumour suppressor genes shown in Table 1.7 also occur in non familial neoplasms. p53, in particular, has a central role in cell cycle control and DNA damage repair (Figs 1.4, 1.5 and 1.6) and somatic mutations of this gene are one of the most frequent in human cancer (Hollstein et al, 1991; Lane, 1994). Other genes involved in negatively regulating growth, inducing apoptosis, DNA repair, and maintaining tissue structure have also been identified as tumour suppressor genes or candidate tumour suppressor genes.

Loss of heterozygosity

In persons with a constitutional karyotype heterozygous for a polymorphic satellite DNA marker, loss of heterozygosity, LOH, of that marker in the tumour reveals the possible position of a tumour suppressor gene closely linked to the marker. LOH is a frequent event in solid tumours indicating the role of tumour suppressor genes in the process of tumourigenesis in these tumours (Hansen and Cavenee, 1987). The term allelic imbalance (Devilee et al, 1991) may be used instead of LOH as being more inclusive, including both allelic change due to amplification as well as deletion of an allele (Chapter 4 section 4.1.1).

1.3.3 GENOMIC STABILITY

Cells have several mechanisms for repairing mutations to the DNA and thus help maintain genomic stability (section 1.2.2). Many of the genes which control these pathways are tumour suppressor genes. If the function of these genes are lost then mechanisms for maintaining genomic stability are no longer present, mutations can occur unchecked and genetic instability may ensue, any mutation which causes an increase in the overall rate of mutation will also serve to accelerate the process of neoplastic progression.

Defective DNA repair

There are some rare autosomal recessive hereditary conditions which exhibit genomic instability due to DNA repair defects.

Bloom's syndrome:

The cells exhibit a high rate homologous recombination and of sister chromatid exchange, and patients are hypersensitive to a wide range of DNA damaging agents and have a predisposition to several types of cancer (German, 1972). It is a defect in the genes producing DNA ligase that is thought to contribute to this syndrome (Barnes

et al, 1992a). Some lines of Bloom syndrome cells have been found to be defective for a radiation induced increase in p53 expression which would normally result in apoptosis (van Laar et al, 1994).

Fanconi's anaemia:

Patients are extremely sensitive to DNA x-linking agents, due to an absence of the enzymes which eliminate these cross linkages, and have a predisposition to develop leukaemia. There are apparently at least four pairs of genes involved in this disease (Strathdee et al, 1992). The pathway of p53 radiation induced apoptosis appears to be deficient in these patients (Rosselli et al, 1995).

Ataxia-telangiectasia (AT):

A genetically heterogeneous disease with at least six different molecular forms. Patients have a defective repair mechanism for dealing with radiation induced DNA damage, and are predisposed to develop lymphomas (Taylor, 1992). Individuals heterozygous for AT mutations have a 5-8-fold risk of developing breast cancer (Swift et al, 1987). (The role of AT gene and breast cancer is discussed in the section 1.4.3)

Individuals with these DNA repair deficiency syndromes are at an increased risk of developing tumours, and a study of the genes involved gives an insight to the role of genomic instability in neoplasia in general.

Defects in genes responsible for mismatch repair, *h*MSH2, *h*MLH1, *h*PMS1, *h*PMS2 have been correlated to microsatellite instability (MSI) in tumours (Bodmer et al, 1994; Nicolaides et al, 1994; Papadopoulos et al, 1994). Microsatellites are repetitive nucleotide sequences scattered through the genome, many of which exhibit individual specific length polymorphisms in normal tissue (Jeffreys et al, 1985). Variation in the number of repetitive sequences at a given locus has been observed in tumour tissue when compared with normal tissue from the same patient (Thein et al, 1987, Matsumura and Tarin, 1992). MSI is marked by expansion or contraction in the number of repeats in the tumour caused by slippage due to strand misalignment during DNA replication and was found in several studies on colorectal carcinoma. (Aaltonen et al, 1993; Thibodeau et al, 1993; Ionov et al, 1993), particularly in younger patients (<35 years) (Liu et al, 1995). MSI has also been recorded in other tumours including breast (Yee et al, 1994; Patel et al, 1994; Field et al, 1995; Watanabe et al, 1995; Shaw et al, 1996) and is reported as being more prevalent in post menopausal breast cancer patients (Fujii et al, 1998).

1.3.4 A MODEL OF GENETIC MECHANISMS INVOLVED IN THE PROCESS OF NEOPLASIA

Colorectal cancer has provided a model of the process of neoplasia in solid tissue, illustrating the multistep hypothesis of tumourigenesis. There are inherited syndromes which predispose individuals to develop colorectal adenomas and carcinomas and these have been useful in studying the genetic changes. (Table 1.8) The mutation of the APC (Adenomatosis polyposis coli) gene in FAP (Familial adenomatous polyposis), and the mismatch repair genes, hMSH$_2$ and hMLH$_1$, in HNPCC (Hereditary non polyposis colon cancer) results in hyperproliferative epithelium. Further mutations in K Ras, deletion of DCC gene (deleted in colon cancer gene), and mutation or deletion of p53, cause the hyperproliferative state to become malignant (Fearon, 1992). The DNA in the malignant cells is now unstable and further mutations occur during the progression of the tumour.

Table 1.8 Inherited syndromes predisposing individuals to colorectal neoplasms

Inherited syndrome	Early genetic changes	Description
Familial adenomatous polyposis FAP	Early LOH of APC, Hyperploidy seen in malignant foci of large adenomas	Hundreds of diploid polyps of which only a small fraction develop to colorectal neoplasms Patients with FAP only develop tumours in the colorectal region however patients with Gardener Syndrome (GS) have symptoms identical to FAP, but in addition also develop tumours in other regions.
Hereditary non polyposis colorectal cancer. HNPCC	Apparent constitutional tendency to tetraploidy[a], MSI[b]	No polyps Lynch syndrome type I develop tumours only in the colorectal region. Lynch syndrome type II develop tumours at other sites in addition to the colorectal region.

a (Lynch et al, 1985) b (Liu et al, 1995)

The observations that FAP and GS, and similarly Lynch type I and type II, only vary from each other by the location of tumours suggests a mechanism responsible for tumour site specificity, the genetics of this mechanism however has yet to be fully explained.

The process of mutation and selection is repeated resulting in the evolution of a neoplastic clone. An observation which supports this is that most adenomas appear to be monoclonal whereas the normal mucosa may have several separate cell lines (Fearon, 1992). Although there is a progression from normal to adenoma (if present) and then to carcinoma the precise sequence of genetic events may vary (Vogelstein et al, 1988), the total sum of events being more critical than the sequence (Fearon and Vogelstein, 1990). Colorectal carcinoma represents a progression from normal to benign and then to malignant, involving oncogenes, tumour suppressor genes and MSI and thus represents a model to which other tumours can be compared.

The characteristics which differentiate a benign neoplasm from a malignant neoplasm are its ability to invade surrounding tissue and to metastasise to distant sites.

1.3.5 INVASION AND METASTASIS

Deregulated cell growth and subsequent clonal expansion gives rise to a mass of cells, a neoplasm, which may be either benign or malignant. The capability to invade surrounding tissue and therefore possibly spread to distant sites (metastasis) is the characteristic which classifies a neoplasm as being malignant, and which has no direct equivalent in normal breast tissue.

Angiogenesis, the microvascularization of the tumour is required if the tumour mass is to exceed 2-3mm in diameter (Ellis and Fidler, 1995). Blocking angiogenesis in mouse models significantly retards tumour growth (Hori et al, 1991). Angiogenesis also increases the risk of metastases (Weidner, 1992), tumours with no micro vascularization rarely invade the lymph or blood vessels (Liotta et al, 1976). The microvessels also secrete degradative enzymes which facilitate the extension of the vessels and their invasion by tumour cells (Rifkin et al, 1981).

During tumourigenesis there is breakdown of normal tissue organisation, and therefore, in epithelial tissue, of the interactions which keep cells attached to a basement membrane and maintain cells in a specific location. The genes for cell adhesion products, basement membrane components, and receptors may therefore be altered, deleted or their function negated resulting in or secondary to the process of invasion. Enzymes such as proteases may also be produced by the malignant cells, and the physical pressure exerted by the growing tumour may result in the breach of the basement membrane. Once this has occurred the process of invasion and possible subsequent metastasis can be said to have begun. Metastasis has three main phases firstly the invasion of the ECM, followed by vascular dissemination and then relocation and growth of tumour cells.

Invasion of the ECM

Initially there is a loss of cell : cell interaction. This is achieved by down regulation of the CAMs, in breast neoplasms particularly E-cadherin (Oka et al, 1993), and α catenin (Rimm et al, 1995) as well as changes in β catenin molecules and plakoglobin (Sommers et al, 1994), which are involved in the functioning of E-cadherin. In breast carcinoma there appears to be a correlation between the density of laminin receptors and the invasiveness (Cioce et al, 1991) whereby loss of receptors can result in a loss of the normal relationship and encourage the ability to spread.

In addition to the mechanical pressure exerted by the growing neoplasm, there is enzymatic degradation of the ECM by proteases (Steeg, 1992). Migration of the tumour cells is stimulated by cytokines such as the autocrine motility factor, produced by the cell and activating receptors on the cell surface (Nabi et al, 1992). The proteinases then attack the basement membrane of the vessels allowing intravasation to occur.

Vascular dissemination

Following intravasation the endothelial cells of the vessel release E-selectin, a carbohydrate binding compound which attracts T-lymphocytes to the site (Lasky, 1992). Once in the circulation neoplastic cells interact with the lymphocytes, and in the blood also with platelets, to form an emboli.

Relocation at a distant site : Soil and seed theory

The formation of emboli may assist the neoplastic cells in relocating since the lymphocytes express L-selectin on their surface which initiates the adherence of leucocytes to vascular endothelium (Ley et al, 1991). Although the metastatic site is to a certain extent governed by the path taken by the emboli, neoplasms have a tendency to be site specific in the establishment of metastases dependant on the location of the primary neoplasm. For example most breast neoplasms tend to metastasise to bone, lungs, liver or brain, this tendency is described by the Soil and Seed Theory (Paget, 1889). The migration to specific sites could be influenced by :

i. Growth factors or hormones produced by the target organ.

ii.The types of CAM's receptors on the surface of the neoplastic cells.

iii. Locations may produce inhibitors which prevent growth of a specific neoplastic cell type (Rusciano and Burger, 1992).

It is however evident that the control of invasion and metastases has many interacting components and is therefore probably not attributable to any single gene. The neoplastic cell is under severe selective pressure during the process of metastasis and

very few cells released into the circulation will actually reach the target organ and become established (Yosida, 1983).

1.4 BREAST CANCER

There are many different parameters which may be measured to indicate the probable course of breast cancer. Prediction of behaviour has been assessed using clinical, pathological, and biological features.

1.4.1 CLINICO-PATHOLOGICAL ASSESSMENT

Breast cancer can be assessed clinico-pathologically according to its stage, histological type and grade:

Stage

The stage defines the extent of spread of the disease. Most of the commonly used staging systems are modifications of the 1989 Pathologic classification of the UICC-AJC(Union Internationale Contre Cancer-American Joint Committee) (American Joint Committee on Cancer 1989). The TNM (Table 1.9) system stages tumours according to size of primary tumour, extent of regional lymph node involvement, and presence of distant metastasis, the stage in the TNM system being defined by the three letters with the appropriate subscripted number.

Table 1.9   T N M   System

Symbol	Description
T	Tumour size; ranging from $T_0 < 20$mm to $T_4 > 100$mm
N	Node status N_0 Node negative N_1 Axillary node positive, mobile N_2 Axillary node positive, fixed nodes N_3 Supraventricula nodes positive
M	M_0 No distant metastases M_1 Distant metastases

Stage may also be numbered I-IV according to clinical findings as described in Table 1.10.

Table 1.10 **International Classification of Stage**

Stage	Description	Equivalent TNM stages[a]
I	Breast lump, lymph nodes not involved, slightly attached to the skin	T1N0M0.
II	Breast lump, lymph node involvement, or attached to the skin	T0N1M0, T1N1M0,T2N0M0, T2N1M0, T3N1M0.
III	Neoplasm extensively attached to the skin, or fixed lymph nodes	T0N2M0, T1N2M0,T2N2M0, T3N1M0, T3N2M0, T4NnM0, TnN3M0
IV	Presence of distant metastases	TnNnM1

n = any number   a : American Joint Committee on Cancer 1989

The individual components of the TNM system are correlated to patient prognosis. Tumour size and axillary lymph node involvement are independent prognostic indicators (Carter et al, 1989). Patient prognosis is inversely related to the number of positive axillary lymph nodes (Ferguson et al, 1982). Tumour size is directly related to the probability of metastasis (Koscielny et al, 1984) the presence of which is a sign of advanced systemic disease, patients with tumours less than 1cm generally having a significantly better survival rate than patients with tumours bigger than 2cm (Peer et al, 1996).

Histological Type

Breast neoplasms can either be in situ or invasive, giving two basic sub-divisions of classification. These are further divided up according to histological appearance and grade (Table 1.11).

In situ ductal carcinomas (DCIS) are confined within the basement membrane although have the potential to invade (Silverstein et al, 1987). Patients with DCIS have a better prognosis than patients with invasive carcinomas. Lobular carcinoma in situ is considered to be an "at risk" lesion.

There are several different identifiable histological patterns of DCIS (Table 1.11). High grade comedo type tumours appear to be more aggressive and associated with a higher rate of recurrence (Lagios et al, 1989; Patchefsky et al 1989) and hence poorer outcome than low grade non comedo type tumours.

Invasive carcinomas can be divided into specialised types and non-specialised. Infiltrating ductal carcinoma not otherwise specified is the most frequent type. Of the specialised types tubular carcinomas are well organised and are associated with a favourable prognosis (Carstens et al, 1985). Medullary carcinoma, a relatively uncommon tumour, also has a favourable prognosis (Rosen et al, 1982) and a low frequency of axillary lymph node involvement (Wargotz and Silverberg, 1988). Two other uncommon variants, mucinous (Rasmussen et al, 1985) and invasive papillary carcinoma (Fisher et al, 1980) are both associated with a favourable prognosis and low rate of recurrence. The knowledge that a patient has a tumour with a favourable prognosis aids in the selection of post-surgical treatment.

Table 1.11 **General classification of breast neoplasms**

	Location/Description
Insitu (non invasive)	ductal carcinoma in situ, (DCIS) in situ tumours are graded high, intermediate and low formally described by histological architecture into comedo, and non-comedo non comedo comprising of other histological patterns such as cribriform, micropapillary, papillary, solid. lobular carcinoma in situ,(LCIS)
Invasive	Infiltrating ductal carcinoma (IDC) not otherwise specified (NOS) and specialised types with characteristic histological patterns medullary, mucinous, invasive papillary, and tubular Infiltrating lobular carcinoma (ILC)

The prognosis for patients with ILC depends on stage at presentation and on the histological sub-type (Dixon et al, 1982). The diagnosis of ILC is clinically relevant however because ILC can be multicentric and 25% of patients will develop a tumour in the contralateral breast (Richter et al, 1967; Lesser et al, 1982). Metastases from IDC tend to occur in the liver, bones, and parenchyma of the brain, whereas ILC has a predisposition to metastasise to the abdominal cavity and gastrointestinal tract (Harris et al,1984), the ovaries and uterus (Kumar and Hart, 1982), and the meninges of the brain (Smith et al, 1985).

Grade

The grade of a neoplasm is a measurement of the extent of which the cells have deviated from the normal. Two main approaches have been used to assess grade, dependant either on nuclear characteristics, or a combination of nuclear, cytological, and structural characteristics. The most widely used system is the modified Bloom and Richardson (Elston and Ellis, 1991) (Table 1.12) which uses a combined approach. Points are allocated for tubule formation, nuclear pleomorphism, and mitotic activity (Fig 1.8).

Table1.12 : **Modified Bloom and Richardson grading system**

Grade	Score	Degree of differentiation
I	3 -5	well differentiated
II	6-7	moderate
III	8-9	poor

The Bloom and Richardson method of grading has been shown to be prognostically significant (Elston and Ellis, 1991).

1.4.2 BIOLOGICAL MARKERS

Biological markers which prove to have clinical significance can provide information about aetiology, assess the probability and possible location of recurrence, and indicate the general prognosis. Some tumour markers may also indicate which treatment is most suitable for the patient.

Steroid Receptors

Much of the normal functioning and development of the breast is controlled by hormones especially oestrogen and progesterone (Forsyth, 1991) (section 1.1.2), and hormones also have an important role in breast neoplasms. It is only in the presence of an appropriate receptor, that a hormone can induce a response in the cell.

Fig 1.8 Histological grading (modified Bloom and Richardson)

Tubule Formation

75%	=	1
10-75%	=	2
<10%	=	3

Nuclear pleomorphism

mild	=	1
moderate	=	2
marked	=	3

*Mitosis / 10 high power fields

eg. (for Nikon LabPhot)

0 - 0.5	=	1
0.6 - 1.99	=	2
>2.0	=	3

TOTAL

3 - 5
Grade I
well differentiated

6 - 7
Grade II
moderately differentiated

8 - 9
Grade III
poorly differentiated
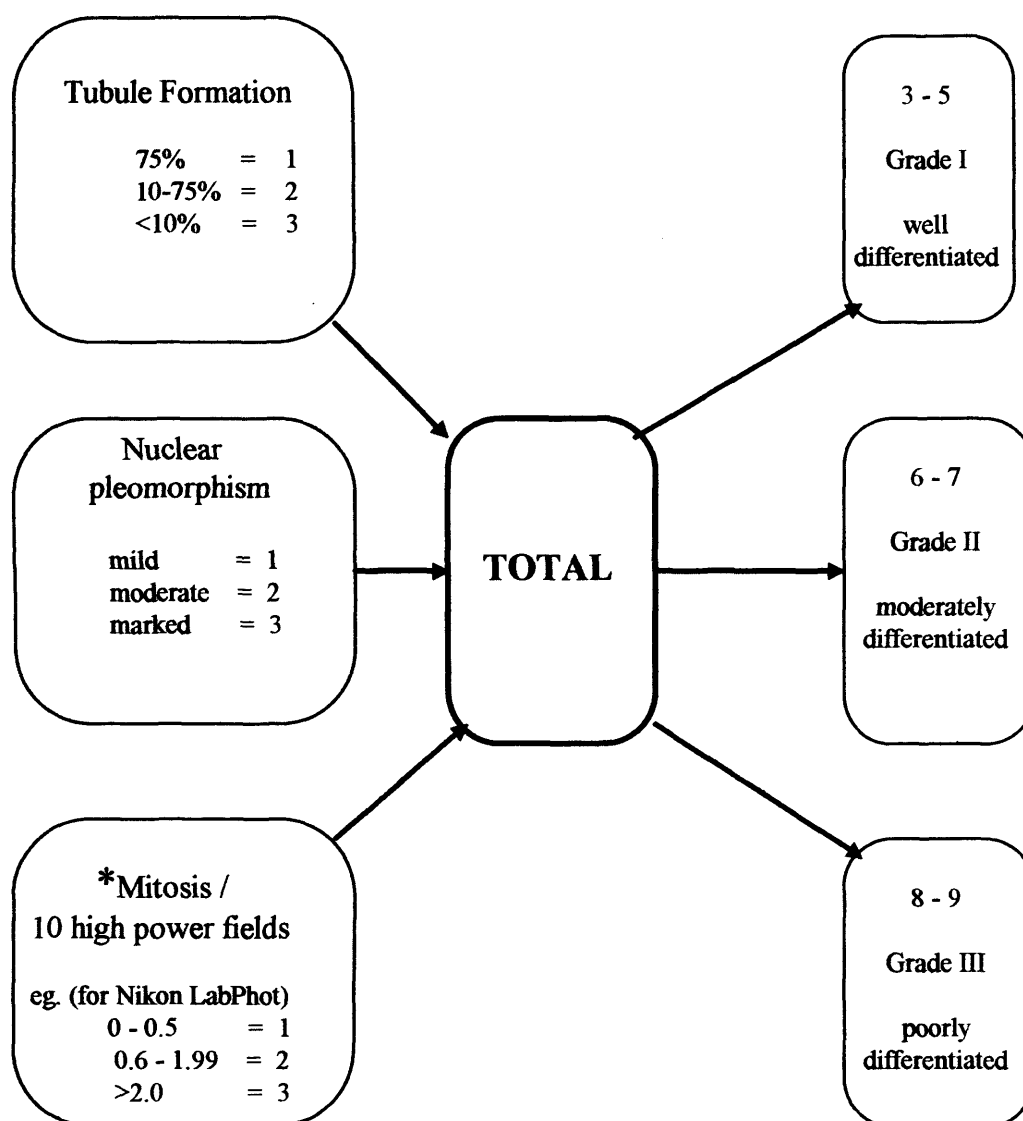
* Now modified in the NHSBSP guidelines for Pathology reporting, 2nd Edition
Mitosis counts are scored on a microscope specific basis, differing according to high power field size

Oestrogen receptor

The oestrogen receptor, ER, is a nuclear protein which has both hormonal and DNA binding domains (Kumar et al, 1986) which are both necessary for normal functioning. (Beato, 1989). In the normal breast ER changes with the menstrual cycle and with age. ER can be found in 60-70% of breast neoplasms, and is more frequently found in better differentiated carcinomas (Walker and Varley, 1993). The percentage of ER+ve tumours increases with age (Nicholson et al, 1995), and is a useful marker for potential response to endocrine therapy. With the identification of a second oestrogen receptor, ER β (Enmark et al, 1997) this is now known as ER α.

Progesterone receptor

The progesterone receptor, PgR, is regulated by oestrogen via the ER. Therefore the majority of neoplasms are either ER+ve/PgR+ve or ER-ve/PgR-ve, there are however some which are ER-ve/Pgr+ve (Clark and McGuire, 1989) The absence of PgR in breast neoplasms may be due to either defects in ER or it the PgR itself. LOH has been recorded about the PgR locus, but does not appear to correlate to loss of expression of the PgR (Walker and Varley, 1993).

Presence or absence of ER and PgR is clinically significant and allows deductions to be made as to the probable course of the disease and response to treatment. Tumours with a high concentration of ER and PgR are more likely to respond to endocrine treatment (Voogd et al, 1998; Castagnetta et al, 1999). There also is a variation in the probable site of metastasis; ER+ve tumours tend to metastasise to bone, whereas ER-ve tumours tend to metastasise to organs such as the brain, and liver (Campbell et al, 1981). Generally speaking ER+ve tumours are correlated with favourable characteristics (e.g. low grade), and hence ER+ve patients tend to have a more favourable prognosis (Blamey et al, 1980).

Growth Factor Receptors

Epidermal growth factor receptor, EGFR, and epidermal growth factor, EGF, are both present in normal epithelial tissue. Approximately 40% of primary breast carcinomas over express EGFR (Sainsbury et al, 1987; Rios et al, 1988) and the overexpression has been found in many studies to be associated with aggressive tumours and poorer prognosis (Koenders et al, 1993).

Cell Proliferation

In addition to the mitotic index described as part of the grading system there are other markers of cell proliferation which appear to add relevant information to patient prognosis.

The proportion of cells in S-phase represents those cells which are actively dividing (section normal cell division). As might be predicted rapidly dividing cells respond better to chemotherapy, and it has been observed that the proportion of cells in S-phase is positively correlated to the response achieved during chemotherapy (Remvikos et al, 1989).

Angiogenesis

The process of angiogenesis gives rise to microvascularization of the tumour, (section 1.3.5). Microvascular density has been demonstrated to have an inverse relationship to patient prognosis in both lymph node positive and lymph node negative patients (Weidner et al, 1992). Increased density also correlates to an increased risk of metastases (Toi et al, 1993). Gasparini et al (1994) finding microvascularization an independent prognostic factor in node negative and Viens et al (1999) in node positive patients. A few studies however have failed to find correlation (Hall et al, 1992; Goulding et al, 1995); this could be due to different detection techniques, or it could reflect the heterogeneity of breast carcinoma.

1.4.3   GENETICS OF BREAST CARCINOMA

A family history of breast neoplasia is known to increase a womans risk of developing the disease, especially at an early age (Houlston, 1992), and possibly accounts for up to 9% of all breast neoplasms (Lynch et al, 1989). Often such families also show a high rate of bilateral breast carcinoma (Thompson et al, 1994).

Predisposing genes in familial breast cancer

BRCA1 and BRCA2

BRCA1 was localised on chromosome 17q21 by linkage analysis of families with early onset breast cancer (Hall et al, 1990). BRCA1 was cloned and more than 40 mutations identified in the early onset breast cancer families (Miki et al, 1994; Simard et al, 1994; Castilla et al, 1994). However, although BRCA1 has been found, it is very difficult to use for screening purposes to identify those at risk for familial breast and ovarian carcinoma, because of the large number of mutations and the size of the BRCA1 gene, over 100 kilobases long (Futreal et al, 1994; Miki et al, 1994).

BRCA2 was identified in familial breast cancer families which contained at least one affected male (Stratton et al, 1994) and located on chromosome 13q12-13 (Wooster

et al, 1994). Together BRCA1 and BRCA2 account for the majority of familial breast carcinomas.

Li-Fraumeni Syndrome and p53

Patients with Li-Fraumeni syndrome have a germline mutation of p53 (Malkin et al, 1990). In a few families no mutation has been identified (Santibanez-Koreg et al, 1991); recently Bell et al (1999) showed that some of these families have germline mutations of the gene hCHK2, a homologue of the yeast Rad G_2 checkpoint control genes. The Li-Fraumeni syndrome is autosomal dominant, and families with this syndrome develop malignancies at a young age (Li and Fraumeni, 1969). The malignancies often occur during childhood and include sarcomas, adrenal cortical carcinomas, osteosarcomas, acute leukaemia, and brain tumours. The mothers of these children are at risk of developing breast carcinoma, approximately half the women with such a mutation will develop breast carcinoma by the age of 30, and yet there is no excess risk of post-menopausal breast cancer (Narod, 1994). The loss of function of p53 has also been shown to result in increased genomic instability in Li-Fraumeni cells (Liu et al, 1996).

Genes predisposing to sporadic breast cancer

BRCA1, BRCA2 and p53

It has been proposed that BRCA1 and BRCA2 act as tumour suppressor genes in familial breast cancer, but it is not clear if they have the same function in sporadic breast cancer (Yang and Lippman, 1999). Reduced expression of BRCA1 has been shown to correlate to high grade (Wilson et al, 1999) and to predict the occurrence of distant metastases (Seery et al, 1999). Over expression of BRCA2 has been correlated to high grade in sporadic breast cancer (Bieche et al, 1999) LOH BRCA1 has been identified in 21-49% and BRCA2 in 30-38% of breast tumours (Beckmann et al, 1996). However somatic mutations have only rarely been identified for BRCA1 (Langston et al, 1996) and BRCA2 (Lancaster et al, 1996; Miki et al, 1996). Somatic mutations in p53 are very common in breast cancer and other cancers (Harris and Hollstein, 1993) and have been associated with an increase in genotypic instability (Eyfjord et al, 1995).

Ataxia telangiectasia

In addition to the genes involved in familial breast cancer, the gene for human ataxia telangiectasia, ATM, may also be involved in breast cancer. Persons heterozygous for ataxia telangiectasia have a predisposition to develop breast carcinoma (section 1.3.3) increasing the risk of breast neoplasm five fold (Swift et al, 1991) approximately 1.2% of the population are heterozygous for AT (Pippard et al, 1988) and this could

account for 7% of breast carcinoma (Easton et al, 1993). The AT gene although only increasing risk slightly has an additive effect due to the relatively high gene frequency. Deficiency in DNA repair, and the resulting genomic instability, may be a predisposing factor for developing forms of sporadic breast carcinoma (Parshad et al, 1996).

Genetic alterations in sporadic breast cancer

Oncogene amplification

Amplification and increased expression of *ERBB2* occurs in about 20 % of invasive breast carcinomas (Van de Vijver and Nusse, 1991). However c-erb-B2 expression in DCIS has been identified in 60% of DCIS of high grade type, indicating that it is an early alteration within a subset of cancers (Barnes et al, 1992b). Increased expression of the oncogene *ERBB2* have been correlated to adverse factors associated with poorer prognosis (Cline et al, 1987; Walker et al, 1989; dePotter et al, 1990).

MYC amplification and increased expression occurs in about 20% of breast carcinoma (Mariani-Constantini et al, 1988) and has been associated with post menopausal breast carcinoma (Escot et al, 1986). Alterations in the *MYC* gene have been associated with aggressive tumours (Garcia et al, 1989) and poor prognosis (Varley et al, 1987), especially in less well differentiated tumours (Adnane et al, 1989).

Karyotypic changes

Involvement of chromosome arm 16q has been noticed to occur primarily in near diploid neoplasms and therefore may be an early event (Hainsworth et al, 1991), deletions in 3p have also been reported (Pandis et al, 1993). Of the few insitu neoplasms karyotyped, most were in the diploid range, showed less complex karyotypes than the more infiltrating cases, and several contained changes in chromosomes 1 and 16 (Pandis et al, 1992; Neilsen et al, 1989).

Loss of heterozygosity

LOH has been found has been found to varying degrees for all the chromosomes but at rates higher than 35% for chromosome arms 6q and 17q and above 50% for 16q and 17p (Devilee and Cornelisse, 1994). Chromosome arm 17p shows LOH in up to 60% of breast carcinomas, many of which have somatic mutations of p53 (Varley et al, 1991). The topic of loss of heterozygosity and tumour suppressor genes in breast carcinoma is discussed further in chapter 4.

1.4.4 MODELS FOR THE DEVELOPMENT OF BREAST CARCINOMA

Although less evidence exists than for the colorectal carcinoma model (section 1.3.4), a model has been proposed for breast carcinoma.

Evidence for this model is derived from:

i. Retrospective histopathological data

ii. Biological data of changes in e.g. *ERBB2* and *ER*.

A study by Devilee et al (1994) (Fig 1.9) describes the theoretical "normal" stem cells going to a predisposed dividing state, and then giving rise to

a. Atypical Ductal Hyperplasia ADH

b. DCIS

c. IDC

all of this being at a sub clinical level.

Further growth and clonal expansion of DCIS and/or IDC would result in clinical manifestations of the disease and continuing neoplastic progression.

Currently it is not possible to identify either stem cells or "premalignant" cells in normal or non-malignant breast. Observations exist however that help support some of the other steps in the model:

ADH to IDC and DCIS

Atypical ductal hyperplasia, ADH, is a proliferative lesion which has some but not all the features of DCIS (NHSBSP Pathology Reporting Guidelines 2nd Edition 1995). Evidence for it being a precursor lesion comes from the following studies:

i. Women with ADH identified in a previous biopsy have a 4-5 fold increase in the risk of developing breast cancer (Dupont and Page, 1985).

ii. ADH is monoclonal in nature (Noguchi et al, 1994; Lakhani et al, 1995)

iii. Molecular changes have been identified in ADH which also occur in DCIS (Lakhani et al, 1995; Chuaqui et al, 1997; Amari et al, 1999).

DCIS to IDC

Knowledge of the natural history of untreated DCIS in human breast carcinoma is limited because it is normally removed once diagnosed. However, long term follow up of women who were originally diagnosed as having a benign lesion and only had a biopsy, who in fact had low grade DCIS, has shown that 10 of 28 women developed breast cancer, mainly invasive, after 24 years (Page et al, 1995). High grade DCIS have a higher rate of invasion (Lagios et al, 1989), if not excised.

Fig 1.9 **Development of breast cancer, Devilee model**
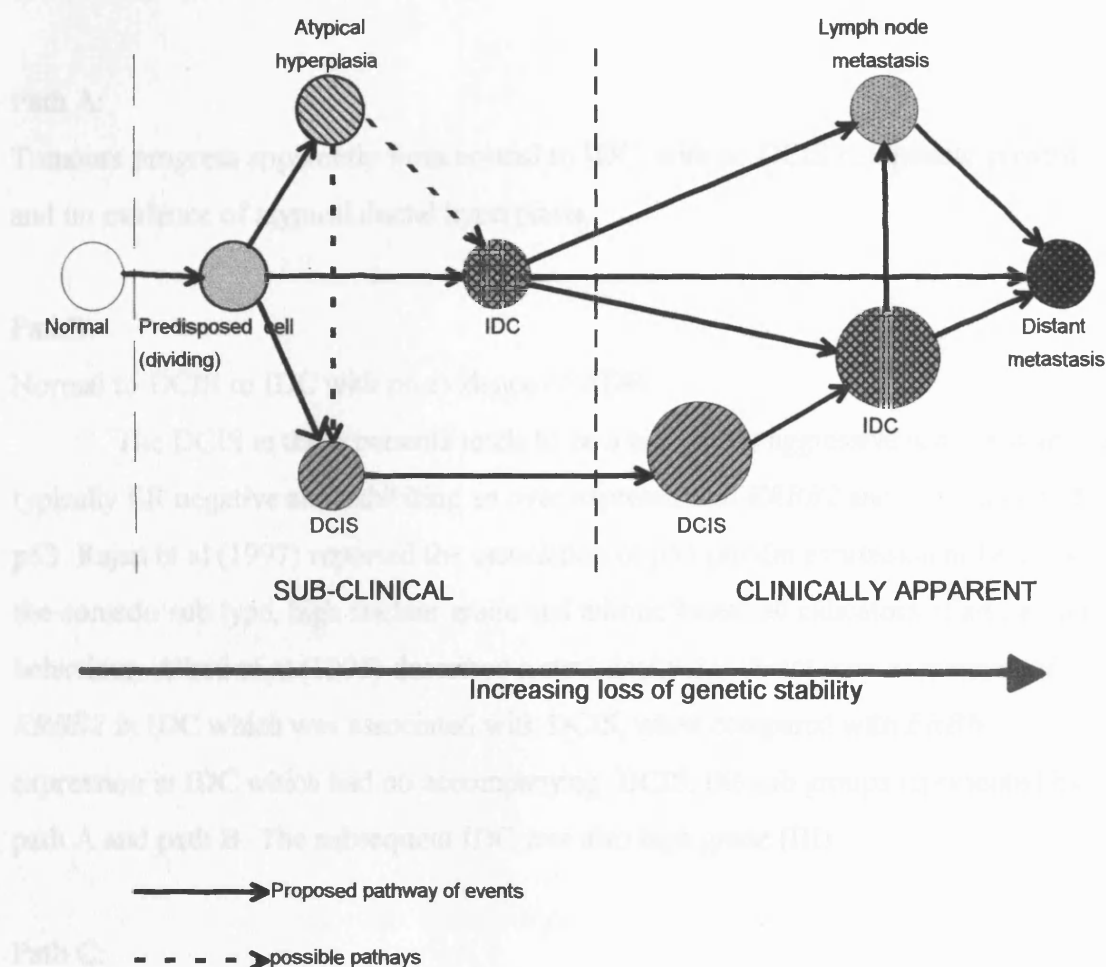
Adapted from Devilee et al 1994

With developments in molecular studies the above model has been extended (Walker, 1997; Lakhani, 1999) . Walker (Fig 1.10) describes three alternate pathways for the development of invasive breast carcinoma, each pathway having different characteristics. The relationship between ADH , DCIS and IDC, as well as that between DCIS and IDC derived from retrospective histopathological data form part of the model, the additional paths and the evidence to support the existence of alternate routes is derived from molecular changes and the clinicopathological behaviour of various tumours (Walker et al, 1991; Walker and Varley, 1993; Munn et al, 1995; Gretarsdottir et al, 1996; Aubele et al, 2000).

Path A:

Tumours progress apparently from normal to IDC, with no DCIS component present, and no evidence of atypical ductal hyperplasia.

PathB:

Normal to DCIS to IDC with no evidence of ADH

The DCIS in these patients tends to be a high grade aggressive tumour with cells typically ER negative and exhibiting an over expression of *ERBB2* and or mutation of p53. Rajan et al (1997) reported the association of p53 protein expression in DCIS with the comedo sub type, high nuclear grade and mitotic index, all indicators of aggressive behaviour. Allred et al (1992) described a statistically significant over expression of *ERBB2* in IDC which was associated with DCIS, when compared with *ERBB2* expression in IDC which had no accompanying DCIS, the sub groups represented by path A and path B. The subsequent IDC was also high grade (III).

Path C:

Tumours from this subgroup appear to have developed directly or indirectly from atypical ductal hyperplasia. The DCIS tends to be low/intermediate grade and less aggressive than that seen in path B, cells are typically ER positive with no overexpression of *ERBB2*. IDC arising in this subgroup are also better differentiated and less aggressive and subsequently are of a lower grade (I,II).

The different pathways discussed in this model may help account for the heterogeneous nature of breast cancer, in which variation is seen in rate and length of time for recurrence to occur, site of metastasis, aggressiveness of the disease etc.

Fig 1.10 **Development of breast cancer, Walker modification**

PATH A	PATH B	PATH C

"Normal" cell   "Normal" cell   "Normal" cell

Regular hyperplasia

Atypical hyperplasia

(direct or indirect)

In situ carcinoma (high grade)   In situ carcinoma (low/int grade)

Grade ?   **Grade III**   **Grade I, II**

I N V A S I V E   C A R C I N O M A

(Each sub group may have different time and soil/seed dependant characteristics)

METASTASIS
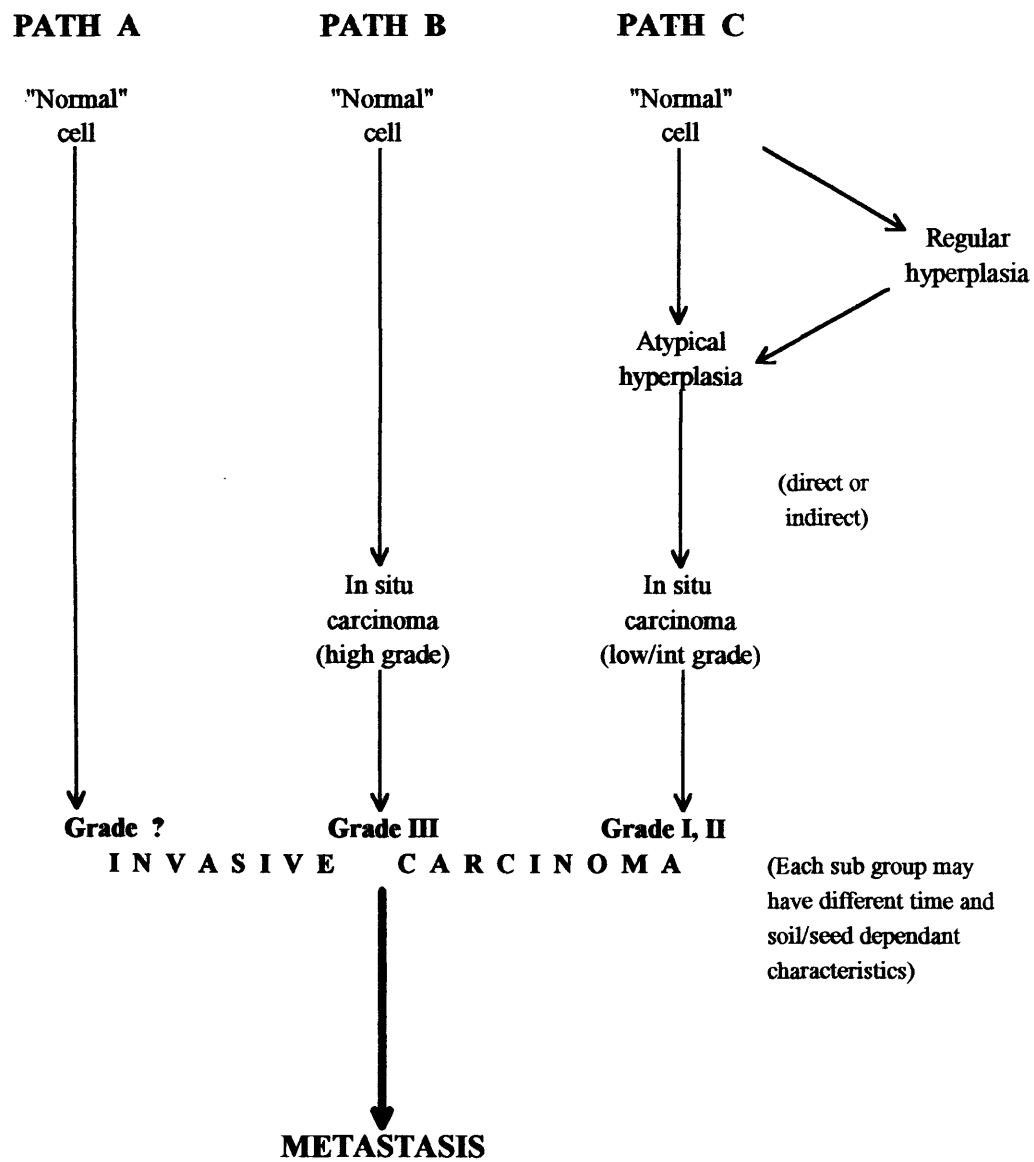
resulting in differing courses of disease. A recent study by Buerger et al (1999a) also suggested the existence of several distinct genetic pathways in the evolution of IDC associated with different tumour subtypes, and provided added evidence that DCIS is a direct precursor lesion of IDC. By understanding the apparent sub groups of breast carcinoma better it should be possible to better tailor treatment to patient needs.

1.5 AIMS OF THESIS

My hypothesis is that breast cancer occurring in different ethnic groups differs in relation to incidence, age of onset, age specific pattern of incidence and aggressiveness. To test this I will investigate:

- Whether any differences observed in the age of presentation and incidence of breast cancer in the study populations reflects differences between low and high breast cancer incidences populations world wide.

- Whether breast cancer in Saudi Arabia and in the Asian community in Leicester occurs in a younger age group and whether it is pathobiologically and or genetically different from that found in the European Leicester breast cancer population.

Chapter Two will analyse age specific incidence rates (ASIR) in the study populations and in populations of high and low incidence of breast cancer. This analysis will attempt to ascertain whether any differences in age distribution can be attributed to possible differences in breast carcinoma or is merely an artefact of the population structure. The relative frequency of breast cancer at different ages and in different ethnic groups, when compared with the incidence of cancer at all sites will also be examined.

Chapter Three will compare nuclear morphometry and histopathological characteristics of breast tumours in the study populations for possible differences in features relating to aggression.

Chapter Four will compare the incidence of genetic alterations at selected chromosomal regions in breast cancers from the different study populations.

Chapter Five will summarise and discuss the results in relation to the different ethnic groups, and possible practical implications arising from these results. Ideas for future work will also be outlined.

CHAPTER TWO

AGE AND ETHNIC DISTRIBUTION OF BREAST CANCER

2.1 INTRODUCTION

Several papers have commented that breast cancer occurs at a lower level of incidence, and at an earlier age, in Saudi Arabia than in the West and yet is still the most common neoplasm effecting women (section 2.1.5). Most of the ethnic differences in cancer incidence have been attributed to environmental risk factors as illustrated by the observations of Haenszel and Kurihara (1968) on the breast cancer incidence among Japanese immigrants to America and more recently among immigrants to Australia and Canada (Kliewer and Smith, 1995). The pattern of age specific incidence rates (ASIR the average annual incidence per 100,000 of the population in specified age groups) of breast cancer also vary between ethnic groups, but these differences have been largely attributed to cohort effects (Moolgavkar et al, 1979; Tarone and Chu, 1992; Winter et al, 1999). However the variation of an individuals genetic ability to cope with exposure to environmental risk factors may contribute to the observed differences, both individual and ethnic, in susceptibility to develop cancer (Feigelson et al, 1996; Neuhausen et al, 1999; Rebbeck et al, 1999).

2.1.1 ENVIRONMENTAL RISK FACTORS INFLUENCING THE INCIDENCE OF BREAST CANCER

Environmental factors which appear to change the overall risk of tumourigenesis include both diet and reproductive behaviour. Normal breast develops and functions under the ebb and flow of oestrogen (section 1.1.2, tables 1.1 and 1.2). Environmental factors which expose the breast to oestrogen have been suspected to increase the risk of developing breast cancer and include both exogenous and endogenous sources.

Exogenous sources of oestrogen exposure

Oral Contraceptives

The major source of exogenous oestrogen is oral contraceptive (OC) use. Findings regarding increased risk have been both negative (Stadel et al, 1985; Beral et al, 1999) and positive (UK National case control study group 1989). The risk appears to be negligible with modern OC, although those who have a history of long term oral contraceptive use, especially before their first full term pregnancy (McPherson et al, 1987; Brinton et al, 1995a; Woodman, 1999) may be at a small additional risk.

Hormone replacement therapy

In addition to its use in OC oestrogen is also used in hormone replacement therapy, HRT, during the menopause. There is an apparent greater risk with increased time of oestrogen use (Briton et al, 1986; Gottlieb, 1999), this risk being higher for

those that have been diagnosed as having benign breast disease (Thomas et al, 1982). There has been some debate recently about the nature of tumours arising in the breast after HRT, some reporting them to be less aggressive (Mayer, 1999) but others disagreeing (Stallard et al, 2000). Overall the benefits of hormone replacement therapy probably override any possible increased risk of breast carcinoma (Henderson et al, 1988; Shoup, 1999).

Endogenous Sources of Oestrogen Exposure

Reproductive factors

Early menarche, late menopause, and few or no pregnancies all increase the body's exposure to oestrogen, resulting in a woman with 40 years of menstruation having twice the risk of developing breast cancer as a woman with only 30 years (Trichopoulos et al, 1972). Since physical activity has been shown to delay menarche, physical activity in the pre-menarche period may reduce the individuals risk of breast cancer (Colditz and Frazier, 1995; Mittendorf et al, 1995).

Generally pregnancy is believed to increase the short term risk by stimulating cell growth but has an overall protective effect on permanent structural changes (Adami et al, 1995). Early pregnancy (before the age of 20) halves the risk of a nulliparous woman (MacMahon et al, 1970) and parity higher than 5 may also add a small additional amount of protection (Yuan et al, 1988). Lactation has been found to have a beneficial effect on breast carcinoma risk only if there is a long cumulative total period of breast feeding. Estimates have been made of a 30% reduction in risk for every five years total of breast feeding (Yuan et al, 1988). The effect is greatest when breast feeding starts below 22 years of age (Brinton et al, 1995b), breast feeding delaying or preventing ovulation, at least in some women.

However a comparison of menstrual and reproductive records in a group of American Asians (Chinese, Japanese, and Filipino), and American Caucasians concluded that the differences observed were not sufficient to explain the lower rates of breast cancer in the American Asian community (Wu et al, 1996a).

Obesity

It has been proposed that high total calorific intake, resulting in obesity, may be related to breast cancer incidence, rather the amount of one particular food type (Rao, 1996). The increased amount of adipose tissue in the body (increased body weight) results in more oestrogen being produced. Studies have revealed that excess weight over the age of 60 adds an additional risk of breast carcinoma, but not excess weight under the age of 50 (Kumar et al, 1995; Ziegler et al, 1996; Honda et al, 1999).

Diet

Many studies have assessed the effect of diet on breast cancer risk.

Fats

An early, widely accepted, hypothesis was that a high fat diet was directly related to an increased breast carcinoma risk (Armstrong and Doll, 1975; Wynder et al, 1986). However recent cohort and case control studies have failed to show a direct relationship between dietary fat and breast cancer (Jones et al, 1987; Phillips et al, 1980; Kinlen, 1982; Cade et al, 1998; Holmes et al, 1999) although the theory is still accepted by some (Walker et al, 1995; La Vecchia et al, 1998).

Dietary fat intake in the form of olive oil has been reported to reduce the risk of breast cancer in populations in Spain and Greece (Trichopoulou et al, 1995; Rose, 1997); this effect was found to apply equally to pre and post menopausal breast cancer. Olive oil forms a major component of dietary fat in the Mediterranean and is proposed to be a factor contributing to the low incidence of breast cancer in this region (Hunter and Willett, 1996). Fish oil has also been found to have a protective effect (Caygill et al, 1996; deDeckere, 1999).

Isoflavones and lignans

Isoflavones, present in tofu and other non fermented soya bean products may lower the risk of breast cancer (Maskarinec et al, 1998). Wu et al (1996b) discusses the correlation between the high rate of tofu consumption by Asian populations and their lower risk for breast, colon and prostate cancer, and suggests that the reduced tofu consumption found in migrant Asian populations to the USA may partially explain the increased breast cancer risk of this population when compared with that in Asia. The apparent protective effect of high levels of tofu consumption applied equally to pre and post menopausal breast cancer (Wu et al, 1996b; Ingram et al, 1997). Synergistic effects have been reported with curcumin in oestrogen receptor positive cell lines reducing cell proliferation (Verma et al, 1997).

Lignans are found in a wide range of foods including legumes, and whole grain and have oestrogenic and anti oestrogenic effect (Verma et al, 1997; Bingham et al, 1998), and may lower the risk of breast cancer (Ingram et al, 1997).

Curcumoids

Curcumin the major biologically active component found in turmeric (*Curcuma longa*), has a cytostatic effect inhibiting cell division at the G2/M check point (Simon et al, 1998). The compound has also been shown to compete with hydrocarbons as a ligand

and substrate for the aryl hydrocarbon receptor pathway, and so actively protects against a wide range of pollutants including pesticides (Ciolino et al, 1998).

Alcohol

A positive relationship between alcohol intake and increased risk of breast cancer has also been reported with even moderate alcohol intake increasing endogenous oestrogen levels (Hunter and Willett, 1996; Viel et al, 1997).

Well cooked meat

Well cooked, or over cooked, meats resulting from very high cooking temperatures are a potential source of heterocyclic amines which are potent mutagens. Regular consumption of such meats have been associated with an increased risk of breast cancer (Djuric et al, 1998; Zheng et al, 1998).

With age the individuals cumulative exposure to oestrogen and carcinogenic agents increases. Relationships between age and breast cancer are discussed further in sections 2.1.2, 2.1.3 and 3.1.2.

2.1.2 MODELS OF BREAST CANCER INCIDENCE

Mathematical models in the study of breast cancer can be used to demonstrate the consistancy of available data with a particular biological hypothesis, or to model the expected out come assuming a given biological hypothesis. For a model to be valid the biological basis for the hypothesis should be as sound as possible, and even then this will be only one alternative, as several biological models could be consistent with the observed data.

The incidence of most epithelial cancers increases steadily with age, cancer being most common in the old, and fits well to the mathematical model

$$\text{incidence} = C(\text{age})^\alpha$$

where C is a constant representing exposure or risk and alpha represents the number of stages in the carcinogenic process (Armitage and Doll, 1961; Doll, 1971). However breast carcinoma does not fit into this simple model since age incidence curves for breast carcinoma do not rise proportionally with age. The rate of increase prior to 50 years steadily increases with age and continues to increase after the age of 50, but at a slower rate, this inflection at the age of menopause is known as Clemmesen's hook (Clemmesen, 1948). Models have been proposed to explain Clemmesen's hook:

De Waard proposed (1969) the possible existence of more than one type of breast cancer, and that the incidence curves for breast cancer seen in different countries comprised the sum of the premenopausal "ovarian" type and the postmenopausal

"extraovarian" type. De Waard, (1979), analysed ASIR data from the first three volumes

of Cancer Incidence in Five Continents and found that data from several countries,

notably Japan and Taiwan, produced ASIR curves different from those seen in data from

Western countries; DeWaard referred to these curves as "oriental" type. The "oriental"

type curve tended to go up until about the age of 50 and then slowly decreased unlike

the classical curve seen in Northern Europe and North America which continues to

increase after the Clemmesens hook inflexion. Southern Europeans, Eastern Europeans,

Latin Americans and Africans were described as having an intermediate type of curve.

De Waard (1979) correlated the "Western" curve to the postmenopausal extra ovarian

type and the "Oriental" curve to the premenopausal ovarian oestrogen type. The degree

that a population is susceptible to premenopausal type breast cancer influences the

position of Clemmesens Hook. The international differences in the incidence of

postmenopausal type breast cancer he related to Western nutrition and in particular total

fat intake.

Hakama (1969) produced a mathematical model of de Waards hypothesis, and

showed that the two disease model fitted the ASIR data from four Nordic countries

(Hakama, 1970). Hakama's model assumes two types of breast cancer, pre and post

menopausal, both of which are represented by a polynomic quadratic equation of the

form:

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2.$$

Where Y is equal to the log of the incidence, X is age and β's are population specific

constants. The position of Clemmesens hook is dependant on the proportion of pre and

post menopausal breast cancer in the population. The model was criticised by Farewell

(1979) because it requires 5-6 hypothetical stages in the process of breast carcinogenesis

to be viable. Manton and Stallard (1980, 1992) in a study of breast cancer of white

females in the United States, however supported the two disease hypothesis, and

estimated that premenopausal breast cancer required seven events for carcinogenesis to

occur and post menopausal breast cancer four events. The, then recent, work on colon

cancer (section 1.3.4) made a multi stage model of breast neoplasia much more

acceptable. Manton and Stallard (1980, 1992) suggested that premenopausal breast

cancer is a more aggressive disease and that genetic factors influencing susceptibility to

cancer were important in its aetiology, whereas post menopausal breast cancer was

influenced more by epigenetic factors such as diet (Manton and Stallard, 1980).

De Lisi (1977) derived a four parameter single disease model (Fig2.1). The model

assumes that the menopause affects the rate of transition of hyperplastic to malignant

Fig 2.1 **De Lisi single disease model for breast cancer**

Pool of N normal cells of which R_0 are at risk of transformation
rate of transformation to hyperplastic state

β_1

Overall transition rate γ_1 from normal to transformed cells in premenopausal patients

Pool of n_1 hyperplastic cells

Overall transition rate γ_2 from normal to transformed cells in postmenopausal patients

IF patient is premenopausal rate of progression to the transformed state equals

β_{2pre}

IF patient is postmenopausal rate of progression to the transformed state equals

β_{2post}

Pool of n_2 transformed cellls

n_2, is the number of normal cells becoming fully transformed, γ_1 the rate at which this occurs before menopause, γ_2 the rate after menopause, and a factor R_0 representing the apparent at risk fraction of the population cells. In the model, N is the pool of normal cells $n1$ become hyperplastic at a rate of β_1 of which n_2 cells then progress to the fully transformed state at a rate of β_2. β_1 is a constant rate, however β_2 varies being β_{2pre} premenopausally and β_{2post} postmenopausally, with β_{2post} being less than β_{2pre}.

cells. This results in a model in which although the rates of pre and post menopausal cancer can vary from population to population, they always pivot around a Clemmesen's hook fixed at the time of the menopause.

Moolgavkar et al (1980) formulated a two-stage single disease model of breast cancer (Fig 2.2). The model does not consider pre and postmenopausal breast cancer to be separate entities and attributes the apparent variation in the age specific incidence curves seen in different ethnic groups to cohort effect (Moolgavkar, 1979; Moolgavkar et al, 1980).

The source of data for the international comparisons from which these models were derived is the national and regional tumour registries of those countries and is therefore dependent on the reliability and accuracy of that registry data.

2.1.3 TUMOUR REGISTRY DATA

Tumour registry data depends on several sources of information, any of which can introduce error and affect the reliability of the data.

Potential sources of error

Population estimation

Incomplete or inaccurate census data can result in artifactual ASIR results. Even relatively recent census data from some regions in the USA have been found to have not been fully reliable, especially data concerning the population over 65 years of age and non whites (Doll and Peto, 1981).

Sources of registry data

Death certificate data is generally a less reliable source of data and particularly prone to under diagnosis in the older age groups, where death may be easily attributed to other causes or a cancer may be so widely disseminated that its primary site is no longer obvious. The reported incidence of new cases is preferable, especially if supported by histopathological data.

Errors in registration

Apart from errors in entering data and inadequate quality control procedure, other sources of error may be caused by patients registering at more than one hospital. The danger is that this may result in aberrations that may be mistaken for trends, which may swamp out any real underlying trends. All of these components vary from registry to registry and directly affect the quality of data produced.

Fig 2.2 **Moolgavkar two stage single disease model for breast cancer**
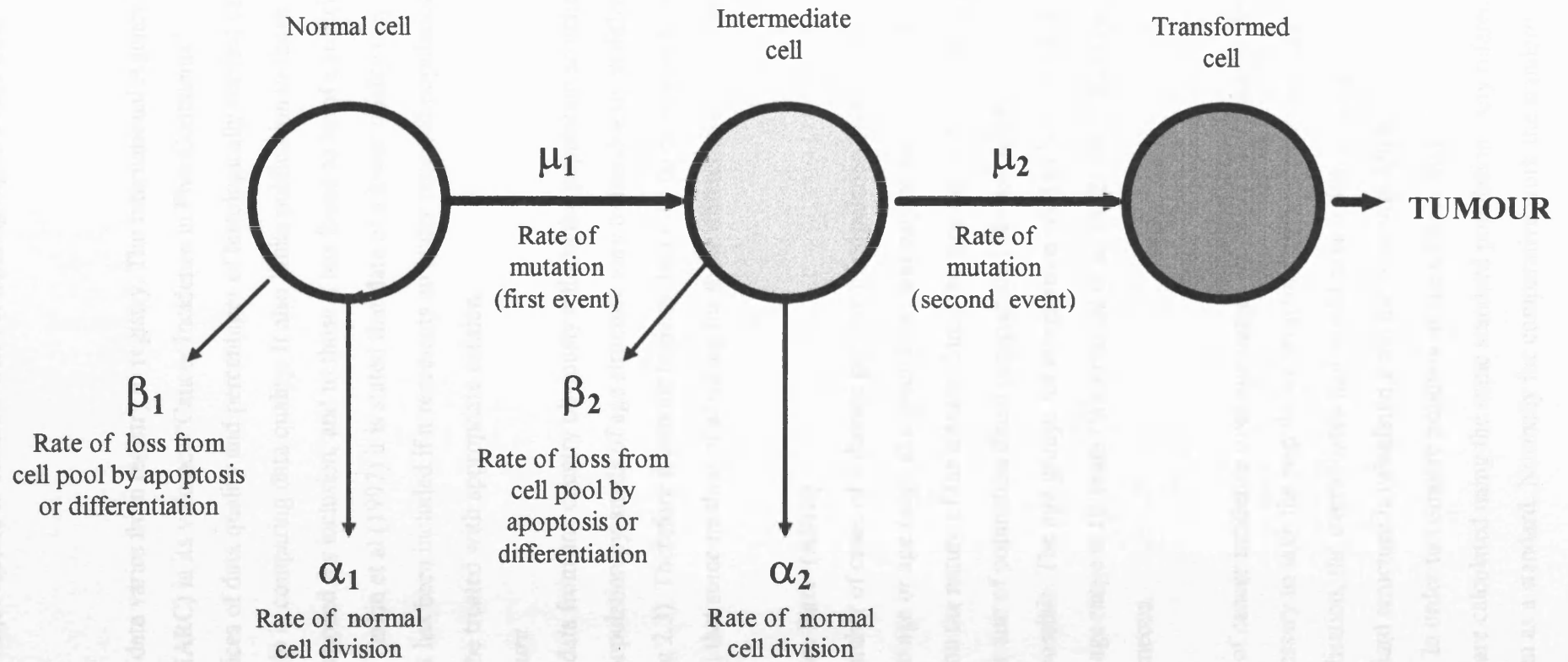
N is the pool of normal cells capable of cell division and thus susceptible to mutation, β the rate at which cells become no longer capable of division (either through apoptosis or differentiation), α the rate of cell division, and μ the transition rate from one stage to another in the model.

Comparison of data between registries

There are two major factors to consider when comparing registry data from different populations

The quality of data

The quality of data varies from registry to registry. The International Agency for Research on Cancer (IARC) in its volumes "Cancer Incidence in Five Continents" includes a table of indices of data quality and percentages of histologically verified cases for each registry to help in comparing data quality. It also limits publication to those registries which have applied for inclusion, and to those it has found to be of a sufficient standard. However in Parkin et al (1997) it is stated that data of a lower quality may be considered valid and it has been included if it represents an under reported population, but it suggests that it be treated with appropriate caution.

The population structure

Crude patient data from any country obviously reflects the population structure of that country. The population pyramids of age structure vary tremendously in different areas of the world (Fig 2.3). Therefore if tumour registry data is to be compared between registries it is essential that some method of allowing for these difference in population structure be utilised.

Age Specific Incidence Rate (ASIR)

ASIR is the number of cases of a disease per unit of population (100,000 individuals) for a given age or age range at a given time, and may or may not be sex specific dependant upon the nature of the disease. Since the incidence rate for each age group is calculated per unit of population direct comparison between ASIR from different registries is possible. The age groups for comparison need to be quite small; the largest recommended age range is 10 years (Waterhouse et al, 1982) since larger ranges might be too heterogeneous.

Age standardisation

If comparison of cancer incidence over age ranges spanning more than 10 years is required then it is necessary to take the population structure of the country or region into account. Age standardisation, the overall rates that would have occurred if the population had a standard structure is calculated from the observed ASIR in the population of interest. In order to compare populations the overall rates for all the countries under study are calculated using the same standard population. Any reference population can be taken as a standard. Normally for comparative work the standard population is either:

Fig 2.3 Standard Populations

Standardised breakdown of a total population of 100,000 into different age groups.
Each standard population having a different age distribution
= 1000 individuals

Age	African	World	European
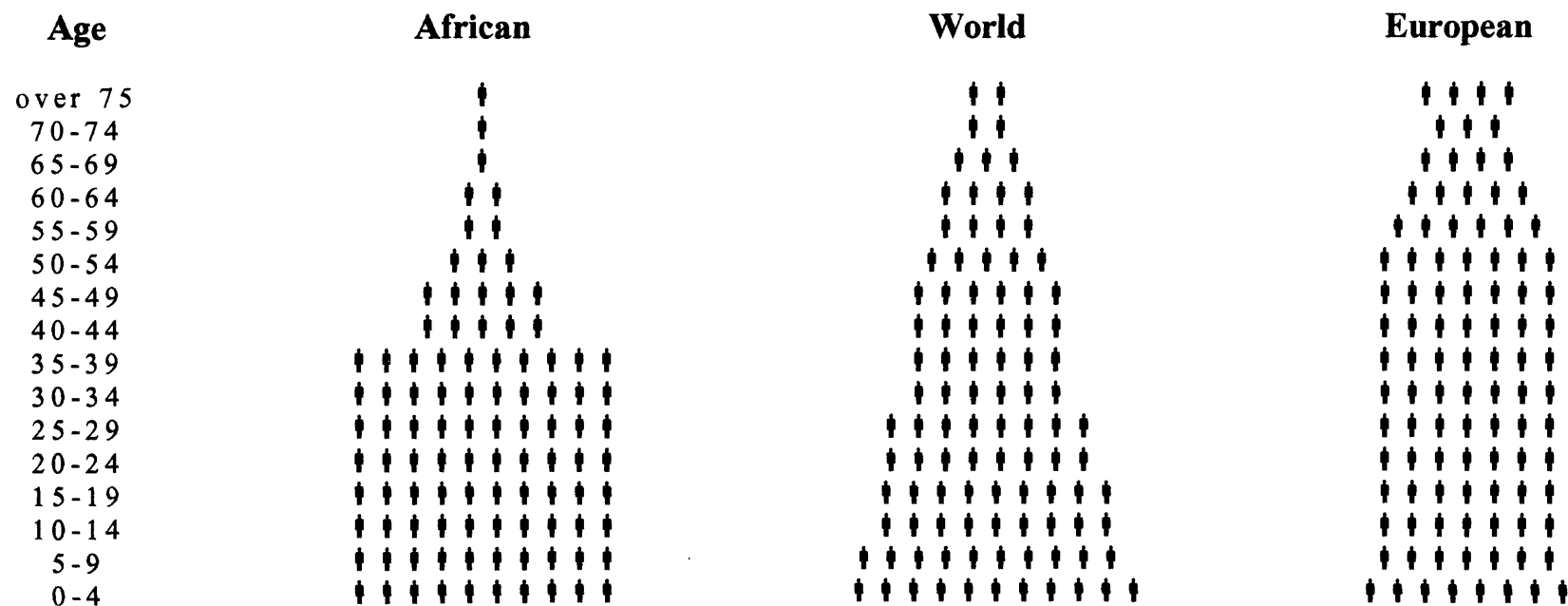
Developing countries have bottom heavy pyramids in with a large young population and relatively few in the older age group
European populations have proportionally more older but less younger population.
(Adapted from Parkin et al 1997)

1.African 0 - 85 years of age;

    a low proportion of persons in the older age groups

2.European 0-85 years of age;

    a low proportion of persons in the younger age groups

3.World 0-85 years of age;

    a population structure intermediate between the African and the

    European.

4. World truncated 35 - 65 years of age;

    a truncated form of the standard world population.

Unless both populations that are being compared come from Africa, or both come from Europe, it is usual (Parkin et al, 1997) to use the World standard or the World truncated standard for comparison.

2.1.4 STANDARDISED INCIDENCE AND AGE SPECIFIC INCIDENCE RATES OF BREAST CANCER IN DIFFERENT ETHNIC GROUPS

Geographic differences in incidence of breast cancer exist (Parkin et al, 1992; Kelsey and Horn-Ross, 1993; Gilliland, 1997) and these differences appear to be both qualitative and quantitative. In areas of low incidence, such as Japan, the disease has a major premenopausal element and slowly decreases after the age of 50. In areas of high incidence such as the USA and UK age specific incidence data (ASIR) (Parkin et al, 1994) shows the disease to be more common in postmenopausal women with an age specific increase premenopausally, followed by a slower rate of increase after menopause to form the classic Clemmesen's hook effect.

De Waard (1979) described Japan and Taiwan as having the lowest incidence and predominantly premenopausal type breast cancer and Northern Europe and North America having the highest incidence and post menopausal type breast cancer. The countries of Southern and Eastern Europe, South America, and Africa were intermediate between these two extremes.

It has been proposed that the ethnic differences might be related to differences in inherited tumour susceptibility (Gilliland, 1997). However the 'Oriental' and 'Western' patterns of breast cancer and ethnic variation in age specific incidence have been discounted by some researchers as being due to differential risks in various cohorts (Moolgavkar et al, 1979; Stevens et al, 1982), which once allowed for statistically result in a more standardised curve, irrespective of the ethnic origin of the population (Moolgavkar et al, 1980). The variation in age of onset and differences in the patterns of

incidence between various countries with high and low breast cancer incidence are examined in detail later in this chapter.

Temporal changes of standardised incidence

Incidence rates of breast cancer are increasing in all countries (Wang et al, 1998). It has been reported that the increased incidence of breast cancer is particularly notable in the developing countries (Kelsey and Horn-Ross, 1993) and in those countries which have previously had a lower incidence of breast cancer (Patavino et al, 1995) and is frequently attributed to environmental risk factors associated with the adoption of a Western life style. Stephens (1997) however reports that breast cancer in the West has increased more than in Asian countries and attributes the difference to one of diet. Some of the increase seen during the last 20 years is probably due to greater public awareness and improved detection methods. This is particularly true in developed countries where asymptomatic breast tumours are now being detected through mammographic screening programmes and may lead to an increase in incidence of breast cancer in the screened population (primarily the 50 - 65 year olds); such an apparent increase was reported in Finland after nation wide screening was introduced (Gastrin, 1980). Cohort effects, when more recent cohorts show a greater incidence of breast cancer which could be due to the reduction of family size, older age at first birth, reduced period of breast feeding, dietary changes etc., have also been used to explain temporal changes, especially the increase in pre menopausal breast cancer (Wang et al, 1998).

Manton and Stallard (1980) however argue that since reproductive factors such as early menopause and early first pregnancy confer a degree of protection against breast cancer into old age, removal or reduction of these factors is unlikely to cause an increase in premenopausal breast cancer. Similarly obesity, caused by dietary changes, has most effect in the post menopausal age group (Kumar et al, 1995).

2.1.5 BREAST CANCER IN SAUDI ARABIA

Saudi Arabia is a large country, approximately 2.15 million square kilometres, (Fig 2.4) comprising of five geographical regions, central, southern, northern, eastern, and western regions. Jeddah, the city from which the detailed study population is drawn is situated in the western region on the Red Sea coast approximately 80kms from Makkah and 400 km from Madinah.

The Ethnic groups in Saudi Arabia

The population in the Western Region of Saudi Arabia comprises tribal Arabs, and other ethnic groups. In the past it used to take many months to reach Saudi Arabia by sea and land and it was not uncommon for pilgrims (Hadji) to stay for a period

Fig 2.4 **Map showing the geographical location of Saudi Arabia**

Legend:

International Boundaries of Saudi Arabia

- - - - Regional Boundaries

Regions
1 Central
2 Northern
3 Southern
4 Eastern
5 Western
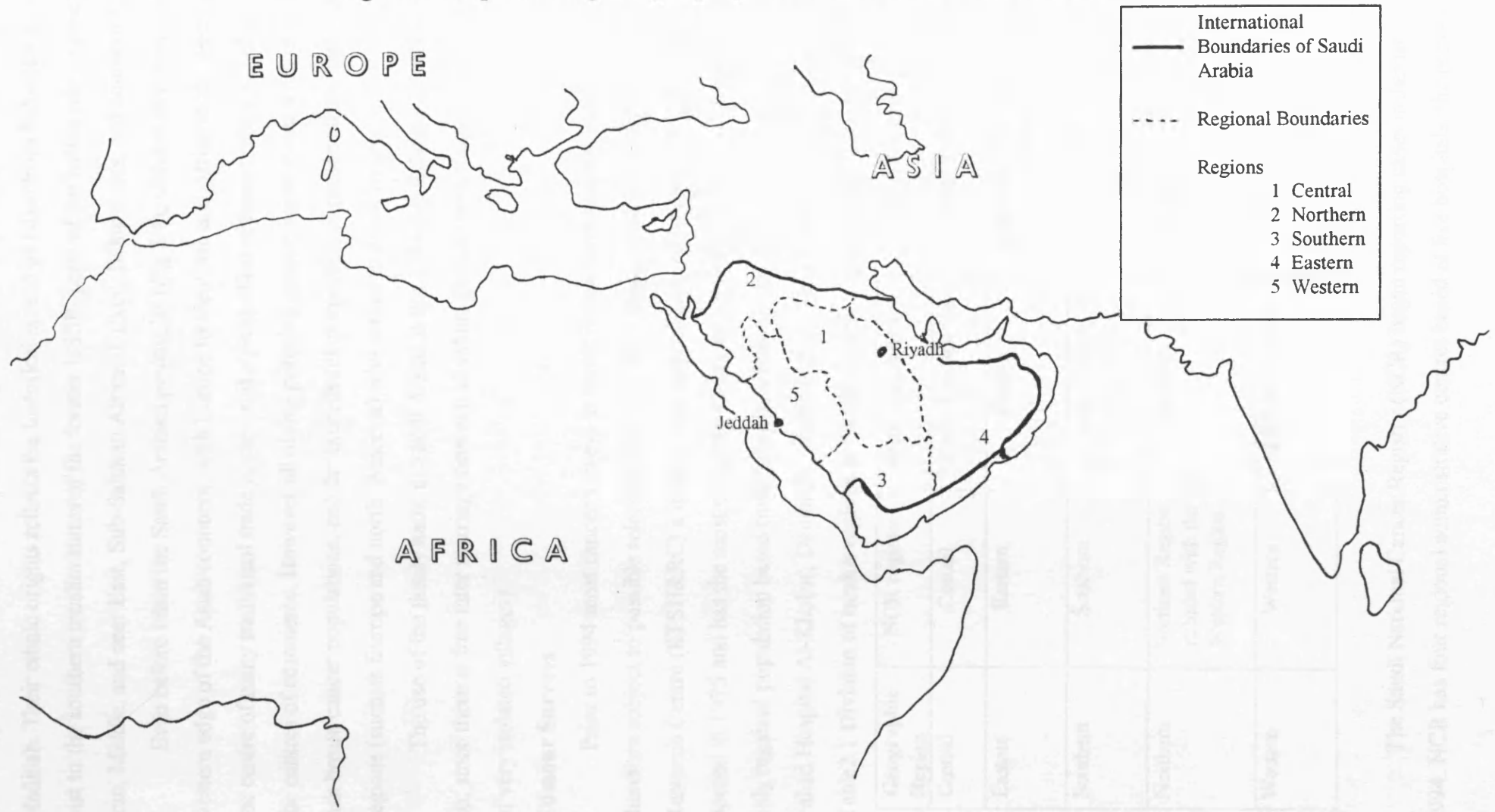
once here, and some settled down permanently to be close to the holy sites of Makkah and Madinah. Their ethnic origins reflects the historical spread of Islam from Indonesia in the east to the southern muslim states of the former USSR, parts of the Indian sub continent, Iran, Middle, and near East, Sub-Saharan Africa, Turkey, Baltic states, and southern Spain.

Even before Islam the Saudi Arabian peninsular (Fig 2.4), situated as it is on the western edge of the Asian continent, with Europe to the north and Africa to the west, was the centre of many traditional trade routes, and a proportion of these traders would settle in the centres of commerce. However all of this potential genetic input comes from other low risk breast cancer populations, and far exceeds the proportion of persons from high risk regions (such as Europe and north America) who settled in Saudi Arabia.

The use of the family name in Saudi Arabia is not a reliable indicator of ethnic make up, since there is free inter marriage between all ethnic groups (with the possible exception of very isolated villages).

Tumour Surveys

Prior to 1994 most cancer surveys in Saudi Arabia were hospital based, and therefore subject to possible selection bias. The King Faisal Specialist Hospital and Research Centre (KFSH&RC) a tertiary care referral hospital located in Riyadh, was opened in 1975 and has the most complete source of hospital based cancer statistics. The only regional population based tumour registry was for the Eastern Region at King Fahad Hospital Al-Khobar, Dammam, established in 1985 (Tamimi et al, 1995).

Table2.1 **Division of health regions in Saudi Arabia**

Geographic Region	NCR regions	Reporting Regions	Health Regions
Central	Central	Riyadh, Qassim, Hail	Riyadh, Qassim, Hail
Eastern	Eastern	Eastern	Dammam, Hasa, Hafr al Batin
Southern	Southern	Asir, Baha, Najran, Jizan	Asir, Bisha, Baha, Najran, Jizan
Northern	Northern Region included with the Western Region	Northern	Northern, Guryat,Jouf, Tabuk
Western	Western	Makkah,Madinah	Makkah, Jeddah, Taif, Madinah

The Saudi National Cancer Registry (NCR) began reporting cases in January 1994. NCR has four regional administrative centres based at five hospitals, the regions

basically the geographical regions of the country (Fig 2.4). These are divided into 11 reporting regions (Fig 2.5) used in the NCR detailed reports, which can be further divided into 19 health regions (table 2.1). Earlier, pre-NCR reports used either geographic areas or health regions for their reports.

National and regional reports show breast cancer to be one of the top ranking female neoplasm in all of the regions (Table 2.2). Breast cancer is quoted in some studies as a percentage of adult malignancy, with no differentiation between male and female cases, and the age range of patients was not always included.

The situation in the Southern Region appears to be different than the other regions, with breast cancer being ranked third or fourth. The local habit, which is equally common among males and females, of chewing shamma (a mixture of tobacco, slaked lime, spices and oils), has been linked to the high level of oral cancer (Khan et al, 1991). Possible explanations as to the high incidence of skin cancer in the Southern region are the high altitude, or that skin cancer is treated locally and so over represented (Willen and Pettersson, 1989). It is probable, therefore, that the differences are due to an increased incidence of oral and skin cancers rather than a reduced incidence of breast cancer.

Saudi Arabia has a crude incidence rate of cancer of 38.1/100,000 (world age standardised rates ASR 80.9) (NCR 1996) compared with 401.8/100,000 (ASR 201.0) in the UK (Parkin et al, 1992). The crude incidence of breast cancer in Saudi Arabia of 6.5/100,000 (NCR 1996) and 88.3/100,000 in the UK (Parkin et al, 1992). Breast cancer in Saudi Arabia still represents about 18% of all female malignancies (NCR 1996).

It has been suggested that reluctance to seek medical attention and environmental risk factors are responsible for the observed pattern of breast cancer in Saudi Arabia (Amer, 1982; Ajarim, 1992). A report by Al Idrissi et al (1992) states that post menopausal patients delay on average 12+/-2.3 months prior to seeking medical attention, whereas in pre menopausal patients the delay is only 4+/-0.57 months, and yet pre menopausal patients were presenting with more advanced disease than the postmenopausal patients. This suggests that any delay in treatment does not explain the observed differences between pre menopausal and post menopausal breast cancer in Saudi Arabia.

With the exception of obesity the female population of Saudi Arabia and the Arabian Peninsula exhibit no obvious high risk environmental risk factors. Obesity in the Saudi female population appears to be mainly pre and peri menopausal women (El Hazmi and Warsy, 1997) and studies have shown that it is excess weight over the age of 60 that results in an additional risk of breast cancer (Kumar et al, 1995).

Fig 2.5 **Geographical and reporting regions in Saudi Arabia**

National Boundaries

Regional Bondaries

Geographical and Reporting Regions

1. **CENTRAL**
   a.Riyadh
   b.Qassim
   c.Hail

2. **NORTHERN**
3. **SOUTHERN**
   a.As.r
   b.Najran
   c.Jizan
   d.Baha
4. **EASTERN**
5. **WESTERN**
   a.Makkah
   b.Madinah

58
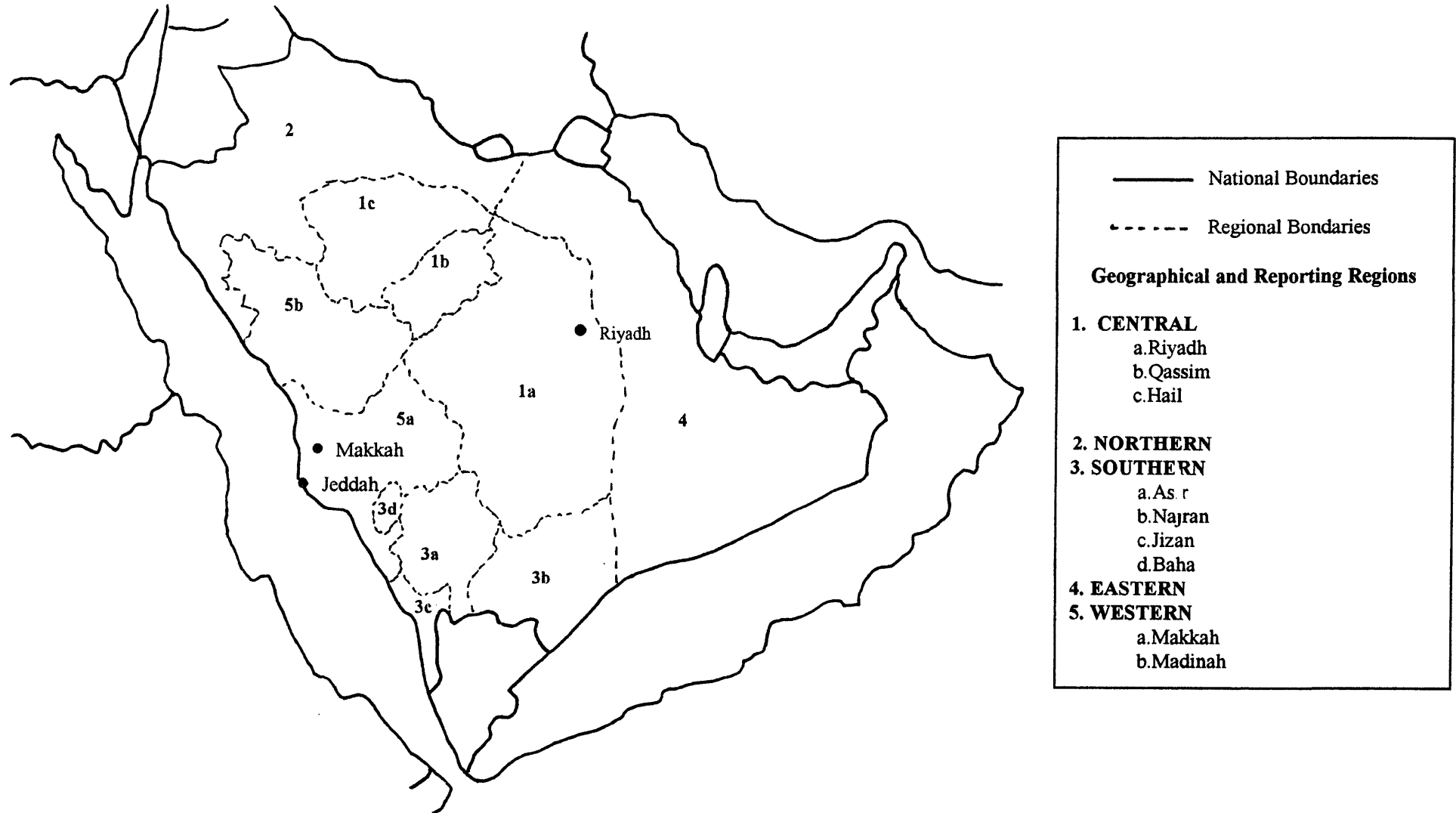
Table 2.2 **Outline of breast cancer in Saudi Arabia, by region**

Geographical region	KFSH&RC Reports 1976-1993 [a]	National NCR Report and Regional Hospital Based Reports
National	1976-1993 most common adult malignancy.39% 15-40 years old, compared to only 11% over the age of 60.	NCR Report: The most common female neoplasm. Mean age at diagnosis 49.6 years crude incidence rate 6.5/100,000
Central Region	most common adult malignancy (9.6%)	most common female malignancy[b], 59% premenopausal[c]
Northern Region	second most common adult malignancy (7.96%), first Lymphoma	No major reporting oncology centre, patients referred to Western or Central Region
Southern Region	Third most common adult malignancy (7.2%), First oral cavity second lymphoma	i. Al Baha 1981-1987[d]: Third most common female malignancy, equal with lymphoma+leukemia (9.8%). First skin, second gastric ii Jizan 1982-1992[e] Fourth most common female malignancy (10%), First oral cavity, second skin, and third lymphoma+leukemia
Eastern Region	most common adult malignancy (11.1%)	most common female malignancy[f] mean age 47.1[g]
Western Region	most common adult malignancy (11.0%)	Madinah 1981-1993: most common female malignancy (20.1%)[h] Jeddah: most common adult malignancy[i]

a:Ezzat et al 1996, b:Koriech and Al-Kuhaymi 1984, c: Al-Salah 1994, d:Willen and Petterson 1989, e:Tandon et al 1995, f:Rabadi 1987, g:Amr et al 1995, h:Al-Saigh et al 1995, i: Mokhtar and Bedeiwy 1992.

This would not be expected to increase the incidence of pre menopausal breast cancer. Increased body size in young women may even decrease the risk of premenopausal breast cancer (Peacock et al, 1999), although others (Coates et al, 1999) have found the situation more complex and this advantage confined to the less aggressive tumours only. Several of the factors exhibited by the Saudi population are thought to protect against breast cancer; therefore environmental risk factors may help explain the low overall incidence of breast cancer in Saudi Arabia and the region, but do not explain the apparent excess of young breast cancer patients.

Table 2.3 **Environmental risk factors**

Environmental risk factors	Assessment in Saudi/Arab population
Reproductive	Menarche 13.07 +/- 0.91 years[a], mean age at first full pregnancy 21 years[b], menopause 49.3 +/- 4.6years[c]
OC use	Of females who use contraception, 43% use the IUD and 6.9% OC, only 25% using contraceptives before the birth of their first baby[d]
Diet	3265 kcal/caput/day of which 10.9 % come from oils and fats[e] Given the strict prohibition laws in Saudi about alcohol consumption it is reasonable to assume that the average consumption per head of female population in Saudi is infinitely smaller than that in the West.
Obesity	Saudi females are more obese than their European counterparts[f]
Breast feeding	In urban areas the majority, 72%, of babies are breast fed for at least 3 months, 11% are fed for at least a year. In rural areas the majority of babies are breast fed for over a year.[g]

a-(Attallah et al, 1990) b: (pers comm. Dr H.Qardi) c: (Qattan and Omu, 1996)

d: (Bakr and Suliman, 1990) e: (Ministry of Agriculture and Water, KSA 1983)

f: (Al Rehaimi and Bjorntorp, 1992) g: (Al-Shehri et al, 1995)

This pattern of high relative incidence of breast cancer against a background of low overall levels of neoplasia and young age of occurrence is not unique to Saudi Arabia and has been noted in other low risk populations.

2.1.6 BREAST CANCER IN OTHER LOW RISK POPULATIONS

Middle East

Studies from Egypt (Richards et al, 1992; Kahan et al, 1997; Soliman et al, 1999), Kuwait (Motawy et al, 1994), Lebenon (Adib et al, 1998; El Saghir et al,1998) and Libiya (Akhtar et al, 1993), all report breast cancer as being the most common

female malignancy, and presenting at a young age with a high proportion of premenopausal patients.

The Israeli tumour registry divides its reports into incidence in Jews and non Jews; the non Jews being primarily Christian and Moslem Palestinians. Data is also available on the ASIR of cancer among Jewish Israelis belonging to different ethnic groups. Incidence rates vary Palestinians having the lowest and Jews born in America or Europe the highest (Steinitz et al, 1989). The incidence for European/American born Jews is four fold that found in the Palestinian population. Peak incidence occurs at 70+ for the Israeli Jewish patients and at 45 years of age in the Palestinian patients (Parkin et al, 1992). In a study of the Jewish migrant populations (Steinitz et al, 1989) the incidence of breast cancer changed only slightly with time, not showing the relatively rapid changes as described by Haenszel and Kurihara (1968) for Japanese migrants to the USA. However Steintz et al (1989) studying female breast cancer cases aged 0 -75+, found Asian immigrants to Israel had the lowest overall incidence rate, but in a study of 3rd generation Israelis 0-29 years (Iscovich and Parkin, 1998) incidence was found to be notably higher among females of Asian descent.

Africa

Studies from Sub-Saharan Africa report a low overall incidence of neoplasia but a high proportion of premenopausal breast cancer (Bjerregaard and Kung'u, 1992; Hassan et al, 1992; Amir et al, 1996; Adebamowo and Adekunle, 1999).

South East Asia

The overall incidence of breast cancer is lower in Japan but breast cancer still represents one of the most common neoplasms affecting females. In a study of ASIR of breast cancer in the Japanese female population and the British female population Kodama et al (1992) described a rising incidence with age for the Japanese population until a break point at 47.5 years when there was a gradual decline, whereas the British population exhibited the classical western pattern of breast cancer described earlier. A study by Hirose et al (1995) described breast cancer in Japan to be primarily a premenopausal disease. There is some evidence to suggest that differences in the classical epigenetic risk factors may not be sufficient to explain differences in the incidence and pattern of breast cancer in Britain and Japan (Chaudary et al, 1991; Tung et al, 1999). Merchant et al (1999) studying British and Japanese breast cancer patients found Japanese patients to be significantly younger.

Breast cancer accounts for about 20% of female malignancy in India (Goel et al, 1995) the majority of patients being premenopausal (Hussain et al, 1994).

Migrants from low risk populations

Migrants from the Near and Middle East to Australia and France have been reported as having a low overall incidence of neoplasia and breast cancer (Khlat et al, 1993; Khlat, 1995). Asian migrants from the Indian sub-continent to New South Wales, Australia were found to have a lower overall incidence of cancer, with an incidence of breast cancer intermediate between that found in the sub-continent and Australia. (Grulich et al, 1995).

USA minority populations

Incidence rates 40 -54 years in White American women (AmW) are higher than those seen in Black American women (AmB), however in women under the age of 40 there is an excess incidence in the AmB population which cannot apparently be attributed to reproductive factors (Brinton et al, 1997). There is also a marked transition from higher to lower incidence rates at the age of 45, not seen in AmW, which cannot be accounted for by other known risk factors (Hankey et al, 1994; Trock, 1996). Generally AmB patients present at a younger age (Aziz et al, 1999) than AmW and the same is true for Hispanic Americans (AmH) and Asian Americans (AmA) (Boyer-Chammard et al, 1999). AmB patients have been reported to be at a higher risk for premenopausal breast cancer than their AmW counterparts, and this increased risk persists even after all known confounding factors have been taken into account (Weiss et al, 1996). Ijaduola and Smith (1998) comparing AmW, AmB and West Africans (WAf) found both AmB and WAf to have a peak incidence of breast cancer below the age of 50 years, whereas that in AmW is postmenopausal.

The lower standardised incidence of breast cancer in the AmH population (Davis et al, 1995) suggests that AmH might still retain some of the lower risk profile of their home despite being exposed to the environmental risk factors prevalent in the USA. However many studies have shown that upon emigration to the USA the risk of breast cancer in Asian women (Japanese, Filipino, Chinese) approaches that of AmW after a few generations (Haenszel and Kurihara, 1968; Buell,1973; Fraumeni and Mason, 1974; Thomas, 1979), emphasising the impact of environmental risk factors, probably associated with western life style, on breast cancer incidence.

2.1.7 BREAST CANCER IN THE BRITISH ASIAN COMMUNITY

Several studies have found the overall incidence of cancer lower in the British Asian than in the non Asian community (Donaldson and Taylor, 1983; Winter et al, 1999) and that breast cancer incidence is lower, but the most common form of cancer affecting females (Potter et al, 1983; Donaldson and Clayton, 1984; Barker and

Baker, 1990; Winter et al, 1999). Potter et al (1983) in a hospital based study in Birmingham concluded that the differences in breast cancer incidence could not be explained by differences in reproductive factors alone and were similar to incidence rates in their countries of origin. Similar results were obtained by Winter et al (1999). Breast cancer incidence in the UK South Asian population was found to be approximately half way between that of the UK and India. ASIR data was examined for cancer at all sites for the same populations, Winter et al (1999) concluded that there are underlying differences in ethnic incidence even after age structure has been taken into account. A high incidence of young breast cancer in the S.Asian UK population, below 29 years, was noted but this was attributed to a probable cohort effect requiring longer follow up.

The study of cancer epidemiology in different ethnic groups will be made easier once the mandatory requirement that ethnic data be included as part of patient admission documentation (Bahl, 1996) becomes effective and such data is routinely included in tumour registries.

2.2 AIMS:

The aims of this chapter are to

Analyse differences in overall standardised incidence and age specific incidence rates (ASIR) in populations of high and low incidence of breast cancer in general, and in the study populations in particular. This analysis will attempt to ascertain whether the larger proportion of young cases found in the Western Region can be attributed to a different presentation of breast carcinoma or whether it is an artefact of the population structure. The relative impact breast cancer has at different ages and in different ethnic groups, when compared with the incidence of cancer at all sites, will also be examined for the study populations.

2.3 MATERIALS

Data was collected from a variety of sources for comparison of the patterns of incidence of female breast cancer in the Western Region of Saudi Arabia and Trent UK with respect to breast cancer incidence in other low and high incidence countries.

2.3.1 INTERNATIONAL DATA

The Israeli Asian/African and Israeli European Jewish data were from Steinitz et al (1989), and other countries were from Parkin et al (1992), Parkin et al (1997) and Waterhouse et al (1976).

2.3.2 BREAST CANCER DATA IN THE WESTERN REGION OF SAUDI ARABIA, TRENT AND LEICESTERSHIRE

i. Western Region of Saudi Arabia

Data was obtained for breast cancer and for all cases of cancer occurring in the Saudi adult female population of the Western Region of Saudi Arabia (Figs 2.4, 2.5) from the Western Region NCR Office, for a three year period, 1994-1996. National incidence rate for Saudi Arabia and ASIR for the Western Region of Saudi Arabia was obtained from the NCR report for 1994-1996. The NCR report gives the incidence of breast cancer in Makkah district, and this was used to calculate the population of the Makkah district. The sex specific age structure was estimated from the census data of 1977 for the Makkah district (see discussion).

ii Trent Health Region In The United Kingdom

Data for the UK and Wales and regional UK data for the Trent Health Region was obtained from Cancer Incidence in Five Continents vol. VI. The data for female breast cancer patients from Leicestershire (population approx.900,000) which forms part of the Trent Region (Fig 2.6) was compiled from the computerised records of the Leicestershire Pathology Service, covering 1994 - 1996. Malignant cases were extracted and classed either as Asians or non Asians, according to name, and their age at presentation age calculated from their date of birth. The total number of malignant cases (Asians + non Asians) was checked on a hospital by hospital basis against the total numbers of malignant cases, previously compiled by Dr Walker, to ensure that no cases had been missed. Data was obtained from the 1991 census data for Leicestershire which gives both total population and ethnic breakdown by age. For the purpose of this study persons of Indian, Pakistani, and Bangladeshi, were treated as a single group as it would be very difficult to distinguish between then by name.

Fig 2.6 **Map showing the location of the Trent reporting region**

Health Regions
(England)

Trent Health
Region

- - - - Leicestershire

● Leicester
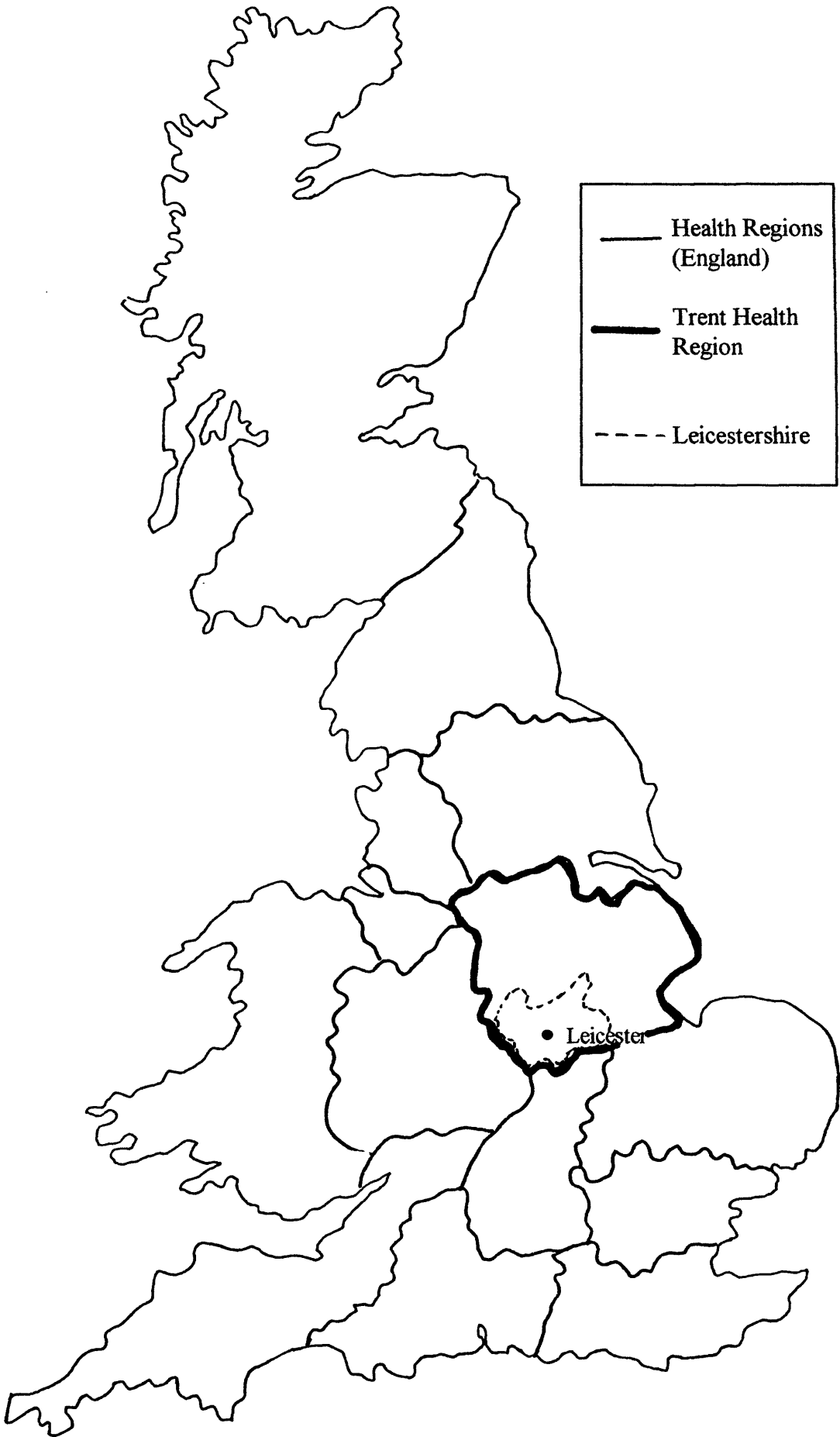
2.4 METHODS

2.4.1 DETERMINATION OF THE COMPARATIVE STATISTICAL MEASUREMENTS ASIR, SIR, relSIR, AND AVERAGE AGE OF ONSET

International data for ASIR was extracted from vols III, VII 'Cancer Incidence in Five Continents' (Waterhouse et al, 1976; Parkin et al, 1997), and from Steinitz et al (1989). SIR was also available from these sources with only minor modifications. ASIR data for the Trent region was obtained from 'Cancer Incidence in Five Continents vol VI' (Parkin et al, 1992).

ASIR and SIR for the Western Region of Saudi Arabia, and ASIR for Leicestershire Europeans and Asians was calculated from patient record data of the NCR and Leicestershire Pathology Service. Rel SIR, and average age of onset was calculated in all cases. Data was entered into Excell 7.

ASIR

To calculate ASIR it is necessary to know, or be able to derive, the total population (affected and non affected) of the study region, and the number and ages of affected persons in that region.

Calculation of ASIR for Saudi woman in the Western Region of Saudi Arabia

The NCR is compiled on EPI INFO 6; this was transferred to Excell 7. Data was sorted according to site, sex and age; breast cases were selected and male cases deleted. This was performed for 1994, 1995, and 1996 and the resulting files merged. Manipulation of the raw data in the spread sheet was performed by creating suitable equations and macros in Excell 7.

Cases from Makkah district are identifiable in the NCR records by the hospital code, and all cases had details of nationality. It was therefore possible to find the number of new Saudi cases of breast cancer in the Makkah district reported for 1994. By utilising this figure and the crude incidence rate for breast cancer in Saudi females in the Makkah district quoted for 1994, the population of Makkah district could be calculated. The age structure for the female Saudi population of Makkah district was calculated from the 1977 population census (Central department of statistics, 1977) and was assumed to be representative for that of the whole of the Western Region. The total population for each age group in the Western Region was calculated. Knowing this and the total number of cases of female breast cancer occurring in each age group over a three year period (1994-1996), the ASIR for breast cancer was calculated.

Calculation of ASIR for the European and Asian Communities of Leicestershire

Trent region is not ethnically homogeneous and includes a sub set of British Asians. The Leicestershire data base has the advantage over the Trent data in that the Asian and European sub populations can be identified. The Leicester Asians (AL) in the database were identified as Asian by name; this method has been shown to be a satisfactory method of identifying Asian patients (Nicoll et al, 1986; Winter et al, 1999). The age specific data for the female populations of Indian, Pakistani, and Bangladeshi ethnic origin in Leicestershire was obtained from the 1991 census and used to produce age specific data for a combined Leicestershire Asian female population. This and the number of breast cancer cases occurring in this population during 1994 to 1997 was used to calculate the ASIR for the AL breast cancer population. The mean and standard deviation for grouped data was calculated for the AL population.

The ASIR for the Leicester European breast cancer population (EL) was also calculated. The population EL from the Leicestershire data base was compared statistically with that for the Trent region. Using the age specific data for the total female European population of Leicestershire and the known number of breast cancer cases occurring in this population during 1995 and 1996 the ASIR was calculated. The mean and standard deviation for grouped data was calculated for the Leicestershire European breast cancer population.

The ASIR data from the three study populations was compared using a modified Mantel-Haenszel test (section 2.4.4).

Standardised Incidence

The standardised incidence rate (SIR) is the rate expected if the observed age specific rates had occurred in a standard population. The standardised incidence was compiled from published data, or, when such data was unavailable calculated according to the method outlined in Parkin et al (1992).

The calculation comprises the following steps:

The ASIR for each age group is multiplied by the number in that age group in the standard population. This is repeated for each age group. The numbers are added and the sum divided by the standard population.

The standard error is then calculated. The varience for each age group is calculated by multiplying the expected number in the standard population by the total number of persons in the standard population at that age group. The product is then divided by the number of years over which the data was collected multiplied by the actual number of persons in that age group in the study population.

This is repeated for all age groups and the results summed. The square root is taken of this figure and divided by the total number of persons in the standardised population to obtain the standard error of the standardised rate.

Standardised Incidence from International data, including the Trent Region

A spread-sheet was compiled from the data in the standardised incidence tables for breast cancer from Steinitz et al (1989), Parkin et al (1992), and Parkin et al (1997). In order to present the maximum data spread, the incidence of breast cancer in a country was calculated plus and minus the standard deviation when available. Where countries had results from more than one registry, the median value was calculated, and also values plus or minus the maximum standard deviation between the contributing registries. Excell 7 was used for data manipulation.

Standardised Incidence of breast cancer in the Western Region

The NCR report only provided the crude incidence rate for female breast cancer in the Western Region, therefore to allow comparison of the total incidence with other registries it was necessary to standardise the rate. The standardised incidence rate for female breast cancer in the Western Region of Saudi Arabia and the estimated standard error was calculated with the world population as the standard (Fig 2.4 section 2.1.3) using the methods described by Parkin et al (1997) utilising the Excell 7 software worksheet.

RelSIR

RelSIR is equal to the SIR of a given cancer divided by SIR for cancers at all sites. The value of relSIR gives a measure of the relative proportion of the tumour burden attributable to breast carcinoma in a given population. RelSIR was calculated for the international data, and at five yearly age intervals for the Trent and Western Region breast cancer populations.

Average age of onset

The mean age, and standard deviation of age was calculated for each group using the standard methods for grouped data (Daniel et al, 1987). Calculations were made for the Western Region, Leicester Asians, Leicester Europeans, Trent and International data.

2.4.2 COMPARISON OF STANDARDISED INCIDENCE RATES

A series of countries was selected for whom age standardised incidence rates were available; all incidence rates were standardised to the world standard population. These included African, Asian, Western European, Eastern European, Mediterranean European and North American. The ASIR data for the Western Region of Saudi Arabia was prepared and the standardised incidence rate calculated.

Calculation of median incidence and allocation of countries into high and low incidence groups

The median standardised female breast cancer incidence rate was calculated and the populations were grouped into low or high incidence categories, according to whether their incidence was above or below the median.The mean and variance was calculated for both categories, and Z-tests performed to compare the categories. Populations were then recorded by geographic or ethnic origin and a chi squared analysis performed to assess the probability of the distribution of the populations found in the two incidence categories to be occurring at random.

Comparison of standardised incidence rates over a twenty year period, between 1976 and 1997, in low and high incidence countries

Populations with records published in both Cancer in Five Continents vol. III (Waterhouse et al, 1976) and Cancer in Five Continents Vol. VII (Parkin et al, 1997) were selected and their standardised incidence rates for female breast cancer tabulated for 1976 and 1997. The data from each volume is identified as 1976 data and 1997 data; this refers to the publication date rather than the date of data collection (which varies slightly from population to population) but the overall time difference is still approximately twenty years.

Utilising the median value, low and high incidence categories for 1997 and 1976 were allocated. Populations were then recorded by geographic or ethnic origin and studied to see whether the same regional distribution was present for the low and high incidence categories as was seen in the initial 1997 analysis. The difference between the rates for 1997 and 1976 between the populations was calculated, tabulated and a Z test utilised to compare any increase in low and high incidence countries.

SIR of cancer of all sites and relSIR of breast cancer between 1976 and 1997

Using the same data set, graphs were compiled of the total cancer incidence for all sites, and the relSIR, in order to get a measure of the relative proportion of the tumour burden attributable to breast carcinoma in the selected countries and how this varied over the twenty year period.

2.4.3 ASIR AND AGE OF ONSET OF BREAST CANCER IN SELECTED COUNTRIES WITH RESPECT TO THEIR LEVEL OF INCIDENCE

ASIR

ASIR data for female breast cancer (NCR, 1996, Parkin et al, 1997), was entered into Excell 7 to form a spread sheet. When countries had entries from more than one registry, with no whole country registry, that with a level closest to the mid value of the

incidence range was selected. A graph of log incidence against age was made for the selected countries, the incidence category to which they belonged being represented by different colours.

Age of Onset

The actual numbers of cases were either counted (for the Western Region of Saudi Arabia), or calculated from the group data in the spread sheets, and used for the calculations of the mean age, and standard deviation of age, using standard methods for grouped data (Daniel et al, 1987) for each group. The data was assessed using Z test analysis to find if there was a significant difference in the mean age of onset in high incidence and low incidence countries internationally.

2.4.4 BREAST CANCER INCIDENCE IN TRENT UK COMPARED WITH THE LEICESTERSHIRE EUROPEAN AND ASIAN SUBGROUPS AND THE WESTERN REGION. OF SAUDI ARABIA

The mean age of onset, the standard deviation and variance was calculated for Trent and the Western Region and a Z test performed to compare the mean age of onset of both populations. The mean age of onset, the standard deviation and variance were also calculated for the Leicester European and Leicester Asian sub groups, and Z tests performed.

ASIR, and relSIR

The rel SIR was calculated from the ASIR for breast and from ASIR for all sites for both the Western Region and Trent and examined graphically .

Mantel-Haenszel test on the ASIR data from the three study populations

The Mantel-Haenszel test method used is that detailed in Esteve et al (1994) for the comparison of incidence rates in two populations. The test takes as the null hypothesis that the age specific rates are equal, against the alternative hypothesis that the rates are proportional. A sample calculation, Western Region tested against Leicester Europeans for all age groups, is shown in fig2.7 using Excell 7.

The test was used to compare the breast cancer populations of

W.Region with EL, W.Region with AL, and AL with EL

for age ranges:

20-69 years, 20-44 years, and 45-69 years

The basic steps were:

1.The age specific frequencies for breast cancer in the two populations, k1 and k2, were added to form combined age specific frequencies K.

Fig 2.7 Sample Mantel-Haenszel test   W.Reg vs. EL

AGE	k1	k2	K	Pop W R	m1	Pop EL	m2	M
20-24	11.00	0.00	11.00	136209.48	408628.45	28656.00	57312.00	465940.45
25-29	39.00	3.00	42.00	118516.23	355548.68	29598.00	59196.00	414744.68
30-34	79.00	20.00	99.00	105796.44	317389.33	27201.00	54402.00	371791.33
35-39	62.00	36.00	98.00	91962.58	275887.75	25908.00	51816.00	327703.75
40-44	72.00	51.00	123.00	81030.83	243092.50	29475.00	58950.00	302042.50
45-49	73.00	83.00	156.00	50870.87	152612.61	24378.00	48756.00	201368.61
50-54	54.00	126.00	180.00	55596.77	166790.32	20898.00	41796.00	208586.32
55-59	55.00	92.00	147.00	26220.79	78662.38	19994.00	39988.00	118650.38
60-64	35.00	90.00	125.00	46854.68	140564.03	20697.00	41394.00	181958.03
65-69	41.00	78.00	119.00	69857.01	209571.04	20480.00	40960.00	250531.04

KEY:

k1	frequency of breast cancer in the Western Region (WR)
k2	frequency of breast cancer in the the Leicester Europeans(EL)
K	k1+k2
m1	population WR X 3 (number of years covered by frequency data) (person years in WR)
m2	population EL X 2 (number of years covered by frequency data) (person years in EL)
M	m1+m2

From this data the expected number in EL (e2) can be calculated for each group
$$e = K * (m2/M)$$
and the varience
$$var(k2) = K * ((m1*m2)/(M*M))$$

AGE	M	m1	m2	K	k2	e 2	Var(k2)
20-24	465940	408628.45	57312	11	0	1.35303	1.186605
25-29	414745	355548.68	59196	42	3	5.99461	5.139005
30-34	371791	317389.33	54402	99	20	14.4861	12.36642
35-39	327704	275887.75	51816	98	36	15.4956	13.04546
40-44	302043	243092.5	58950	123	51	24.0061	19.32077
45-49	201369	152612.61	48756	156	83	37.7712	28.62593
50-54	208586	166790.32	41796	180	126	36.0679	28.84074
55-59	118650	78662.38	39988	147	92	49.5425	32.8455
60-64	181958	140564.03	41394	125	90	28.4365	21.96743
65-69	250531	209571.04	40960	119	78	19.4556	16.27478
SUM		2348747	494570	1100	579	232.609	179.6126
						sqrt	13.40196

The value of the test statistic

Z = sum observed - sum expected / square root of the sum of vark2

Z = sum k2 - sum expected 2 / square root of the sum of vark2

Z = 579 - 232.6 / 13.4

Z = **25.85 more**   **than 1.96**   **therefore not same**

Adapted from: Walker RA
Chp 16 The breast, General and systemic pathology
Underwood JCE (Ed), Churchill Livingstone (pub) 1992

2. The number of person years in the general population for each age range was calculated for the two populations (i.e. the population at a given age multiplied by the number of years covered by the frequency data), m1 and m2, which were then added to form combined person years for each age group M.

3. The number of cases expected for population 2, if proportionality existed
   with population 1, were calculated (e2).

$$e2 = K \times (m2/M)$$

4. The variance for cases in population 2 were also calculated

$$var(k2) = K \times (m1 \times m2) / M^2$$

5. The test statistic Z was calculated

   Z = sum of observed - sum expected / square root of the variance

$$Z = \Sigma k2 - \Sigma e2 / \sqrt{var(k2)}$$

6. The test statistics were compared with the calculated critical value of Z
   of 1.96 for a two tailed test at 95% confidence level.

2.4.5 AGE SPECIFIC RELATIVE RISK OF BREAST CANCER IN THE LEICESTERSHIRE ASIAN , LEICESTER EUROPEAN, AND WESTERN REGION BREAST CANCER POPULATIONS COMPARED TO THAT IN TRENT

For any cancer, dividing the ASIRs of two populations gives a measure of comparison of the relative risk of that cancer in the those populations (Breslow and Day, 1980). The ASIR of the three study groups was compared to that of Trent. ASIR are drawn on a log scale and the separation of the two lines gives a measure of relative risk (subtraction of the logs of two numbers is equivalent to dividing those numbers). If there exists, e.g. between two populations, a situation of constant relative risk irrespective of age, then the ASIR graph would be expected to show two parallel lines (Esteve et al, 1994), the difference between them being constant for all age groups.

In order to investigate any age specific differences in relative risk for breast cancer, the three populations were compared graphically with the Trent region. The ASIRs of the Western Region, the Leicestershire Europeans, and the Leicestershire Asian breast cancer populations were each divided by the ASIR for female breast cancer for the Trent region and the log of the quotient plotted against age.

2.5 RESULTS

2.5.1 ASIR AND STANDARDISED INCIDENCE OF FEMALE BREAST CANCER IN THE WESTERN REGION OF SAUDI ARABIA

ASIR

The ASIR data in table 2.4 shows a steady increase the 30-34 years old age group after which there is a slight decrease followed by a rise, until a peak is reached at the 55- 59 years old age group, and a subsequent decrease in ASIR. The data in Table 2.4 was used to plot Fig 2.13 section 2.5.5.

Table 2.4 Population structure and ASIR of female breast cancer in the Western Region of Saudi Arabia

Age range	total	male	female	male : female ratio	total number female breast cancer cases 1994-96	ASIR of female breast cancer
0-4	651698.9	330073.4	321625.6	1:0.97		
5-9	644486.3	328802.5	315683.8	1:0.96		
10-14	504942.8	263022.2	241920.7	1:0.92		
15-19	391647.7	204303.2	187344.4	1:0.92	1	0. 18
20-24	281848.8	145639.3	136209.5	1:0.94	11	2.69
25-29	232658.7	114142.4	118516.2	1:1.04	39	10.97
30-34	207447.4	101651	105796.4	1:1.04	79	24.89
35-39	192367.4	100404.8	91962.58	1:0.91	62	22.47
40-44	171521.7	90490.9	81030.83	1:0.90	72	29.62
45-49	120326.3	69455.39	50870.87	1:0.73	73	47.83
50-54	119481.8	63884.99	55596.77	1:0.87	54	32.38
55-59	65576.74	39355.95	26220.79	1:0.67	55	69.92
60-64	105018	58163.29	46854.68	1:0.81	35	24.90
65+	147776.7	77919.66	69857.01	1:0.90	41	19.56

Standardised incidence rate

Using the data in table 2.4 and the world standard population (Parkin et al, 1997), the standardised incidence rate for female breast cancer in the Western Region of Saudi Arabia was calculated as 14.89 per 100,000 females with a standard error of 0.01.

2.5.2 INTERNATIONAL INCIDENCE DATA FROM HIGH RISK AND LOW RISK POPULATIONS

Table 2.5 **Incidence of female breast cancer in different countries**

Low incidence countries	upper limit	mean	lower limit
Korea	8.6	7.1	5.6
Algeria	10.39	9.5	8.61
Mali	11.35	10.2	9.05
Thailand	15.9	11.5	7.1
Saudi Arabia(Western Region)	14.9	14.89	14.88
Vietnam	19.03	18.2	17.37
India	26.07	19.27	12.47
Zimbabwe, African	22.81	20.4	17.99
Uganda	22.87	20.7	18.53
China	34.7	20.8	12.5
Palestinian Israelis	22.66	21.3	19.94
Japan	31.58	26.28	20.98
La Reunion	30.91	29.4	27.89
Belarus	29.9	29.6	29.3
Kuwait	35.29	32.8	30.31
Latvia	34.3	33.7	33.1
Hongkong	34.48	34	33.52
Singapore	39	35.1	31.2
Asian Jewish Israelis	35.9	35.9	35.9
Estonia	37.32	36.5	35.68
Poland	45.2	36.6	28
Croatia	37.74	37.2	36.66
Slovakia	39.09	38.6	38.11

Table 2.5(cont.)   Incidence of female breast cancer in different countries

High incidence countries	upper limit	mean	lower limit
Yugoslavia	44.37	43.6	42.83
Czech Republic	45.45	45.1	44.75
Slovenia	47.03	46.2	45.37
Spain	53.9	46.9	39.9
Philippines	48.58	47.7	46.82
Germany (Eastern States)	48.65	48.2	47.75
Norway	54.83	54.2	53.57
Germany (Saarland)	62.73	61.5	60.27
Eire	66.31	64.2	62.09
Austria	66.68	64.9	63.12
Finland	65.56	65	64.44
Italy	74.5	65.2	55.9
Switzerland	76.1	68.9	61.7
UK	77	72.7	68.4
Sweden	73.39	72.9	72.41
Denmark	73.94	73.3	72.66
Netherlands	78.9	74.6	70.3
France	82.9	74.9	66.9
Canada	77.1	76.8	76.5
Iceland	82.45	79	75.55
USA SEER Black	80.41	79.3	78.19
Malta	84.1	79.9	75.7
European Jewish Israelis	89.45	87.9	86.35
USA SEER White	91.09	90.7	90.31

The standardised incidence, based on the standard World population (Table 2.5) in different countries ranged from a mean of 7.1 in Korea to 90.7 in the SEER study of USA whites. The median incidence value was calculated as 43.6; countries with an incidence value less than this were classed as low incidence risk countries, and those with an incidence equal or greater than the median as high incidence risk countries.

To assess if the difference in incidence was statistically significant, the mean incidence and variance of breast carcinoma in low risk countries and high risk countries was calculated (Table 2.6).

Table 2.6 **Mean incidence and variance of breast carcinoma in low risk and high risk regions**

	mean	Standard deviation	Variance
low risk	25.18	10.7	104.57
high risk	65.98	14.1	198.5

A Z-test for the comparison of two means resulted in a Z value of 11.39 which is greater than the critical Z value, at the 95% confidence level of 1.96, and there is therefore a significant difference between the incidence in the two groups.

Figure 2.8 is a graphical representation of the data in Table 2.5 and illustrates the level and ranges of incidence in the selected countries. The countries are ordered according to incidence rates and there is an apparent polarisation of Asian and African countries to the lower end of the incidence scale and European countries to the higher end. Populations were then recorded by geographic or ethnic origin and a chi squared analysis performed to assess the probability that the distribution of the populations into high and low incidence was occurring at random (Table 2.7).

Table 2.7 **Chi squared analysis of the distribution of high and low incidence countries**

Countries	expected in high incidence group	expected in low incidence group	observed in high incidence group	observed in low incidence group
Mediterranean + European = 28	14	14	22	6
African +Asian = 19	9.5	9.5	2	17

The chi squared value for the above data equals 19. 51 with one degree of freedom, this is greater than the critical value of chi squared of 3.84, and the observed distribution can be considered non random, there being an association between the incidence group and ethnic origin of the population.

Fig 2.8 **Breast cancer incidence in selected countries**

Incidence

Countries

Korea
Algeria
Mali
Thailand
Saudi Arabia
Vietnam
India
Zimbabwe, African
Uganda
China
Palestinian Israelis
Japan
La Reunion
Belarus
Kuwait
Latvia
Hongkong
Singapore
Asian Jewish Israelis
Estonia
Poland
Croatia
Slovakia
Yugoslavia
Czech Republic
Slovenia
Spain
Philippines
Germany (Eastern)
Norway
Germany (West)
Eire
Austria
Finland
Italy
Switzerland
UK
Sweden
Denmark
Netherlands
France
Canada
Iceland
USA SEER Black
Malta
European Jewish Israelis
USA SEER White

upper limit
Mean
lower limit

2.5.3 COMPARISON OF STANDARDIZED INCIDENCE RATES OVER A TWENTY YEAR PERIOD

Table 2.8   **Standardised incidence rates for breast cancer in 1976 and 1997**

Based on the median value for each data set the low or high incidence group is indicated by the letter (L) or (H).

Country	1976	1997	difference
Zimbabwe(African)	13.8 (L)	20.4(L)	6.6
Israel(Palestinians)	11(L)	21.3(L)	10.3
Japan (Osaka)	12.1(L)	24.3(L)	12.2
India (Bombay)	20.1(L)	28.2(L)	8.1
Singapore	20.8(L)	35.1(L)	14.3
Spain (Zaragoza)	30.6(L)	40.4(L)	9.8
Poland (Warsaw)	31.5(H)	43.2(L)	11.7
Slovenia	28.3(L)	46.2(L)	17.9
Germany (Eastern States)	33.4(H)	48.2(L)	14.8
Norway	44.4(H)	54.2(L)	9.8
Finland	32.9(H)	65(H)	32.1
Sweden	52.4(H)	72.9(H)	20.5
UK (South West)	52.1(H)	73(H)	20.9
Denmark	49.1(H)	73.3(H)	24.2
Switzerland (Geneva)	70.6(H)	77.8(H)	7.2
Iceland	49.5(H)	79(H)	29.5
Malta	39.6(H)	79.9(H)	40.3
USA black (Detroit)	51(H)	80.8(H)	29.8
Canada (British Colombia)	80(H)	84.3(H)	4.3
Israel (European/USA Jews)	60.8(H)	87.9(H)	27.1
USA white (Detroit)	65.7(H)	91.9(H)	26.2
median value	31.05	65	

N.B. The median in this smaller 1997 data set is different from that in the larger 1997 data set shown in Table 2.5 where the median is 43.6.

Table 2.8 shows a clear rise in the median value of incidence between 1976 and 1997. Figure 2.9 was plotted from the table and appears to show a greater increase in incidence in the high incidence countries than in the low incidence countries. The
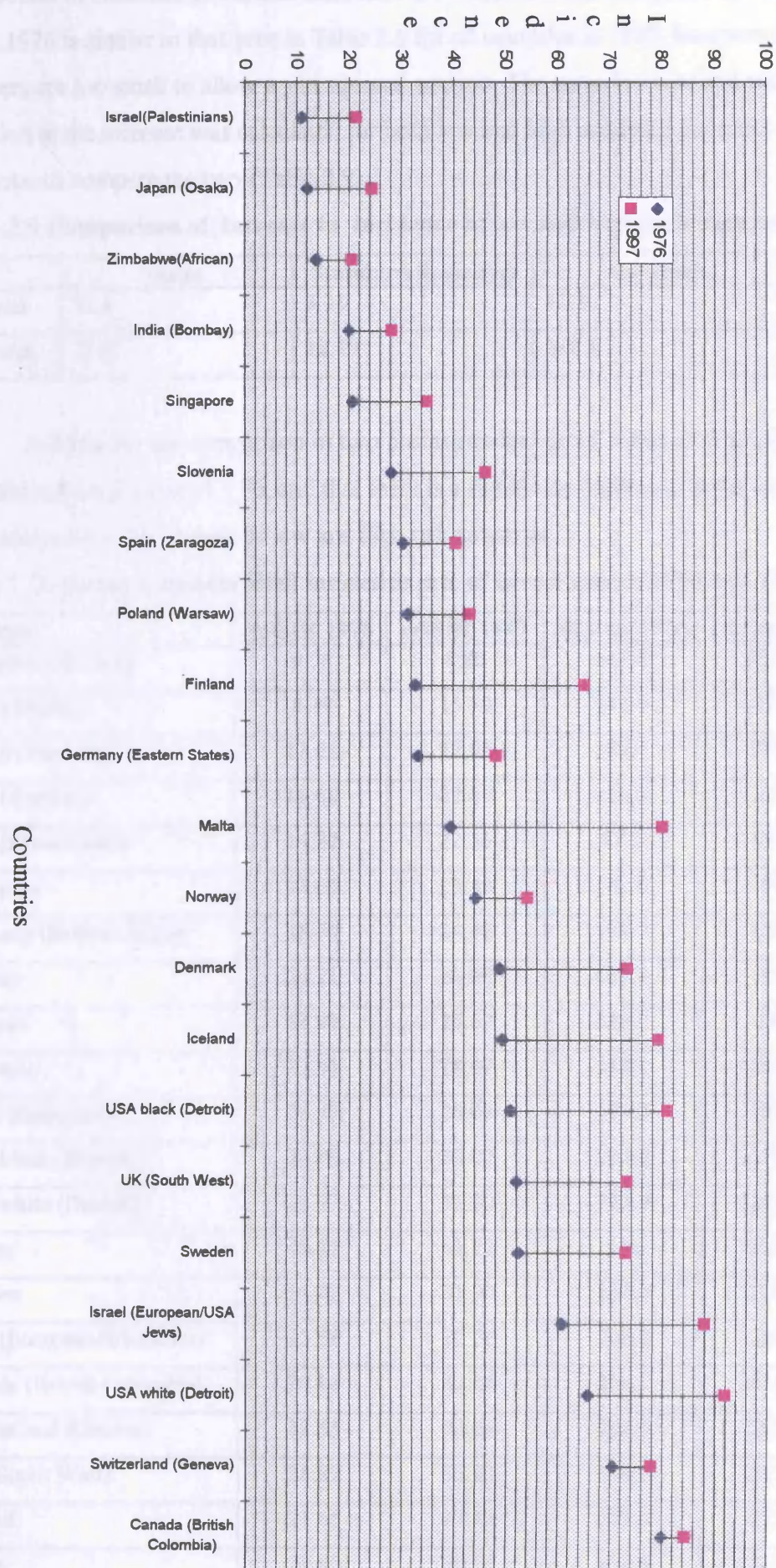
Fig 2.9 Changes in breast cancer incidence in selected countries 1976 - 1997

distribution of countries of African and Asian low incidence and European high incidence in the 1976 is similar to that seen in Table 2.5 for all countries in 1997, however the numbers are too small to allow a chi squared analysis. The mean increase and standard variation in the increase was calculated for both low and high incidence countries and a Z test done to compare the two (Table 2.9).

Table 2.9 **Comparison of increase in incidence in low and high incidence countries**

	mean	**Standard deviation**	**variance**
low risk	11.4	3.38	11.4
high risk	23.8	10.49	110.1

A Z test for the comparison of two means resulted in a Z value of 3.67, greater than the critical Z value of 1.96, and that there is a significant difference in the increase in incidence seen in this sample of low and high risk countries.

Table 2.10 **Relative standardised incidence rate of breast cancer 1976 and 1997**

Country	relSIR 1976	relSIR 1997	all sites 1976	all sites1997
Zimbabwe(African)	9.36	8.80	147.4	231.9
Japan (Osaka)	8.49	15.70	142.6	154.8
Poland (Warsaw)	17.02	22.02	185.1	196.2
India (Bombay)	16.68	22.49	120.5	125.4
Israel(Palestinians)	14.27	22.56	77.1	94.4
Singapore	14.65	23.40	142.0	150.0
Germany (Eastern States)	18.59	24.42	179.7	197.4
Norway	24.21	24.98	183.4	217
Slovenia	18.97	25.67	149.2	180
Denmark	22.41	28.02	219.1	261.6
Spain (Zaragoza)	22.97	28.08	133.2	143.9
USA black (Detroit)	22.83	29.02	223.4	278.4
USA white (Detroit)	28.77	31.30	228.4	293.6
Finland	19.82	31.72	166	204.9
Sweden	24.92	31.90	210.3	228.5
Israel(European/USAJews)	25.68	32.76	236.8	268.3
Canada (British Colombia)	30.88	33.14	259.1	254.4
Switzerland (Geneva)	32.85	33.65	214.9	231.2
UK (South West)	28.82	33.91	180.8	215.3
Iceland	21.65	37.18	228.6	212.5
Malta	29.60	39.55	133.8	202

Graphs were plotted from the data in Table 2.10 to illustrate the standardised incidence of cancer for all sites 1976 and 1997 (Fig 2.10), and the changes in relSIR for breast cancer between 1976 and 1997 (Fig 2.11).

The standardised incidence of cancer for all sites shows varying degrees of increase in all countries except Iceland and Canada (British Colombia). Iceland however, together with Finland and Malta, shows a marked increase in the relative proportionate burden of breast cancer when the rel SIR is considered. Apart from Switzerland, Canada (British Columbia), USA white (Detroit), Norway, and Zimbabwe (African) which show relatively little increase in relSIR, all other countries show a similar rise in the relative proportionate burden of breast cancer.

2.5.4 AGE SPECIFIC INCIDENCE OF BREAST CANCER IN SELECTED COUNTRIES WITH RESPECT TO THEIR LEVEL OF INCIDENCE

Using the full range of countries from the 1997 data a log ASIR against age Fig 2.12a was plotted, the lines being coloured according to the level of breast cancer incidence, high incidence dark blue, and low incidence red. When more than one registry existed the registry with the standardised rate closest to the mean value was chosen. Figure 2.12a shows the complete set of curves for 1997, including three very irregular curves towards the bottom of the graph, shown in pink. These were identified as being for Mali, Korea, and Algeria; it was decided to omit these from subsequent calculations (see discussion). The data was replotted (Fig 2.12b) without these three data sets.

The ASIR curve for low incidence countries is lower than that of the high incidence countries throughout the age range except before the age of 27 years when the ASIR for low incidence countries is either equal to or more than that in the high incidence countries. The low and high incidence curves are approximately parallel until the 30-35 year old age group when there is an inflexion of the low incidence curves at about 30-40, where as a similar inflexion of the high incidence curves does not occur until 40-50 years of age. This difference results in the subsequent diversion of the two sets of lines.

Table 2.11 **Mean age of onset in high and low incidence groups**

countries	mean age of onset (years)	Z test score	result critical Z value = 1.96
high incidence vs low incidence	55.99 50.84	1.25	no significant difference
top 10 incidence vs bottom 10 incidence	56.24 48.7	2.07	significant difference
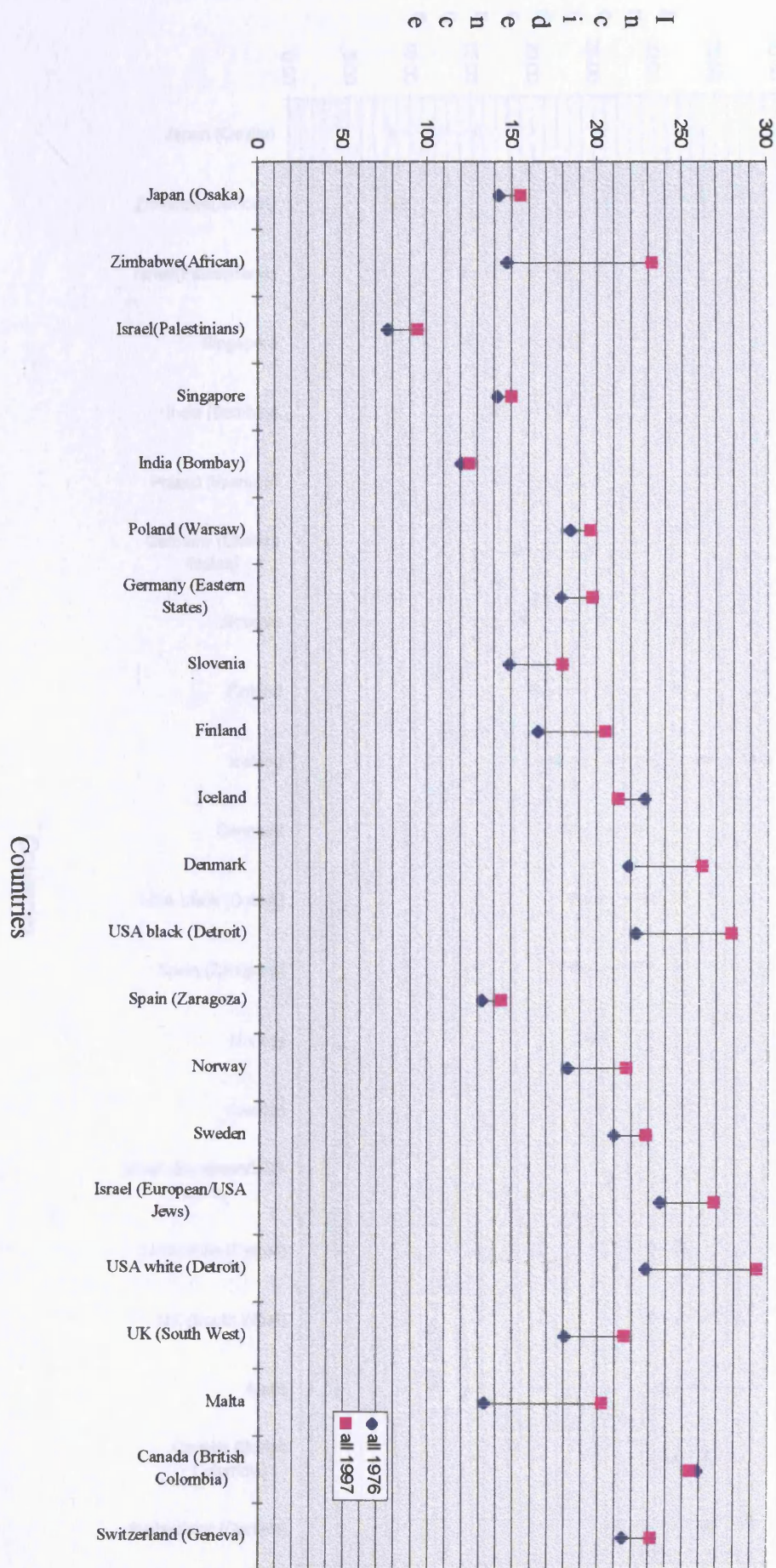
Fig 2.10 **Standardised incidence cancer all sites in selected countries 1976 - 1997**

Countries

Incidence

Japan (Osaka)
Zimbabwe(African)
Israel(Palestinians)
Singapore
India (Bombay)
Poland (Warsaw)
Germany (Eastern States)
Slovenia
Finland
Iceland
Denmark
USA black (Detroit)
Spain (Zaragoza)
Norway
Sweden
Israel (European/USA Jews)
USA white (Detroit)
UK (South West)
Malta
Canada (British Colombia)
Switzerland (Geneva)

◆ all 1976
■ all 1997
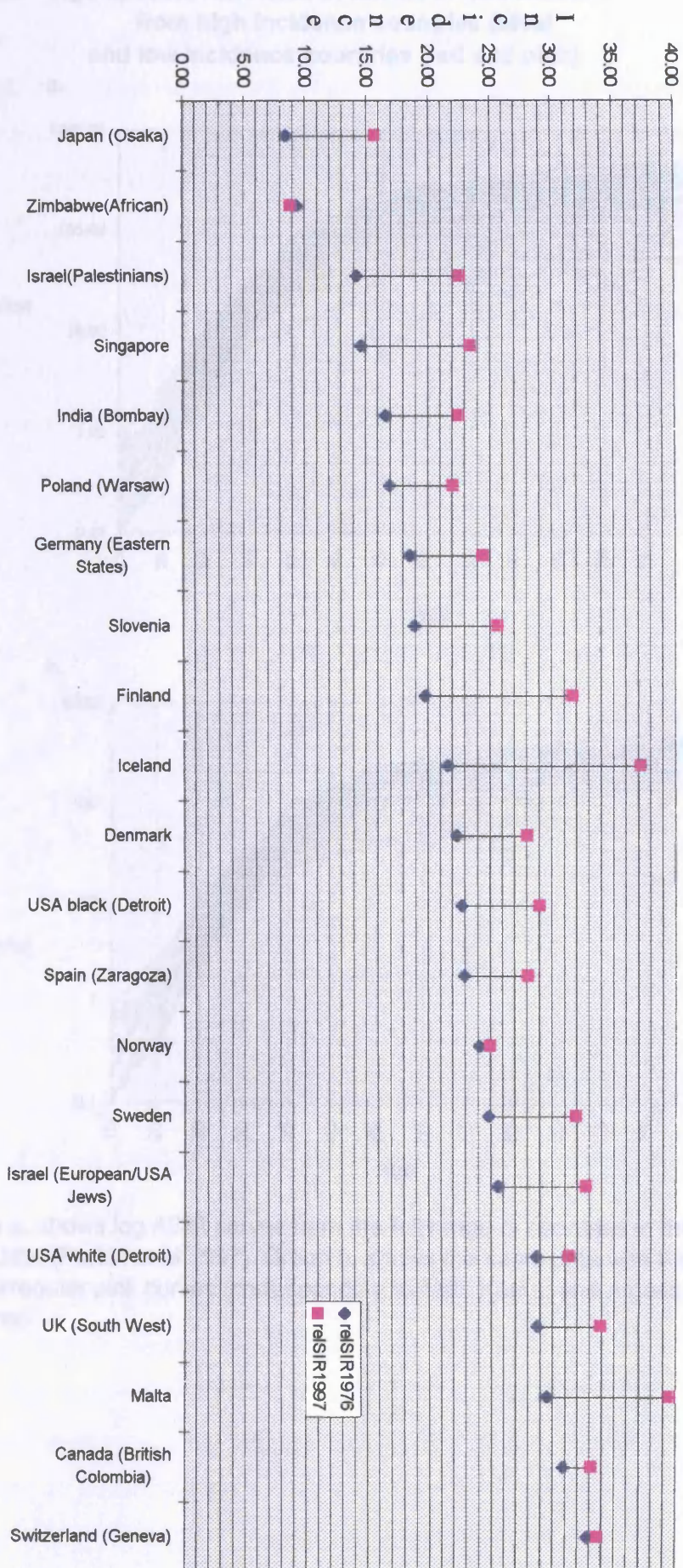
Fig 2.11 RelSir for breast cancer in selected countries 1976 - 1997

Fig 2.12 Age specific incidence of female breast cancer from high incidence countries (blue) and low incidence countries (red and pink)

a.

b.
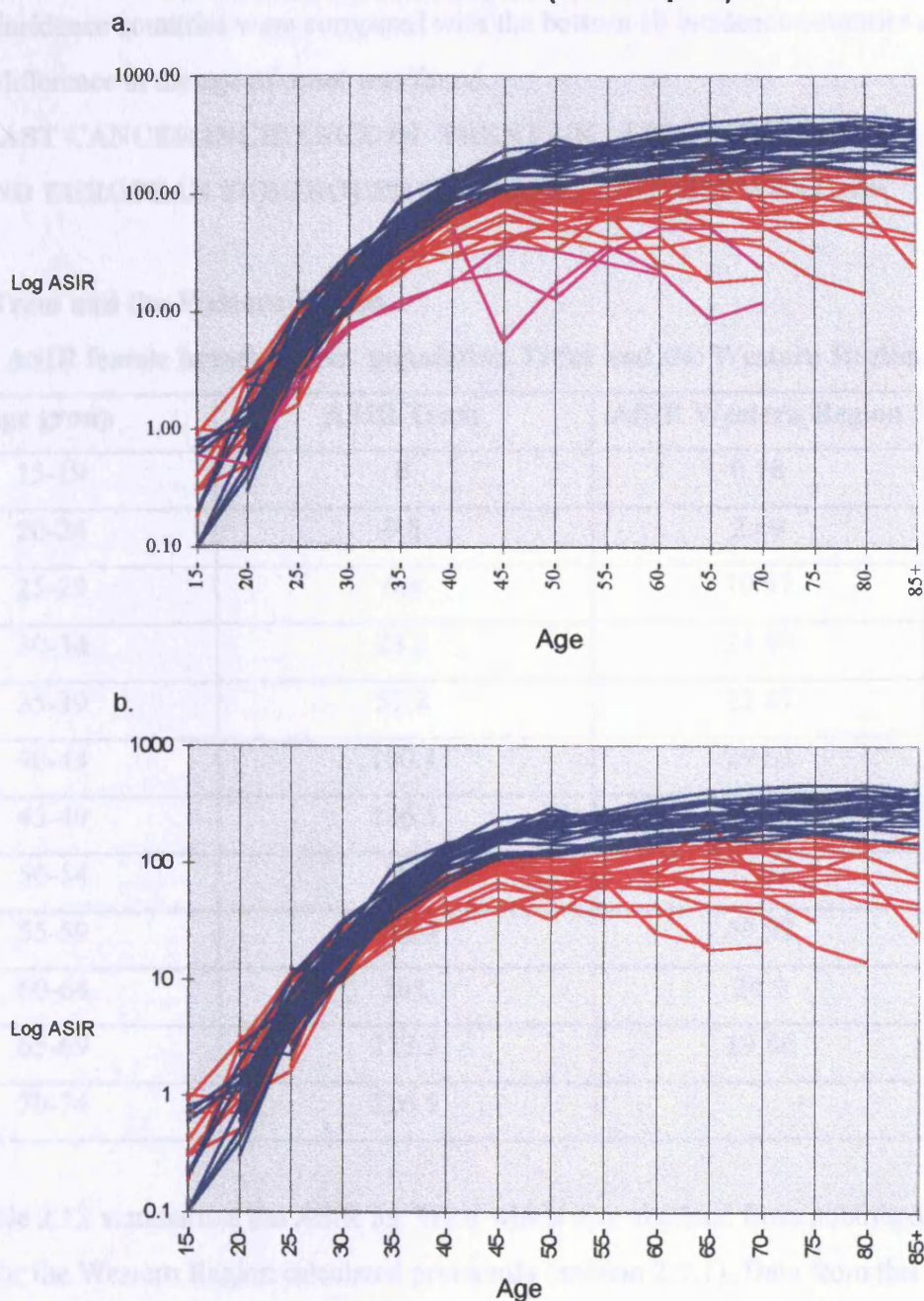
Graph a. shows log ASIR curves from the full range of countries in the 1997 data (Parkin et al 1997). Graph b. shows the same data with the three irregular pink curves (corresponding to Mali, Korea, and Algeria) removed.

The Z test when all countries were considered failed to show significant difference in mean age of onset between high and low incidence groups. However when the top 10 incidence countries were compared with the bottom 10 incidence countries a significant difference in the age of onset was found.

2.5.5 BREAST CANCER INCIDENCE IN TRENT UK , LEICESTERSHIRE ASIAN AND EUROPEAN SUBGROUPS OF TRENT, AND THE WESTERN REGION

ASIR of Trent and the Western Region

Table 2.12 ASIR female breast cancer population Trent and the Western Region

age group	ASIR Trent	ASIR Western Region
15-19	0	0.18
20-24	0.8	2.69
25-29	6.4	10.97
30-34	23.3	24.89
35-39	52.8	22.47
40-44	100.1	29.62
45-49	146.5	47.93
50-54	167	32.38
55-59	176.3	69.92
60-64	201	24.9
65-69	223.2	19.56
70-74	226.5	

Table 2.12 summarises the ASIR for Trent which was obtained from published data, and for the Western Region calculated previously (section 2.5.1). Data from this table is presented in Fig 2.13.

ASIR of the Leicester Asian population

Table 2.13 shows the ASIR of breast cancer in the Leicester Asian population increases steeply to reach a peak at 45-49 years of age, after which there is a decline rising again after the age of 55-59 to reach possibly a second peak at 60-65 year old age group. There were no reported cases younger than 25 years of age. The data from table 2.13 is represented graphically in Figure 2.13.

Table 2.13 ASIR Leicester Asian female breast cancer population

age group	no. females	no. breast cancer	ASIR(5 years)	ASIR
25-29	3708	2	53.94	10.79
30-34	3898	4	102.62	20.52
35-39	3425	3	87.59	17.52
40-44	2525	5	198.02	39.60
45-49	1821	17	933.55	186.71
50-54	1691	8	473.09	94.62
55-59	1325	13	981.13	196.23
60-64	1040	16	1538.46	307.69
65-69	682	4	586.51	117.30
70-74	482	6	1244.81	248.96
75-79	315	1	317.46	63.49
80+	222	4	1801.80	360.36

ASIR of the Leicester European population

Table 2.14 ASIR Leicester European female breast cancer population

age group	no. females	no. breast cancer	ASIR(2years)	ASIR
25-29	29598	3	10.14	5.07
30-34	27201	20	73.53	36.76
35-39	25908	36	138.95	69.48
40-44	29475	51	173.03	86.51
45-49	24378	83	340.47	170.24
50-54	20898	126	602.93	301.46
55-59	19994	92	460.14	230.07
60-64	20697	90	434.85	217.42
65-69	20480	78	380.86	190.43
70-74	17070	72	421.79	210.90
75-79	15573	56	359.60	179.80
80+	20569	66	320.87	160.44

The ASIR of breast cancer in the Leicester European population increases
steeply to reach a peak at 50-54 years, after which there is a gradual decline rising again

slightly in the 70-74 year old age group. There were no reported cases younger than 25 years of age. The data from table 2.14 is represented graphically in Fig 2.13.

Comparison of ASIR in Trent UK, the Leicestershire Asian and European subgroups of Trent and with the Western Region

Figure 2.13 shows a graphical comparison of all three populations with that of Trent region. The ASIR curve for Trent rises steadily until the 45-49 year old age group after which the rate of increase lessens until a peak at 65-69 years. The Leicester European population has an ASIR curve which closely follows that of Trent except in the 30-39 year and 50-55 year age ranges when the incidence is notably higher than that of Trent, and even higher than both AL and AA groups. Generally the curve rises to a peak at 50-55 years and then gradually declines. The ASIR of the Asian population however is somewhat different. With the exception of the very young age group, the incidence is always less than that for Trent rising slowly from 25-40 years and then sharply to peak at 45-49 years of age. After this age the curve is somewhat erratic with possible peaks at 60-65 and 70-75 years of age but the general tendency is one of gradual increase. The ASIR of the Western Region is higher than that of Trent from 15 - 30 years of age and after that is lower. The ASIR rises most steeply at 17- 22 years of age after which the rate drops slightly but continues to rise steadily until the 35-39 years old age group when a sharp drop in the rate of increase occurs, the point of inflexion of the curve described by Clemmesen. The curve then continues to rise, with some fluctuation, at this lower rate until a maximum ASIR is reached at the age 55-59 years, after which the ASIR decreases continually to the 65+ age group. The ASIR curves for the Western Region and for the Leicester Asian population increase at a similar rate until the 40-45 year old age group when the lines cross and diverge, the Leicester Asian population ASIR curve having a general rate of increase similar to that of Trent after the 45-49 years old age group.

Table 2.15 **Z test to compare mean age of onset**

Populations (mean age, variance)	Z test results
Trent (56.7,114.6) vs AA (45.9,165.9)	Z = 0.65 < 1.96 the critical test value at 95% confidence no significant difference of mean age of onset
Trent (56.7,114.6) vs AL (50.93, 156.5)	Z= 0.35 < 1.96 the critical test value at 95% confidence no significant difference of mean age of onset
Trent (56.7,114.6) vs EL (56.9, 145.5)	Z=0.009 < 1.96 the critical test value at 95% confidence no significant difference of mean age of onset

Fig 2.13   **ASIR in Trent UK, the Leicestershire European and Asian subgroups of Trent, and the Western Region of Saudi Arabia**
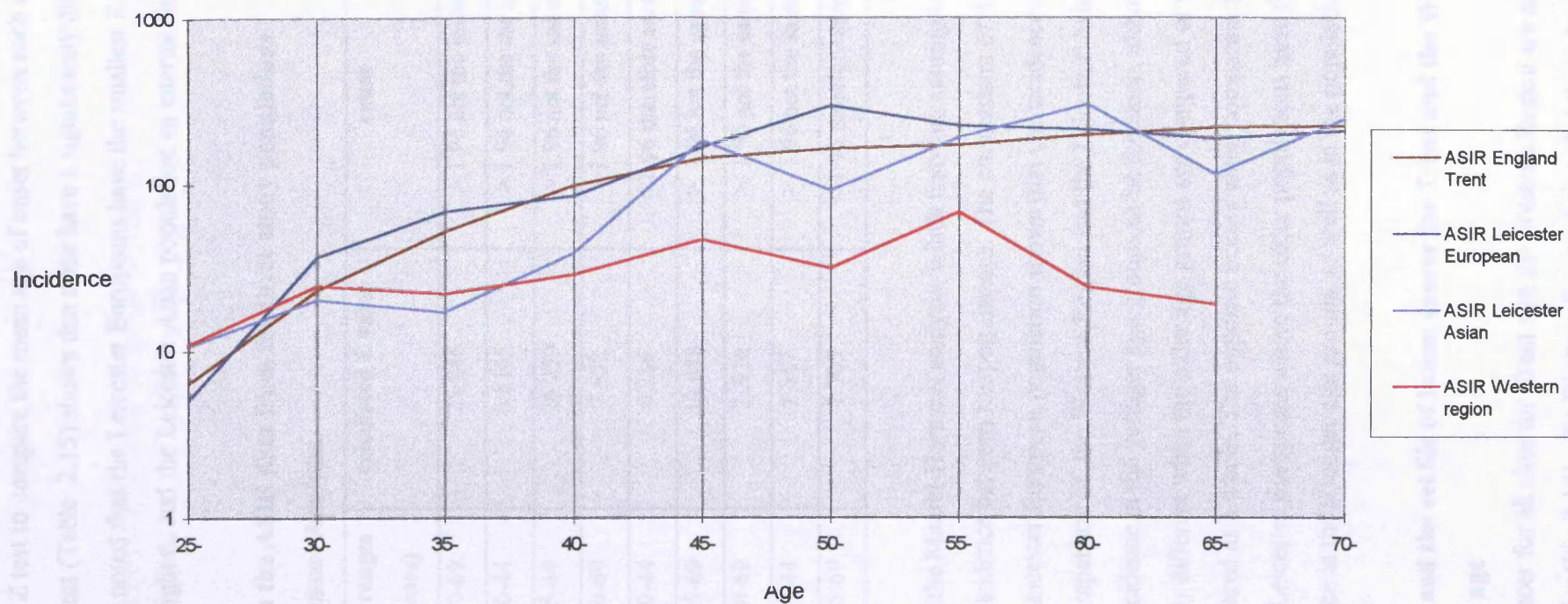
The results of the Z test to compare the mean age of onset between each of the study populations and Trent (Table 2.15) shows that none have a significantly different age of onset. It should be noted that the Leicester Europeans have the smallest Z value, the Western Region the highest, and the Leicester Asian population an intermediate value.

Mantel-Haenszel test on the ASIR data from the three study populations

Table 2.16 Mantel-Haenszel Analysis

Populations	age ranges (years)	calculated Z value	result
W.Region&EL	20-69	25.846	> 1.96 not the same
	20-44	6.8105	> 1.96 not the same
	45-69	26.259	> 1.96 not the same
W.Region&AL	20-69	7.652	> 1.96 not the same
	20-44	0.236	< 1.96 therefore same
	45-69	10.888	> 1.96 not the same
AL&EL	20-69	2..916	> 1.96 not the same
	20-44	2.857	> 1.96 not the same
	45-69	1.705	< 1.96 therefore same

Table 2.16 shows the Mantel-Haenszel analysis, which tests the assumption that there is no difference in incidence between the populations. The comparison of the Western region and the Leicester European population shows that the incidences are significantly different. Comparison of the Western Region and the Leicester Asian population showed the incidence in the younger age groups to be the same, although the incidences are significantly different when the older age groups are compared or when the populations are compared as a whole. The opposite occurs when comparing the Leicester Asians and the Leicester Europeans where the older populations have the same incidence, but the incidence in the younger age groups, as well as in the population as a whole, is different.

ASIR cancer of all sites and the rel Sir of breast cancer for Trent and the Western Region, with respect to age

The ASIR for cancer for all sites for Trent and the Western Region are notable in that they are very similar (Fig 2.14), the Western Region being slightly higher before the age of 35 and Trent being higher after 35 years of age, until the age of 57 when the two lines diverge. Figure 2.15 illustrates the relative tumour burden attributable to

Fig 2.14 **ASIR for cancer for all sites for Trent and the Western Region**
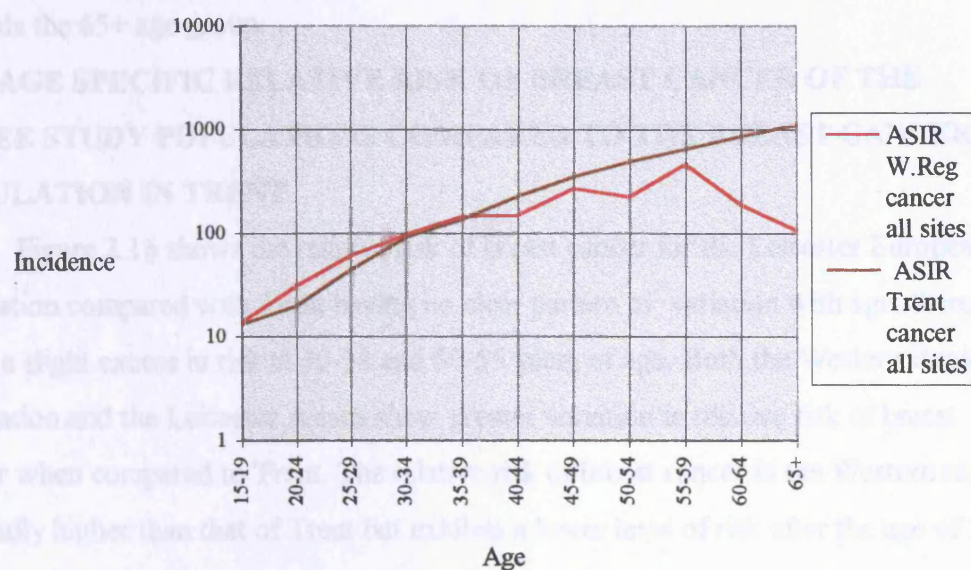
Fig 2.15 **relSIR for female breast cancer for Trent and the Western Region**
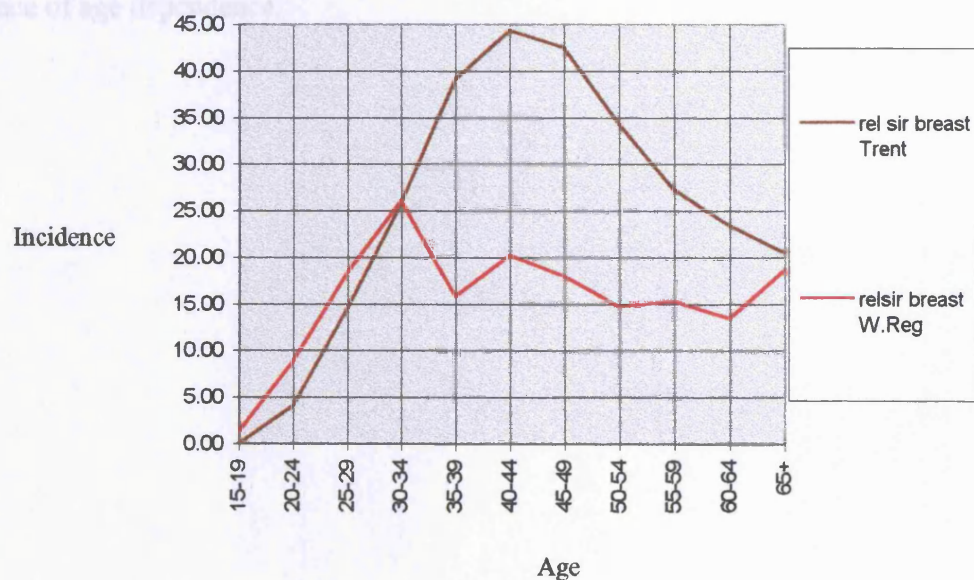
breast carcinoma in different age groups in Trent and the Western Region. In Trent there is a steady rise in relSIR to a peak at 42 years of age and then a slightly slower decline. The Western region shows a similar increase, but to a lower extent, which peaks at 32 years and then shows a much slower, slightly irregular, decline, possibly rising again towards the 65+ age group.

2.5.6 AGE SPECIFIC RELATIVE RISK OF BREAST CANCER OF THE THREE STUDY POPULATIONS COMPARED TO THE BREAST CANCER POPULATION IN TRENT

Figure 2.16 shows the relative risk of breast cancer for the Leicester European population compared with Trent having no clear pattern of variation with age, there being a slight excess in risk at 30-34 and 50-55 years of age. Both the Western Region population and the Leicester Asians show greater variation in relative risk of breast cancer when compared to Trent. The relative risk of breast cancer in the Western region is initially higher than that of Trent but exhibits a lower level of risk after the age of 30-34 years. The reduction in relative risk compared to Trent appears to relate approximately with age, apart from a slight elevation in risk at the 55-59 age group. The relative risk of breast cancer for the Leicester Asian population also declines almost directly with age until the 45-49 year age group when relative risk rises almost to one; after that the relative risk approximates to a constant level just below one with no evidence of age dependence.

Fig 2.16   **Relative risk of breast cancer in Leicester Asian and European populations and the population of the Western Region compared to that of Trent**
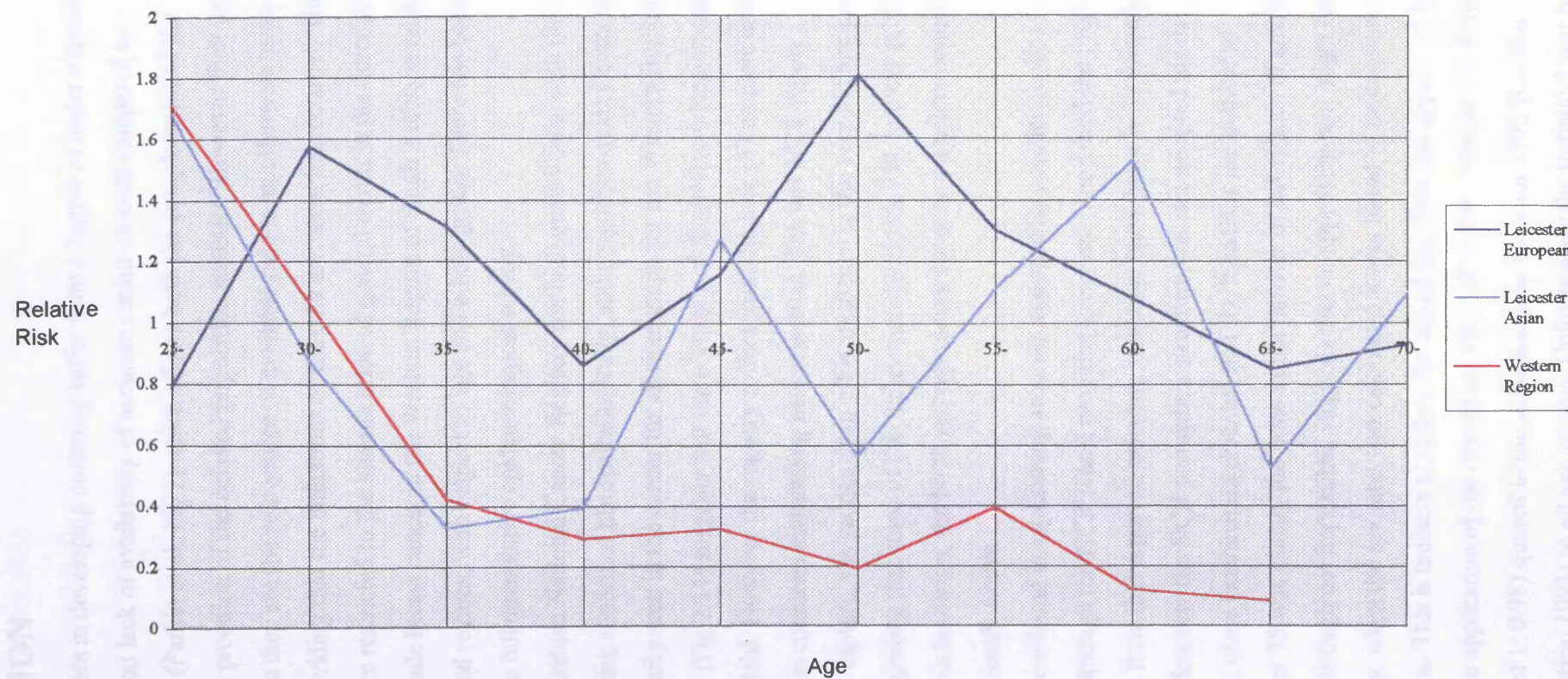
2.6 DISCUSSION

Many registries in developing countries suffer from a degree of under diagnosis, under registration, and lack of availability of accurate census records compared to developed countries (Parkin et al, 1997) which affects both the completeness of the registry data and the precision of the detailed population statistics. However their use can be justified when they are the sole source of available data in an otherwise under reported region, providing that the limitations of the data are taken into account. This chapter was therefore restricted to the identification of general trends in the descriptive epidemiology of female breast cancer in the Western Region of Saudi Arabia in relation to other countries and regions, with respect to age and ethnic group. There has been no attempt to perform a full quantitative epidemiological survey.

The Saudi Arabian National Cancer Registry has high quality data, with the majority, 96.1%, being confirmed histopathologically, and a rigorous quality control program. The similarity seen in the cancer for all sites graph for the Western Region and that of Trent region (Fig 2.14) suggests that the accuracy of the Western Region data, is also of a good standard. However the registry suffers from the lack of complete data a problem often seen in countries establishing new registries. The last NCR report estimated only 70% capture, the sudden drop off in incidence in the very old age groups seen in fig 2.14 suggesting that some of this under registration may be in these groups. However, despite this limitation, the NCR still represents the most complete record of cancer incidence in Saudi Arabia.

Problems were found in establishing accurate age specific population data for the Western Region. Although the NCR report provides such data I only used the total population estimate from that report to calculate incidence rates, since it was found to be the best available. However the NCR population structure was not adopted for the analysis since firstly, close examination revealed that the figure for the number of individuals aged over 75 was exactly the same as the number of individuals of unknown age group, which although not impossible, suggests that an approximation might have been used. Secondly, when the sex ratio was calculated it was found to be constant across all age groups. This is unlikely to reflect the actual situation and appears to have been obtained by the application of the overall sex ratio of 50.5% male: 49.5% females (male:female ratio of 1: 0.98) quoted in the statistical year book for 1993 (Central department of statistics, 1993) to all age groups. Unfortunately the statistical year book for 1993 does not give the population structure broken down by age and sex. Thirdly the percentage breakdown into age groups used, male and female together for the whole of

Saudi Arabia, was from the 1974 census, a rather old document. In preference to these figures the population structure for the Makkah region from the 1977 population census was taken and applied to the population figure for the Western region calculated from the crude incidence rate quoted in the NCR report, and the known number of cases (table 2.4). Although this report was not much more recent than the 1974 census, Makkah is geographically close to Jeddah and both are part of the Makkah reporting region (figs 2.4, 2.5) and so is preferable to more generalised national data. In addition the quality of the data was known to be of a good standard, and nothing more recent was available.

The ASIR for the Western Region breast cancer population (Fig 2.13) shows the initial inflection at 35-39 year age group which is much earlier than the classical Clemmesens Hook seen in Western populations and resembles that described by De Waard for oriental populations. The maximum incidence appears to be reached at the 50-55 year age group and then declines. The standardised incidence rate for breast cancer was calculated to be 14.9/100,000 female population; age and incidence are both much lower than those occurring in the West e.g. England and Wales which has a standardised incidence of 72.7 /100,000 female population with the incidence peaking in the 60-64 age range and then continuing to climb slowly (the world population being used as the standard for both cases). The pattern of breast cancer in Saudi Arabia therefore appears to be different from that seen in the West both in terms of overall incidence and in age specific incidence.

The fact that geographical differences exist in the incidence of breast cancer is well documented. De Waard (1979) described Far Eastern populations as having "Oriental" type, and North American and North Europeans as having "Western" type with Eastern and Southern European, Latin American and African populations having an intermediate pattern of disease. In De Waards study, due probably to the data available, Asian countries were restricted to Japan, Taiwan, and Singapore. African data was also of limited availability. In the present study representative registries from all African, Asian, European and North American countries published in Parkin et al (1997) were included, with the exception of the Zimbabwe white population. This population had an extremely high incidence of breast cancer, and after consideration of the notes given in Parkin et al which indicated probable artefacts due to low population numbers it was decided to exclude it from the analysis, to avoid skewing the data in favour of the hypothesis. The analysis revealed that the difference in incidence observed between low incidence and high incidence countries was significant (table 2.6) and that the geographical distribution

of low and high incidence countries was not random (table2.7), there being a tendency for Asian and African countries to have a low incidence of breast cancer and Mediterranean and European (including North American whites) to have a high incidence. It was noted that the Eastern European countries appear to be equally distributed about the median (five in the low incidence group and four in the high incidence group). This study confirms that the geographical variation reported using earlier data (De Waard, 1979; Kelsey and Horn-Ross, 1993; Gilliland, 1997) still exists and is statistically significant, despite the reported increase in breast cancer in developing countries during recent years (Kelsey and Horn-Ross, 1993; Patavino et al, 1995) and that it can be demonstrated in the countries selected. The non random assortment of countries into regions of high, intermediate and low incidence breast cancer risk by geographical region and the continuous distribution from low to high incidence (Fig2.8) suggests the possibility of a gradient of breast cancer incidence which is related to geographic location.

The results presented also show that there has been little change in the geographical composition of low incidence and high incidence countries during the twenty year period. There is still a common tendency for Asian and African countries to have a low incidence and Northern American and Northern European and Mediterranean countries to have a high incidence. Breast cancer has been reported to be increasing in all countries for which data is available (Wang et al, 1998) and countries studied here also showed this trend. However the findings for the change in standardised incidence rates over a twenty year period (Fig 2.9) shows that although the increase is seen in most of the countries, there is significantly a greater increase in those countries which already had a high incidence of breast cancer (table 2.9). This contrasts with the findings of Kelsey and Horn-Ross (1993) and Patavino et al (1995) who reported the greatest increase to be in low incidence countries but agrees with the findings of Stephens (1997). Possible explanations for this difference may be the selection of countries, and the time span selected for comparison. I concentrated on countries in Europe, Africa, and Asia (with the addition of the North American black and white communities who are considered to be of African and European descent) and excluded those countries from South America and the Pacific Region which were included in the paper by Kelsey and Horn-Ross (1993). The time period quoted in Patavino et al (1995) of 1950 - 1988 is also different from the 1977 to 1997 which I used.

In order to understand the temporal changes observed, cancer incidence for all sites was examined for 1977 and 1997 for the same group of countries (Fig 2.10). All

the countries, during this period, showed an increased incidence of cancer at all sites with the exception of Iceland and Canada (British Colombia); particularly large increases were shown by Malta and Zimbabwe. Using the incidence values for cancer at all sites and the incidence values for breast cancer it is possible to calculate the relSIR for breast cancer, this being a measure of the relative tumour burden in a population attributable to breast carcinoma. The relSIR for breast carcinoma (Fig 2.11) was examined over the twenty year period from 1977 -1997 in relation to cancer incidence for all sites. All countries showed an increase of relSIR for breast carcinoma with the exception of Zimbabwe (African) which exhibited a very small decrease. The notable increase in the incidence of breast cancer in Finland during this period has been attributed (Gastrin, 1980) to the establishment of mammographic screening. The increase in relSIR for breast carcinoma seen in Iceland however is probably attributable to the overall decrease in cancer incidence at all sites thus increasing the relative tumour burden due to breast carcinoma. Malta however exhibits a large increase both in cancer incidence at all sites and in relSIR, although the latter is less than that seen for Finland or Iceland, which could indicate overall improvement of cancer awareness and diagnostic methods.

Increase in relSIR appears to vary throughout the incidence range of countries, suggesting that this might be a general phenomenon even in regions experiencing only a moderate increase in standardised incidence of breast carcinoma (fig 2.9) such as the low incidence countries of Asia and Africa, although the extent to which the increase is due to an increase of breast cancer or a reduction of other cancers may vary from country to country.

Epigenetic explanations for the world wide increase in breast cancer mainly relate it to an increased exposure to oestrogen, due to:

changes in reproductive behaviour (e.g. tendencies for an older age at the birth of the first child, and reduced breast feeding)

diet, the intake of food substances of high calorific value result in weight gain and an increase in risk of breast cancer.

Another factor possibly contributing to the rise observed in high incidence countries (which correspond primarily to developed countries of the Western world) is increased detection due to mammographic screening. Since breast cancer has a natural progression of several years screening may discover asymptomatic lesions which may not have presented within the life span of the patient. Such findings could result in an overall increase in incidence in post menopausal breast cancer.

Reproductive factors which decrease oestrogen exposure have been shown to give a reduced risk of breast cancer through out the life time (Manton and Stallard, 1980). The effects in Asian populations are similar to those for European populations and are not enough to explain ethnic differences (Wu et al, 1996a). Incidence in Asian populations being modified by diet has a firmer biological foundation and tofu consumption probably accounts for at least some of the lower incidence of breast cancer seen in the far east and Indian sub-continent. However it can not account for the low incidence seen in the Middle East, or the relatively low levels of breast cancer seen in Southern and Eastern Europe since tofu is not part of the basic diet. Similarly olive oil might contribute to the lower incidence of breast cancer in Southern Europe and the Levant but not for the rest of the Middle East or the Far East. In regions of the former and overlapping into the Indian sub continent there is widespread, almost daily use of turmeric and consumption of a range of legumous plants not traditionally found in the diets of Northern Europe. Such a diet would probably be high in curcumoids and flavenoids, both compounds believed to influence the risk of breast cancer. In some Indian dishes there may even be an enhanced effect caused by the synergistic combination of tofu with turmeric. Turmeric, although not such a major part of Far Eastern cooking is a major ingredient of traditional herbal remedies such as those used in Indonesia. There appear to be protective dietary overlapping 'foot-prints' stretching from the Far East to the Mediterranean: Tofu from the Far East to the Indian sub continent, legumes and turmeric from the sub continent to the Middle East, and olive oil and legumes in the Mediterranean.

The age of mean onset analysis performed enabled a comparison to be made of the mean age of the patient presenting with breast cancer in the different countries. When the entire range of low and high risk countries were analysed the mean age of onset of a patient presenting with breast cancer in low incidence countries was not found to be significantly different than that for patients from high incidence countries. However when the ten countries with the highest incidence were compared with the ten countries with the lowest incidence then the mean age of onset was found to be significantly younger (table 2.11).

Analysis of total incidence will not determine whether there are age specific variations in incidence between different geographic locations. Comparing the ASIR of the different populations however solves this problem. Since breast cancer is rare before the age of 15 and the peak in incidence is reached before the age of 75 years in both high and low incidence countries a truncated range was chosen of 12 age groups from 15

years to 75 years (when available) in five year periods and ASIR studied within these limits. The data from as many countries as possible was pooled (fig 2.12) to help reduce the effect of fluctuations from individual registries due to cohort effect or registration practices and to allow general trends in the ASIR of high and low incidence countries to become apparent.

The log incidence vs. age plot of different populations should result in parallel lines if the populations have the same change in incidence with respect to age, with the curves only varying from each other by a proportionality factor (the relative risk) which is constant for all age groups. However figure 2.12 shows two slightly divergent groups of lines. It can be concluded that there is a tendency for ASIR of breast cancer in countries with a low and high incidence to vary with respect to age, the low incidence countries having a higher incidence before the age of 30 years and an increasingly lower incidence after the age of 45 years. The pattern in these countries is a possible explanation for the apparently contradictory results presented in the studies of Jewish migrants and their descendants (Steinitz et al, 1989; Iscovich and Parkin, 1998). In a study (Iscovich and Parkin, 1998) of a young age group, 0-29 years, of 3rd generation Israelis of Asian descent the rate of breast cancer was much higher than in the previous study by Steinitz et al (1989) of first generation Asian immigrants aged 0 -75+ years. An alternative explanation is that this difference is due to environmental risk factors brought about by changes in life style. Neither of these explanations can be confirmed or excluded until the 3rd generation Israeli cohort reach 75+ years of age.

Differences in ASIR curves for breast carcinoma in individual countries have been explained by cohort effect (Moolgavkar et al, 1979; Tarone and Chu, 1992). Although the differences in exposure to external risk factors experienced by individual cohorts within a population will introduce fluctuations in the shape of the ASIR curve, there appears to be a degree of uniformity of shape attributable according to whether the country has high or low incidence (fig 2.12). For fluctuations in environmental risk factors to cause the observed changes in relative synchrony there would be either:

    i. a range of different risk factors leading to similar changes, or

    ii. a smaller number of risk factors affecting all the populations studied.

Evidence from dietary studies shows that certain foods may lower the risk of breast cancer and so the overall incidence, but gives no evidence to account for the apparent differences in ASIR of high and low incidence countries.

The two study regions for this project, Trent UK and the Western Region of Saudi Arabia, are located in high (Northern Europe) and low (Asia) regions of breast

cancer incidence and so serve as a case study for a comparative analysis of the pattern of breast cancer incidence between regions of different incidence. A comparison of the ASIRs for female breast cancer in Trent and the Western Region (fig2.13) show that there is higher incidence in the Western Region than in Trent until the age of 30-35 years, after which the converse is true. The ASIR curves for cancer for all sites for the two regions (fig 2.14) show close similarity, there only being a slight excess in incidence in the Western Region before 30 years of age. The ASIR curve for the Western Region after the age of 40 years is slightly erratic; this may be due to actual differences relating to cohort effects or an be an artefact of age registration. The overall curve is slightly divergent from that of Trent until after the age of 60 years when a marked decrease in incidence occurs. This decrease in incidence over the age of 60 years may again be actual, or due to cohort effects; but more likely is related to, at least in part, under registration in the older age groups.

The relSIRs of the Trent and Western Region present two very different situations (fig 2.15), although under the age of 35 years, the relative tumour burden for breast cancer is similar with only a slight excess in the Western Region. It is interesting to note that the peak relSIR for the Western Region and Trent for the 30-34 year age group is the same. For the oldest age group of 65+ years there is again a common relSIR for breast cancer (although figures related to older age groups and in particularly open ended categories must be treated with caution). The graph indicates that the difference in tumour burden attributable to breast cancer in the Western Region compared to Trent comprises two components, firstly an excess of incidence in the under 35 years and a marked decrease of incidence at over 39 years of age. Also of note is the finding that the graph for the Trent Region shows a peak relSIR at 40-44 years indicating that this is the age group where the tumour burden attributable to breast is at its greatest and not at the age of 65+ years when the incidence is greatest. If alternative approaches to mammographic screening could be found which had a high efficacy in this age group or younger, with a view to detecting breast cancer in the in situ or even possibly pre-malignant stage, then even a modest reduction in the cases of breast cancer would result in a significant improvement in the total tumour burden of this age group.

Trent contains a sub group of British Asians; by utilising the data from Leicestershire (Fig 2.6) a sub region located within Trent, it was possible to consider European and Asian patients separately and compare them to that of the Western Region and Trent. Z tests comparing the mean age of onset (table 2.15) shows that none are significantly different from that of Trent although the difference was greatest for the

Western Region and least for the Leicester Europeans. Comparison of the ASIR curves for breast cancer for the four populations (Trent, Leicester Europeans, Leicester Asians, and Western Region) (fig 2.13) shows the Leicester European population follows closest that of Trent. This is to be expected since the majority population in Trent is European. The ASIR for breast cancer for the Western Region is higher that that of Trent under the age of 30, but after that age has a lower incidence. The curve for the Leicester Asian population appears to have two phases, following closely the curve for the Western Region until the 45-49 age group, after which it is closer to the curve for the Leicester European population. To compare the differences in incidence between the Leicester European, the Leicester Asian and Western region populations Mantel-Haenszel analysis was performed, which tests the assumption that there is no difference in incidence (table 2.16). Because the ASIR curves for the Leicester Asian population differed whether under or over 45 years, 20-44 year and 45-69 year ranges were considered as well as 20-69 years.

The incidences for the age range from 20-69 years showed the three populations to be significantly different, with the biggest difference being between the ASIR for the Western Region and the Leicester European population. This concurs with the study of Winter et al (1999) who found the incidence of breast cancer in the S.Asian UK population to be 46.6 compared with 72.9 in the non-Asian population, which would place the S.Asian population in the low incidence group and the non Asian in the high incidence group as defined for this thesis (sections 2.4.2 and 2.5.2). When the younger (20-44years) and older (45-69 years) age groups for the Western Region and the Leicester European populations were analysed the incidences were significantly different for both groups. However the Leicester Asian population was found to have no significant difference in incidence in the 20-44 year age range when compared to the Western Region population, and no significant difference in incidence in the 45-69 year age range when compared to the Leicester European population, confirming the statistical significance of the observations made from fig 2.13.

The age specific relative risk of the three study populations using Trent as a standard was also compared (fig 2.16). There appears to be a greater relative risk of breast cancer in the Leicester European population compared to Trent in the 30-34 years and 50-54 year age groups. Whether this is a reflection of population differences or a transient feature is not addressed here but warrants further study. The Western Region has a relative risk higher than Trent in the under 30 year olds, but a much lower risk after the age of 35. An interesting feature of the graph is how the relative risk of the Leicester

Asian populations follows closely to that of the Western Region population until the 40-44 age group after which it is closer to that of Trent. It was not possible to compare the three study populations with the S.Asian study group of Winter et al (1999) since detailed 5 yearly ASIR data was not provided. However they did find that although the overall incidence of breast cancer was lower in S.Asians, young S.Asians (under 29 years) had a higher incidence of breast cancer than their non Asian counterparts. This is not inconsistent with the results found in my research.

I have already discussed that reproductive factors and obesity are unlikely to have an effect on premenopausal breast cancer. However there may be similarities in the diet of Western Region and Leicester Asians which may be relevant, and there is evidence that these dietary factors lower the overall risk of breast cancer. There appear to be ethnic differences in breast cancer incidence which cannot be fully explained by known environmental risk factors, and there is the possibility that this may be due to a genetic component (Chaudary et al, 1991; Davis et al, 1995; Trock et al, 1996).

Since the Leicester Asian population is closer genetically to the population of the Western Region than to that of the Leicester European population and yet experiences an environment (although not necessarily diet) similar to the Leicester European population, the pattern invites the speculation that breast cancer under the age of 44 years is dominated by genetic components and after this age environmental risk factors, for example those related to reproduction and weight gain become important.

The observation that the excess of very young cases appears to be a robust characteristic of low incidence countries being evident even in immigrant populations that have moved to a Westernised high incidence environment, adds weight to the argument for a significant role of genetic predisposition in this group.

I have demonstrated graphically in this chapter differences in the ASIR curves, with low incidence countries having an earlier point of inflexion (Clemmesens hook) than high incidence countries, in the populations studied. This is in addition to the apparent difference in the age of onset of breast cancer patients between those populations.

The De Lisi (1977) model requires a fixed position of Clemmesens hook and Moolgavkar (1980) makes no allowance for differences in ASIR, considering observed ethnic differences to be due to cohort effect. It is therefore proposed that the study presents data which is not inconsistent with the model of Manton and Stallard (derived from the de Waard model) that breast cancer comprises two diseases, one

the proportion of pre menopausal type and post menopausal type cases of breast cancer present in the population.

CHAPTER THREE

COMPARISON OF MARKERS OF TUMOUR BEHAVIOUR WITH REFERENCE TO AGE AND ETHNICITY IN THE THREE STUDY POPULATIONS

3.1 INTRODUCTION

Aggressive is an adjective frequently utilised in papers describing the behaviour of breast cancer in Saudi Arabia (Al Idrissi et al, 1992) and some other low risk countries e.g. Egypt (Richards et al, 1992), Kuwait (Motawy et al, 1994). Some publications suggest that there may be underlying biological differences between ethnic groups (Huang et al, 1995; Hartmann et al, 1996). Breast tumours in the Indian sub continent are reported to be of early onset and late stage (Hussain et al, 1994; Goel et al, 1995), but whether this is due solely to the limitations of public health education and facilities, or to the aggressive nature of the tumour has not been studied. The British Asian breast cancer population are also at low risk for breast cancer, but full details on survival, disease free interval etc. are still limited for the minority ethnic groups in the United Kingdom. In the United States early age at onset, irrespective of ethnic origin, has also been associated with aggressive tumours and poorer prognosis (Fowble, 1994).

3.1.1 TUMOUR BEHAVIOUR

Differences in the relative behaviour of breast tumours have been described between different ethnic groups, and between age groups.

Ethnic variation

Generally Black Americans (AmB), Hispanic Americans (AmH), and Asian Americans (AmA) present at a younger age, higher stage and have more extensive lymph node involvement than American Whites (AmW) (Aziz et al, 1999; Boyer-Chammard et al, 1999). Krieger et al (1997), comparing AmA, AmW, and AmB, found that AmA and AmB have more tumours over the size of 2cm, independent of socio-economic status. In addition AmB have been reported as having lower survival rate within each stage, which cannot entirely be accounted for by delay in diagnosis (Vernon et al, 1985). Edwards et al (1998) in a large study of 115,838 patients comparing race, age and stage found that the ratio of regional disease to localised disease decreased with age in AmW, but remained approximately constant in AmB. They concluded that age and race had a significant association with tumour stage and an independent impact on survival. This appears to be present even when there is equal access to health facilities as in a study by Wojcik et al (1998) in the US Military. Wojcik's study found AmB had a younger age of onset of breast cancer, and even when this was adjusted for still had a worse survival than AmW. Similar results were found in other studies (Hunter et al, 1993; Eley et al, 1994) so socio-economic differences are not the only explanation.

Ijaduola and Smith (1998) found both AmB and West African women (WAf) present with breast cancer at a later stage and larger tumour size than AmW. Hassan et

al (1992), also noted an excess of young WAf patients with a much lower than expected survival rate and postulated that this was due not only to the delayed presentation of the patients and lack of efficient treatment regimes but also to the aggressive nature of the tumours.

The differences in presentation and course of disease therefore appear to indicate ethnic variation in the biological nature of breast cancer (Newman and Alfonso, 1997), although this is disputed by some (Weiss et al, 1995; El-Tamer et al, 1999; Yood et al, 1999).

Age related variation

Several studies have found a young age at diagnosis to be an independent negative prognostic factor (Aaltomaa et al, 1992a; de la Rochefordiere et al, 1993; Nixon et al, 1994), including studies from Saudi Arabia (Al Idrissi et al, 1992; Ezzat et al, 1998). Younger breast cancer patients have been reported to have a higher proportion of grade three tumours (Walker et al, 1996; Gillett et al, 1997; Fisher et al, 1997) a generally poorer prognosis irrespective of grade (Albain et al, 1994; Chung et al, 1996), and more frequent occurrence of lymph node involvement, even when compared with tumours of the same size in postmenopausal women (Peer et al, 1996; Gillett et al, 1997).

Kollias et al (1997), concluded that the worse prognosis for breast cancer in the young was due to the higher proportion of poorly differentiated tumours and not directly attributable to age, Azzat et al.(1999) describing breast cancer cases in Saudi Arabia made a similar conclusion. Bertheau et al (1999), though considered that breast cancer in young women often has the features of high grade malignancy, even if some have good survival, and that young breast cancer is different. Generally young, premenopausal, breast cancer is associated with markers associated with aggressive, less well differentiated tumours (section 1.4.5 Devilee/Walker model) including overexpression of erbB2 (Allred et al, 1992), and higher modal chromosome number (Pandis et al, 1996). These studies provide support to the proposal that premenopausal breast cancer may be biologically different to post menopausal breast cancer.

It is apparent that heterogeneity also exists in the biological nature of breast cancer within the premenopausal age group. Walker et al (1996) found tumours occurring in patients under 35 year old to more poorly differentiated, have higher rates of proliferation, and more frequently p53 positive than tumours in premenopausal patients aged over 35 years. Patients under 30 years also had a lower incidence of ER and PgR positive tumours. The majority of cases were sporadic (18% of the 35-44 years age

group and 11% of the under 30 years age group reported a family history of breast cancer). However some studies have failed to find a poorer prognosis or evidence of a more aggressive tumour type in these younger premenopausal breast cancer patients (Anderson et al, 1995; Sariego et al, 1995; Sananes et al, 1996), finding breast cancer in the older age group to be an equally aggressive disease (Hakama and Rihimaki, 1974). Others describe young breast cancer as an aggressive disease but quote a more favourable long term prognosis for these patients (Gillett et al, 1997). These apparently contradictory results may be attributable to differences in methodology and classification, or may simply reflect the heterogeneous nature of the disease.

The higher incidence of larger tumours may partly be explained by the reluctance or inability of certain ethnic groups to obtain prompt medical attention. There is also the tendency of some clinicians not to suspect malignancy when presented with a breast lump in a patient under 35 years of age (Menon et al, 1992). However the majority of studies indicate that young patients and certain ethnic groups have a higher than expected risk of developing high grade aggressive tumours (Velentgas and Daling, 1994) and there is increasing evidence for the existence of actual biological differences in the tumours of these groups.

3.1.2 INDICATORS OF TUMOUR BEHAVIOUR

The disease free interval (DFI) and overall survival reflects the biological dynamic characteristics of the tumour providing similar treatment protocols are followed. In the absence of adequate patient follow up data to compare survival or disease free interval in the study populations, this chapter seeks to use histopathological tumour data, and nuclear morphometric characteristics as surrogate markers of tumour behaviour.

Stage of Disease

TNM, which comprises tumour size, lymph node involvement and presence of distant metastasis, gives information about the extent of invasion spread and is of prognostic value (section 1.4.1). Generally the further the progression, the worse the prognosis, with the presence of distant metastases being a sign of advanced systemic disease. However the TNM stage alone gives a rather static picture of the existing state of affairs and not the full picture of the biological nature of the tumour and its aggressiveness.

Tumour Size

A larger tumour size is associated with an increased incidence of node involvement and distant metastasis (Koscielny et al, 1989). However a proportion of

small tumours (<1.0cm), as reported by Carter et al (1989), may have a lower survival rate than some larger tumours with the same extent of lymph node involvement.

Node Status

Generally lymph node negative patients have a better prognosis than lymph node positive patients, however 20-30% of lymph node negative patients still go on to develop metastases within the first 10 years. This may indicate a subset of more aggressive tumours (Sears et al, 1982), or the presence of occult metastases not revealed by routine examination.

Histopathological grade

The histopathological grade, comprising tubule formation, mitotic activity, and pleomorphism of cells (section 1.4.1), is known to be of prognostic significance in breast cancer (Elston and Ellis, 1991). Grade has been shown to be a prognostic factor for long term tumour behaviour (Elston and Ellis, 1991), as well as for overall survival independent of lymph node status (Davis et al, 1986; Contesso et al, 1989). In post menopausal patients grade has been shown to exert influence on the outcome of adjuvant therapy (Contesso et al, 1989). Henson et al (1991) described cases where, within each TNM grouping, grade was related to survival irrespective of lymph node status, e.g. patients with grade 1 tumours had a consistent survival advantage even in stage IV disease.

Prognostic indices

The use of histopathological and biological markers can help differentiate TNM stages into prognostic groups by providing information about the biological nature of the tumour (Baak et al, 1985).

Two documented prognostic indices are:

1.The Nottingham Prognostic Index, NPI, was formulated for patients with primary breast carcinoma based on those factors that influenced prognosis. Nine prognostic factors were analysed retrospectively and after appropriate weightings from multivariate analysis the following formula was derived:

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

NPI scores maybe categorised: <3.4 good prognosis. 3.4 -5.4 moderate prognosis, and >5.4 poor prognosis (Pinder et al, 1995). NPI has successfully been used to differentiate patients with good prognosis (80% 15 year survival), moderate prognosis (42% 15 year survival), and poor prognosis (13% 15 year survival) (Galea et al, 1992).

2.The Yorkshire Breast Cancer Group (YBCG) used similar methods to derive a modified prognostic index YBCGPI (Brown et al, 1993):

YBCGPI = 0.1 x clinical tumour size (cm)+ 0.5grade score + 0.6 nodal involvement

Using this equation it is possible to group patients into three prognostic groups (good< 1.21, moderate1.21<>1.82 and poor > 1.82). The YBCGPI and the NPI show close correlation when Kaplan-Meier survival curves are plotted for the prognostic groups (Brown et al, 1993).

3.1.3 QUANTITATIVE MICROSCOPY

Quantitative techniques have the advantage of greater reproducibility and objectivity than qualitative judgements, and may reveal differences in comparative studies, not readily identified by qualitative description (Baak and Oort, 1983).

Tumour Fraction

Measurement

Percentage of tumour area or volume (a/A or Vv) occupied by invasive tumour cells is required for the calculation of the volume corrected mitotic index VMI. It also gives a measure of cellularity and has been termed cellularity mean index CMI (Ambros and Trost, 1990)

Applying the principals of Delesse (1847), the average area of any component of the tissue on a histological slide will be proportional to its volume:

$$V_v = A_A$$

However Delesse assumes that A_A is a constant through out the sample and therefore identical in each section, as would be the case of a cube with a face parallel to the cut surface. Since nuclei approximate to spheres this assumption does not hold true. Instead it is necessary to use an alternative estimator of volume fraction derived by Mayhew and Cruz-Orive (1974).

$$V_v = a/A$$

a is the mean area of the component being measured

A is the mean area of the surrounding area.

The fractional areas (A and a) may be estimated directly from the microscope using an eye piece graticule and by point counting (Ahearne and Dunhill, 1987), (section 3.4.2).

Prognostic value

Ambros and Trost (1990) found CMI (Vv) to correlate to lymph node involvement and VMI, and to indicate poor prognosis. A study by van Diest et al (1992) concluded that CMI was a good prognosticator of the response to adjuvant chemo - therapy in pre menopausal lymph node positive patients.

Mitotic index

Reproducibility

The reproducibility of the components of histopathological grade, including the mitotic count, have often been questioned (Dufer et al, 1993), but if strict criteria are adhered to, good inter observer correlation can be obtained (Frierson et al, 1995).

There are two potential sources of variation when making quantitative mitotic counts, associated with:

Counting methodology:

It is necessary to strictly define the selection of the area on the slide to be counted, the method of counting, and what constitutes a scorable mitotic figure. In choosing the area it is important as far as possible to avoid selection bias. Baak and Oort (1983) summarised the optimal sampling procedure:

Mitotic figures should be scored in the area showing most mitotic activity.

10 fields should be counted at random within the area.

The mitotic index is conventionally taken as the number of mitotic figures per 10 high power fields selected from the most cellular area of the tumour (Baak et al, 1985). Robbins et al (1995) suggested that by counting fours groups of 10 high power fields and recording the highest mitotic score adds to the reproducibility of the counting procedure. However this method does not allow for differences in tumour cellularity, cell size, nor size of the high power field of the particular microscope. These differences can be standardised by measuring the ratio of the number of mitoses to the number of malignant cells (Ellis and Whitehead, 1981; Simpson et al, 1992). If the ratio of mitoses per tumour volume, the volume corrected mitotic index M/V, is measured this will also allow for variation in the thickness of sections (Haapasalo et al, 1989). M/V has the same prognostic value as the mitotic index (Jannink et al, 1996), and the advantage of increased reproducibility, especially important when comparing samples from different institutions.

Whatever counting method is chosen only definite mitotic figures should be counted, Baak and Oort (1983) suggested certain criteria to ensure that pyknotic nuclei are not included:

The nuclear membrane must be absent, and either a distinct stage of mitosis should be recognisable or the 'hairy' projections of the chromosomes visible. The latter contrasts with pyknotic or deformed nuclei that tend to appear 'spiky'.

ii. Stability of mitotic figures

Delay in fixation may affect mitotic count by decreasing the number of mitotic figures, since during the delay it is possible for dividing cells to complete their cycle of division (Bullough, 1950). Bergers et al (1997) found that although a delay prior to fixation decreases the quality of the section the number of identifiable mitotic figures and reproducibility was not affected.

Prognostic value

The mitotic frequency has been found in several papers to be a significant independent parameter for prognosis (Biesterfeld et al, 1995; Laderkal and Jensen, 1995) particularly the risk of recurrence (Aaltomaa et al, 1992b). The volume corrected mitotic index (m/v) allows identification of patients with poorer prognosis among ER+ve tumours (Aaltomaa et al, 1991). The number of mitotic figures and nuclear atypia were strongly correlated with the copy number of c-erbB-2, an independent prognostic factor associated with poor prognosis and aggressive tumours (Heintz et al, 1990; Tsuda et al, 1990).

Nuclear morphometry

Measurements

The function of nuclear morphometry, or planimetry, is to derive descriptive statistics of populations of tumour nuclei from two - dimensional measurements. Any nucleus in a histological section will appear approximately circular, but even if all the nuclei were uniformly sized spheres these circles would vary in size according to the point of sectioning (fig 3.1). The thickness of a histological section is less than the diameter of the tumour nuclei being studied, and so all nuclei within the section will be transected, this being at random. Hence then the mean size and standard variation in dimensions of the resulting circles will be dependent upon the size of the population of nuclei from which they were cut. Also, measurements that rely on quotients or ratios (shape and ratio of axis) should remain relatively constant for all the sections of the nucleus.

Reproducibility

The main sources of error when making morphological measurements of nuclei can be attributed to:

i. Selection of nuclei:

Baak et al (1982) suggested using microphotographs taken at 1000 x magnification randomly in the most cellular area of the tumour and measuring 25-50 nuclei, whereas

Fig 3.1 Sections through spheres in a theoretical matrix

Plane of section through spheres

Cross section through spheres
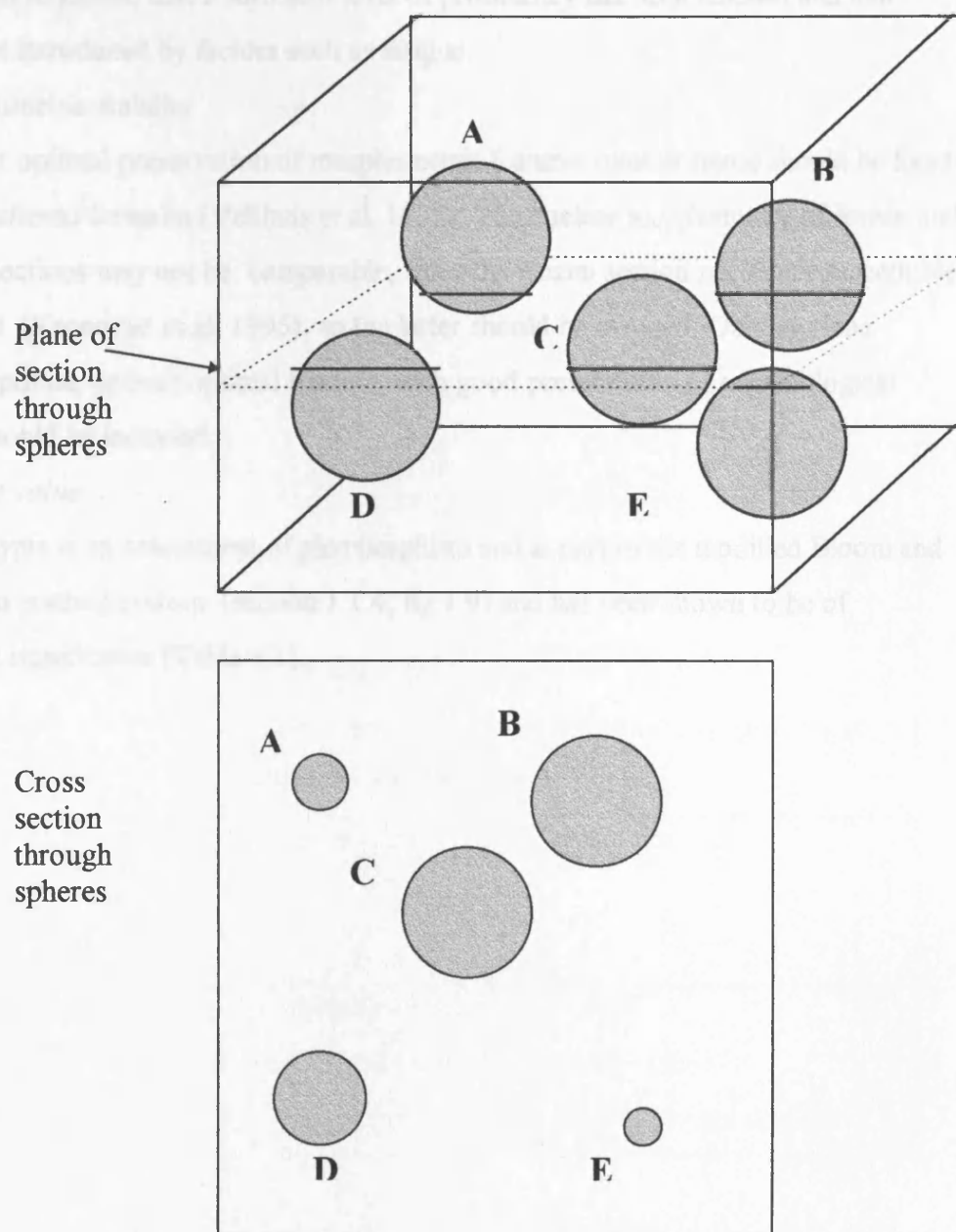
Pienta and Coffey (1991) selected 30 fields at random from across the specimen and measured 150 nuclei. In order to achieve meaningful results care must be taken at all stages to be consistent and avoid selection bias.

ii.Reproducibility of measurements:

Measurements should be done, when possible, by the same person to avoid inter observer variability. Even if only one person does the measuring, reproducibility tests must be run to ensure that a sufficient level of proficiency has been reached and that error is not introduced by factors such as fatigue.

iii. Morphometric stability

For optimal preservation of morphometric features tumour tissue should be fixed in 4-5% buffered formalin (Velthuis et al, 1978). The nuclear morphometry of frozen and unfrozen sections may not be comparable, since the frozen section nuclei are susceptible to collapse (Kronqvist et al, 1995), so the latter should be avoided. Only sections showing optimal, or near optimal fixation, with good preservation of morphological features should be included.

Prognostic value

Nuclear atypia is an assessment of pleomorphism and is part of the modified Bloom and Richardson grading system (section 1.1.4, fig 1.9) and has been shown to be of prognostic significance (Table 3.1).

Table 3.1: **The prognostic significance of selected morphometric measurements**

Measurement	Research findings
standard deviation of nuclear perimeter (SDNP)	independant prognostic value[a] and a predictor of DFI [b]
mean nuclear area (NA)	Significant prognostic factor[c] correlated to higher rate of proliferation[d]
intra sample varience of nuclear area (VNA)	node +ve pts marked increase in VNA compared to node -ve[e]
standard deviation of nuclear area (SDNA)	predicts DFI in young node positive patients[b]
nuclear area for the 10 largest nuclei in the field (NA10)	predicts survival and DFI in young node -ve patients[b]
max nuclear diameter (Ndmax)	Significant prognostic factor[c] correlates to ER status (ER-ve > 10μ for 75% of premenopausal patients)[f]
ratio of max and min nuclear diameter (ND1:ND2)	gives a measure of pleomorphism[f]
shape coefficient (Sh)	measurement of pleomorphism[e] calculated as 4π X Area/Perimeter predicts 5yr survival in stage III breast cancer[g]

a: Aaltommaa et al 1992 b: Eskelinen et al 1992 c: Kronqvist et al 1998 d: Mikuz and Dietze 1982 e: Pienta and Coffey 1991 f: Baak and Oort 1983 g:Tamura et al 1988

3.2 AIMS

The aims of this chapter are to:

Assess if any differences in the potential biological behaviour of tumours from the three study populations could be detected by use of chosen prognostic indicators.

Determine if any differences found could be attributed to age or ethnic origin .

3.3 MATERIALS

3.3.1 PATIENT AND TUMOUR DATA

Jeddah study group (AA)

A total of 198 breast cancer patients with invasive carcinomas were identified in five major hospitals in Jeddah, to form the AA study group. Ethnic origin and age of the patient, tumour size, type, and grade, and the number of lymph nodes examined plus the number involved by metastasis, was extracted manually from pathology records for three hospitals (149 patients). At the remaining two hospitals (50 patients) a computer search was made and the required patient and tumour data copied.

Leicester European (EL) and Leicester Asian (AL) study groups.

Two groups of Leicestershire patients, 78 EL and 42 AL, were identified from either a database of patients compiled by Dr Rosemary Walker, or from the computerised records of the Leicestershire Pathology Service. Data was provided about age, tumour size, tumour type, grade, and lymph node status (including number of total lymph nodes involved).

Complete patient and tumour data.

This comprised:

Patient data: the name or ethnic group and age.

Tumour data: type, grade, size, lymph node status and number of affected lymph nodes.

Minimum patient and tumour data.

This was at least the ethnic group and age of the patient and the type of the tumour. Although not all cases had complete patient and tumour data, cases were considered for inclusion if at least minimal patient and tumour data was available.

3.3.2 TISSUES

All tissues had been fixed in formal-saline (4% formaldehyde/saline) and processed through to paraffin wax. The samples from the hospitals in Jeddah comprised specimens that had been immersion fixed for periods of time which could not be determined from the records, prior to selection of blocks for processing. In the review

of material by Dr.Walker only blocks with adequate preservation were selected. Samples that had been frozen prior to fixation were avoided for morphometric work.

All of the specimens from Europeans in Leicestershire had been received fresh, and samples of tumour selected, generally no more than 2.0 x 1.5 x 0.5 cm, for fixation for 18-24 hrs prior to processing. Approximately half of the tissues from Asians in Leicestershire had been treated in the same way, while the rest consisted of specimens which had been immersion fixed for 18-48 hrs prior to selection of the tumour blocks.

3.4 METHODS

3.4.1 COMPILATION OF THE STUDY GROUPS

Comparison of the Saudi and non Saudi female breast cancer populations in the Western region

Although for the ASIR calculations for the Western Region in Chapter two only Saudi patients were considered, the NCR contains data on both Saudi and non-Saudi patients, the nationality of all individuals being included.

Inclusion of the non-Saudis would provide a larger pool of cases for study. Therefore a comparison was made to find if there is a significant difference in the ages of Saudi and non Saudi female breast cancer patients in the Western Region, or whether they could statistically considered to be elements of the same population with respect to age. A t-test was performed between Saudi and non-Saudi cases over the truncated age range of 20 -65 years (see discussion). A Z-test was performed to compare the two patient populations to determine if the mean age of onset varied significantly between the two.

Patients were only selected for the AA study group if they were Saudi nationals or nationals of low incidence countries sharing a similar ethnic background as the Saudi population (section 2.1.5.).

Investigation of the Jeddah data as a sub group of the Western Region tumour registry data

The detailed data for the Saudi study group came from 5 major hospitals in Jeddah. The age distribution of this study group was statistically compared with that of the Western Region database to test whether it is a representative sample.

The age data for the Jeddah database and the Western Region Tumour Registry were subjected to a t-test to investigate whether the Jeddah data base can be considered

a statistically representative sub-group of the Western Region Tumour Registry female breast cancer population, with respect to age.

Selection of the Leicestershire European (EL) and Leicestershire Asian (AL) study groups

The European patients were selected from patients diagnosed between 1993 and 1995 by Dr Walker, and were age matched with the Saudi groups for whom both data and samples were available. The Leicester Asian group, due to the relatively low incidence and smaller overall population, were selected from a wider time span (1983 - 1997) in order to collect sufficient cases and included all suitable available cases. For both EL and AL cases with impalpable tumours diagnosed by mammographic screening were excluded, since no such routine service is available in the Western Region.

Comparison of the three study groups and data encoding

After selection of AA, AL, and EL cases t - tests were performed to test for any significant difference in age between these three groups.

Once collected the data was coded according to ethnic origin (AA, AL, or EL) age at diagnosis, and source, and given an identification number.

3.4.2 CLINICO-BIOLOGICAL CHARACTERISTICS

Haematoxylin and eosin stained sections of all cases collected from hospitals in Jeddah were reviewed by Dr Rosemary Walker for pathological type, using the classification detailed in the NHS BSP Pathology Reporting Guidelines 2nd edition (1995), and for histological grade using the modification of the Bloom and Richardson grading system (Elston and Ellis, 1991) (See 1.4.1 Fig 1.9). The same classification and grading system was used for all cases from Leicestershire. All tumours selected were Infiltrating Ductal (IDC) of no special type or had a major IDC component.

Patient and tumour data were analysed for the three study groups (Saudi Asians, Leicester Asians, Leicester Europeans)

In addition two prognostic indexes were calculated for those cases where sufficient information was available:

i. The Nottingham Prognostic Index (NPI) (Galea et al, 1992; Pinder et al, 1995)

NPI = 0.2 x tumour size(cm) + lymph node stage(1-3) + histological grade(1-3) where no affected lymph nodes equals lymph node stage 1, 3 or less involved lymph nodes stage 2, and 4 or more axillary lymph nodes and/or internal mammary lymph nodes involved, stage 3.

ii. Yorkshire Breast Cancer Group Prognostic Index (YBCGPI) (Brown et al, 1993) which is

YBCGPI = 0.1 x clinical tumour size + 0.5grade score + 0.6 nodal involvement (1 or 3)

Where grade score equals 1 for the modified Bloom and Richardson grade 1 and 2 for

grades 2 or 3, and lymph node involvement equals 1 for LN -ve and 3 for LN+ve.

Statistics

For the *continuous* variables the mean, variation, standard deviation and

coefficient of variation was calculated for each of the study groups. Variation within a

study group between tumours from patients under and over 44 years of age, and also

those from pre and post menopausal patients was assessed using t-tests, two tailed

assuming unequal variance, for each of the study groups. Menopause was taken as

occurring at 50 years (Morabia and Costarza, 1998).

Each study group was sorted according to age and the mean value for each

variable or PI (prognostic index) was calculated for each age.

The variable or PI was then plotted for:

i. Yearly age intervals

ii. Seven selected age groups :

<div style="text-align:center">

20-29 years

30-39 years

40-44 years

45-49 years

50-54 years

55- 59 years

60-69 years

</div>

To assist comparison of trends between the different graphs polynomic trend lines were

inserted using the trend line facility in Excell 7. A comparison was then made between

pairs of study groups, the following age ranges were tested by means of t-tests, two

tailed assuming unequal variance:

i. Entire age range

ii. Pre menopausal (less than 50 years of age)

iii. Post menopausal (more than or equal to 50 years of age)

iv. 44 years or under

v. over 44 years

for AA and EL, AL and AA, and AL and EL.

For the *discrete* variables variation within a study group, between tumours from

patients under and over 44 years of age, and between pre and post menopausal patients

was assessed using χ^2 tests, for each of the study groups. A comparison was then made

of the same specified age ranges, as for the continuous variables, between pairs of study groups, by means of χ^2 tests, i.e. AA and EL, AL and AA, and AL and EL study groups. Some of the categories used contained less than five and so caution was used in interpreting these, the results affected are marked with an asterisk.

3.4.3 MITOTIC INDEX AND TUMOUR FRACTION

Preparation of sections

4μm sections were cut for 107 breast carcinomas identified from the Saudi Asians, the criterion for selection being the availability of blocks and sufficient material being present within the blocks. These were dewaxed, rehydrated and stained in Mayer's haemotoxylin (1 min) and eosin (10secs), dehydrated and mounted in Xam. Leicester European and Leicester Asian cases were brought from the UK ready cut as 4μm sections onto slides and were then prepared in the same way.

Calculation of tumour fraction and volume corrected mitotic index

The most cellular area of the section was selected and within this area fields were chosen at random. Prior to relocation to a new field the microscope was defocused slightly. This allowed the selection of the field within the cellular area without the mitotic figures being visible, thus helping to ensure the random nature of the selection and minimise selection bias. The microscope was then brought back into focus and using an ocular graticule (1 mm index grid 19mm x 19mm) counts were made utilising the intersections of the grid. Scores were classified as malignant epithelium or non-epithelium, and counted using a manual haematological cell type counter.

A total of 10 fields were counted and the number of mitotic cells recorded counted according to the recommendations of Baak and Oort (1983). This procedure was repeated for another three groups of 10 fields, a total of 40 fields, and the highest recorded number of mitotic figures used for the calculation of the mitotic index as suggested by Robbins et al (1995).

The volume corrected mitotic index VMI was calculated for each case (Theoretical background given in Section 3.1.3):

- Estimation of tumour volume fraction Vv

$$Vv = \Sigma (P_{epi}) / \Sigma (P_{epi} + P_{conn})$$

for a total of ten fields.

P_{epi} is the number of points counted on epithelium and P_{conn} is the number of points on stroma and other non epithelial structures.

- Calculation of volume corrected mitotic index

M/Vv index (VMI)   =   k Σ MI / ΣVv

MI is the number of mitotic figures, Vv the volume fraction estimated by Aa

expressed as a percentage, and k is the fields constant equal to $100/\pi r^2$

(r being the radius of the microscope field measured in mm). Counts were done using

the x40 objective lens.

Reproducibility

VMI and the tumour fraction Vv on a series of three samples, one from each

study group taken at random, was measured. The same samples were measured again

after one month. A paired t-test was performed on the two groups for VMI and Vv.

Analysis

The volume corrected mitotic index, VMI, was calculated for each case and

mean, variation, standard deviation and coefficient of variation for VMI was calculated

for each of the study groups. Variation within a study group between tumours from

patients under and over 44 years of age, and also those from pre and post menopausal

patients was assessed using t-tests, two tailed assuming unequal varience.

A comparison was then made of specified age ranges between pairs of study groups. The

following age ranges were tested by means of t-tests, two tailed assuming unequal

variance for AA and EL, AL and AA, and AL and EL:

Entire age range

Pre menopausal

Post menopausal

under 44 years

over 44 years

3.4.4 NUCLEAR MORPHOMETRY

Photography

Microphotographs were taken of the prepared H&E stained tumour sections,

using a Zeiss Axiophot photomicroscope at 1000 x magnification under oil, following

the guidelines for field selection of Baak and Ort (1983). The most cellular region of the

tumour was located at low power. Microphotographs were taken at random within this

region; a slight defocusing of the microscope when relocating the field ensured that the

field was still within the selected region of highest cellularity but kept the selection of the

fields random, avoiding the possibility of observer biased selection. Using Konica VX100

36 exposure colour print film ISO 100/21°, between 3 - 8 microphotographs were taken

for each case according to the cellularity of the tumour, a minimum of 100 tumour nuclei

per case being recorded. Developing was done commercially using a Konica "Nice Print QSS" developer model 808 (exposure time 2.5 min/picture, set magnification factor of 4.3) to obtain 10 x 15 cm pictures. A 1mm stage micrometer slide was also photographed at x 1000 magnification under oil and subjected to the same processing, for use in calibration.

Image analysis

The microphotographs for each case were scanned on an Apple flat bed scanner using OFOTO version 2 software (Light Source Computer images inc.1993) and a Macintosh power PC 8100/110. The images were stored in TIFF format and exported to the Public Domain NIH programme version 1.57 (written by Wayne Rasband at the U.S. National Institute of Health, part number PB93-504868). The programme will be referred to as NIH Image.

Calibration

The microphotograph of a 1mm stage micrometer slide was scanned into the computer and exported into NIH Image for use in calibration. Within the NIH Image programme the distance between 5 divisions on the scale was measured in pixels. "Set scale" was selected from the "Analyse" menu and the measured length and the equivalent number of pixels entered (in addition to ratio of horizontal to vertical which was set at 1, and the units measured which was set at μm). The resulting scale was 6.9 pixels per μm. in the image.

Nuclear measurements

The tumour cells were identified, after instruction from Dr Walker, by their large nuclei, frequently abnormal nuclear shape, chromatin pattern and nucleoli. The densely staining compact stromal fibroblasts, lymphocytes and macrophages were avoided. Using the free hand drawing tool, guided by the mouse, the perimeter of each tumour nucleus was outlined and a macro (written by Dr P Furness Leicester University) was utilised to measure the basic nuclear features (Table3.2). NIH Image has a series of predefined arrays (including area, length, major axis, and minor axis) and two unnamed arrays (user1 and user2) which can used to store macros to record and display derived results

A minimum of 100 nuclei per case were measured (Baak and Oort, 1983). However in the situation where measurement was continued on a new microphotograph image all tumour nuclei on that image were then measured to avoid selection bias. This total was in some cases as high as 150 nuclei.

The nuclear features measured by the NIH Image programme were (fig 3.2):

> perimeter
>
> area
>
> shape factor (4 π x area / (perimeter)^2)
>
> shortest axis
>
> longest axis
>
> ratio of max and min nuclear diameter

Reproducibility and specificity

Reproducibility was checked by repeating the nuclear measurements of a set of photographs one week and three months after the first assessment. A t-test was then applied. Specificity was checked by:

i. Comparing the nuclear measurements of two cases selected at random to test inter tumour variability.

ii. Comparing the nuclear measurements of two cases selected due to contrasting appearance, to test sensitivity

iii. Taking twice the number of photographs from an individual case, shuffling the prints and dealing them into two groups. The nuclear measurements of these were then compared, and a t-test applied to test intra tumour variability.

Data processing

Raw data was imported from NIH Image into Excell 7 and the following morphometric measurements calculated, which have been found to be of prognostic value in some studies (Pienta and Coffey, 1991; Eskelinen et al, 1992). (Summary data appendix A).

> a. Standard deviation of the nuclear perimeter (SDNP)
>
> ii. Mean nuclear area (NA)
>
> iii. Variance of nuclear area (VNA)
>
> iv. Standard deviation of nuclear area (SDNA)
>
> v. Nuclear area for the 10 largest nuclei in the field (NA10)
>
> vi. Ratio of max and min nuclear diameter (ND1:ND2)
>
> vii. Max nuclear diameter (Ndmax)
>
> viii. Shape coefficient (Sh)

Fig 3.2 Nuclear feature measured with NIH image

Measurements made by NIH Image

A   Perimeter

Area

B   Shortest axis

C   Longest axis

B/C   Ratio of shortest and longest axis

Nucleus
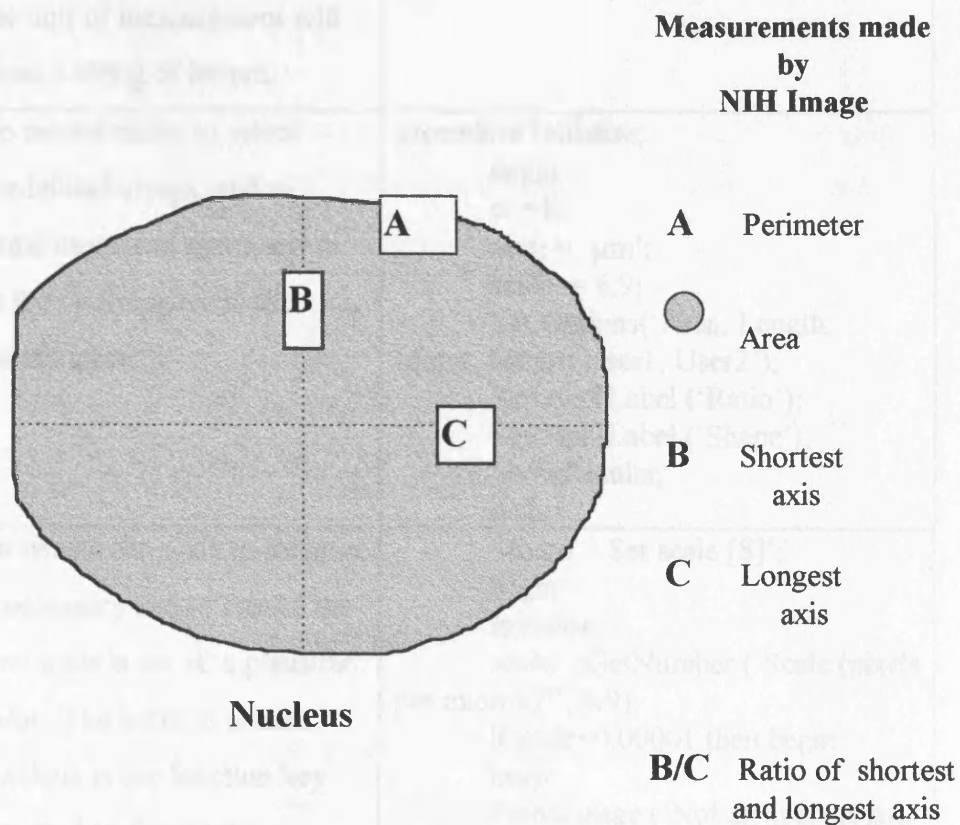
Table 3.2 **Macro used to measure nuclear features**

Sections of Macro	Function	Macro (alignment of lines as shown essential to correct functioning)

Definition of global variables	To inform programme that the unit length and scale will be entered as real numbers and the unit of measurement will be as a string of letters.	Var
		c : integer;
		      scale : real;
		      unit : string;

Definition of procedures	To record scale, to select predefined arrays, and to name user1 and user2 arrays as they will appear in the results table.	procedure Initialise;
		    begin
		    c: =1;
		    unit: = 'μm';
		    scale: = 6.9;
		    Set Options('Area, Length,
		Major, Minor, User1, User2');
		      SetUser1Label ('Ratio');
		      SetUser2Label ('Shape');
		      ShowResults;
		      end;

Macro to set scale	To enable the scale to be reset if necessary and to ensure the new scale is set at a plausible value. The letter in square brackets is the function key assigned to the macro.	Macro ' Set scale [S]';
		    begin
		    Initialise;
		    scale: =GetNumber ('Scale (pixels per micron)?', 6.9);
		    if scale<0.00001 then begin
		    beep;
		    PutMessage ('Not an appropriate scale figure.');
		    exit;
		    end;
		    SetScale (sale, unit);
		    end;

Macro to calculate ratio of major and minor axis and shape	Checks scale, calculates user1,the ratio of the major and minor axis, and calculates user2 shape (4 π x area / perimeter^2). The value 12.5663706 in the macro being the value of 4π. [M] is the function key assigned to the macro.	Macro 'measure nucleus [M]'
		begin
		if c<1 then begin
		Initialise;
		end;
		SetScale(scale,unit);
		Measure;
		rUser1[rCount]
		:=rMajor[rCount]/rMinor[rCount];
		rUser2[rCount]
		:=(12.5663706*rArea[rCount])
		/(rLength[rCount]*rLength[rCount]);
		UpdateResults;
		end;

Analysis

For each study population the following were calculated and compared; for all ages, at intervals of one year, under and over 44 years, pre and post menopausal and for the seven selected age ranges used previously. Polynomic trend lines were included and used throughout for comparison. T tests were performed to compare the entire age range, under and over 44 years, and pre and post menopausal cases for the three study groups.

Morphometric measurements

For each study population, cases were arranged in ascending order according to age and the morphometric measurements tabulated in the summary data plotted against age. AA was plotted twice; once with all available data and again after omission of a single extreme result (see discussion).

i. Variation in the nuclear area

The coefficient of variation and the difference between the coefficient for the under 44 and over 44 year old groups was calculated for each study population. The results were tabulated.

ii. SDNP, and MeanNDmax .

iii. Mean ratio of maximum to minimum tumour cell diameters (ND1:ND2).

iv. The mean shape

Values of the mean variance, and standard deviation of shape were calculated for the entire age. The coefficient of variation was also calculated for each study population.

3.5 RESULTS

3.5.1 NUMBER OF AVAILABLE CASES FOR EACH STUDY

The number of cases available for each study, for each of the study groups is shown in table 3.3

Table 3.3 **Summary of case availability**

	AA	AL	EL
Total screened	198	42	78
Tumour size	132	35	70
LN status	151	37	75
Grade	181	42	78
NPI	107	34	65
YBCGPI	110	34	69
Vv and VMI	77	39	73
Nuclear Morphometry	107	41	76

3.5.2 COMPARISON OF THE CRUDE FREQUENCY OF SAUDI AND NON SAUDI FEMALE BREAST CANCER PATIENTS IN THE WESTERN REGION

Data for breast cancer is shown in Table 3.4. Comparison of the values for standard deviation and coefficient of variation for the Saudi and non-Saudi populations are presented in table 3.4. The age of the non Saudi group is more homogeneous than the population of Saudi nationals. A t-test performed on truncated data (see discussion) between the ages of 20 -65 years yielded a t statistic of 0.398 which is less than the critical value of 1.96 at the 95% confidence level and so the Saudi and non-Saudi samples can be considered as coming from a single population with respect to age.

Table 3.4 **Comparison of breast cancer in the Saudi and non Saudi population**

	Saudi	Non-Saudi
mean age	46.56	44.3
standard deviation	13.9	11.62
coefficient of variation	30.06	26.23

The Z-test for the comparison of two means resulted in a Z value of 0.124 which is less than the critical Z value, at a 95% confidence level, of 1.96, and therefore there is no significant difference between the two populations with respect to mean age of onset.

In view of this breast cancers from both the Saudi and non-Saudi patients were used in the AA study group.

2.5.3 INVESTIGATION OF THE JEDDAH DATA BASE AS A SUB GROUP OF THE WESTERN REGION TUMOUR REGISTRY DATA

Since the data for the Western Region in Chapter two was restricted to only Saudi patients a t-test was performed to assess if there is any significant difference between the Jeddah data (which includes both Saudi and non-Saudis) and the Western Region data with respect to age, table 3.5.

Table 3.5   t-Test: Two-Sample Assuming Unequal Variances

	W.Region	Jeddah
Mean	46.92	46.90
Variance	168.43	170.82
t Stat	0.99	

Since the t statistic is equal to 0.99, for the two tailed test, and is less than the critical value of 1.97 the Jeddah AA group can be considered to be a statistically representative sub group of the Western Region Tumour Registry database with respect to age.

3.5.4 CLINICO-BIOLOGICAL CHARACTERISTICS

Tumour size

The coefficient of variation was calculated for tumour size (table 3.6) for each of the study groups. Over the entire age range the coefficient of variation of the tumour size is less in the AL study group than in the other two groups. The AA study group tended to have a higher mean tumour size than EL, with AL intermediate between the two.

Table 3.6 **Variation in tumour size within the study groups**

Study Group	Range (mm)	Mean (mm)	Var	SD	Coeff Var
AA	8 - 130	40.93	487.59	22.08	53.95
AL	15 - 85	34.1	254.9	15.96	46.76
EL	10 - 80	26.43	187.28	13.68	51.77
	(10-135)*	(27.99)*	(352.94)*	(18.78)*	(67.13)*

* the value in brackets represents the inclusion of one out lying case in EL

The results of t-tests between different age groupings of the same study group, table 3.7., show that there is no significant difference in mean tumour size between pre or post menopausal patients, nor between patients under or over 44 years of age, in any of the study groups.

Table 3.7 **Comparison of tumour size within study groups**

Study group	Age grouping (% over2cm, number of case)	t value	χ^2 <2cm>	calculated t value compared to critical value of t (degrees of freedom)	calculated χ^2 value compared to critical value of χ^2 = 3.8 for a 2X2 table
AA	pre (82,76) and post menopausal (82, 56)	1.09	0.007	1.09 < 1.99 (77) Same	Same
	under(84,56) and over(80,76) 44 years	0.40	0.29	0.40< 1.98 (119) Same	Same
AL	pre (94,17)and post (74,18) menopausal	1.83	2.46*	1.83< 2.04 (33) Same	Same*
	under (90,10) and over (80,25) 44 years	1.70	0.50*	1.70< 2.03 (33) Same	Same*
EL	pre (61,38) and post (53,32) menopausal	0.47	0.39	0.47< 2.00 (65) Same	Same
	under(64, 25) and over(53,45) 44 years	0.10	0.75	0.10< 2.00 (63) Same	Same

NB *: At least one cell contained less than 5 entries and a total less than 40

The mean tumour size was calculated for each of the three study groups at age intervals of one year and the resulting values plotted, (figure 3.3). The AA study group tended to have a higher mean tumour size than EL, with AL intermediate between the two. All three study groups showed a marked degree of variation in mean tumour size.

Table 3.8 **Tumour size in the three study groups over seven selected age ranges**

AGE	t size AA (mm)	t size AL(mm)	t size EL(mm)
20-29	42.9	30.0	
30-39	37.9	24.0	27.0
40-44	48.9	33.8	28.6
45-49	31.5	53.3	31.2
50-54	44.1	26.0	26.5
55-59	36.7	28.6	22.8
60-69	53.1	36.4	29.7

Fig 3.3 **Tumour size at age intervals of one year**
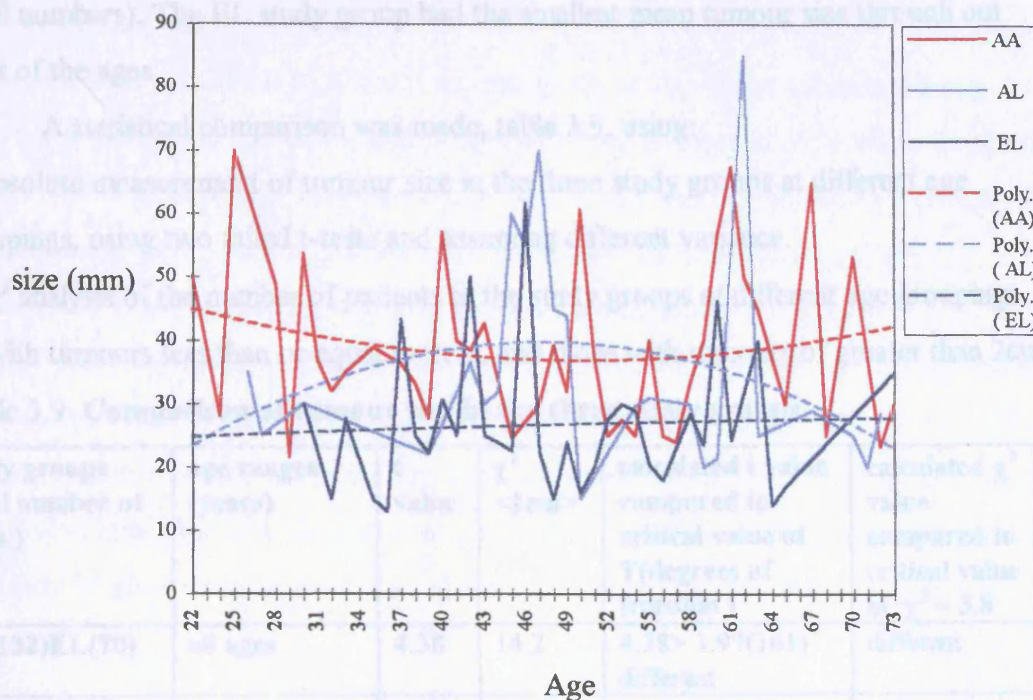
Fig 3.4 **Tumour size at seven selected age ranges**
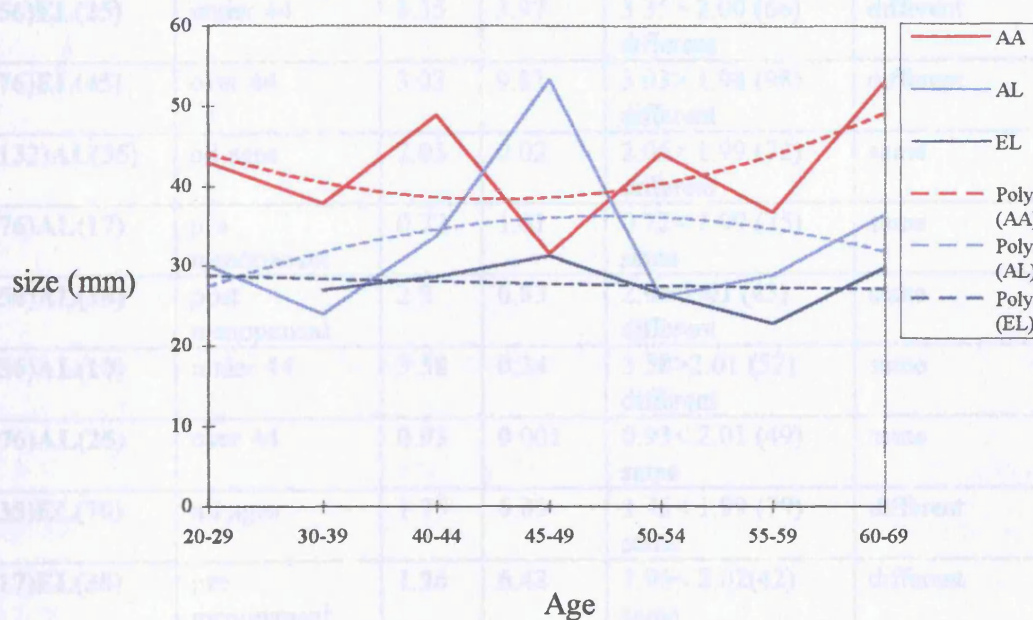
The data for the seven selected age ranges is shown in Table 3.8 and Fig 3.4 .The AA study group had the largest mean tumour size, except between the ages of 45 and 49 years when there was a peak in tumour size in the AL study group, (this may be due to small numbers). The EL study group had the smallest mean tumour size through out most of the ages.

A statistical comparison was made, table 3.9, using:

1.Absolute measurement of tumour size in the three study groups at different age groupings, using two tailed t-tests and assuming different variance.

2. χ^2 analysis of the number of patients in the study groups at different age groupings with tumours less than or equal to 2cm, and those with tumours of greater than 2cm.

Table 3.9 **Comparison of tumour size in the three study groups**

Study groups (total number of cases)	age ranges (years)	t value	χ^2 <2cm>	calculated t value compared to critical value of T(degrees of freedom)	calculated χ^2 value compared to critical value of $\chi^2 = 3.8$
AA(132)EL(70)	all ages	4.38	14.2	4.38> 1.97(161) different	different
AA(76)EL(38)	pre menopausal	2.39	5.9	2.39>1.99 (71) different	different
AA(56)EL(32)	post menopausal	4.04	8.43	4.04> 1.99 (85) different	different
AA(56)EL(25)	under 44	3.35	3.97	3.35> 2.00 (66) different	different
AA(76)EL(45)	over 44	3.03	9.81	3.03> 1.98 (98) different	different
AA(132)AL(35)	all ages	2.05	0.02	2.05> 1.99 (72) different	same
AA(76)AL(17)	pre menopausal	0.72	1.61	0.72< 1.99 (75) same	same
AA(56)AL(18)	post menopausal	2.9	0.83	2.9> 2.01 (45) different	same
AA(56)AL(10)	under 44	3.58	0.24	3.58>2.01 (52) different	same
AA(76)AL(25)	over 44	0.93	0.001	0.93< 2.01 (49) same	same
AL(35)EL(70)	all ages	1.75	6.85	1.75< 1.99 (79) same	different
AL(17)EL(38)	pre menopausal	1.96	6.42	1.96< 2.02(42) same	different
AL(18)EL(32)	post menopausal	0.59	1.75	0.59< 2.03 (34) same	same
AL(10)EL(25)	under 44	0.38	2.37*	0.38< 2.03 (33) same	same*
AL(25)EL(45)	over 44	1.68	4.89	1.68< 2.00 (55) same	different

NB *: At least one cell contained less than 5 entries and a total less than 40

There was a significant difference in the absolute tumour size of the AA study group and the EL study group regardless of the age divisions used. This difference was also significant and independent of age when the number of patients with tumours above and below 2cm were considered.

In the comparison of AA and AL there was no significant difference between the two study groups for any of the age categories when the number of tumours above and below 2cm were analysed. However when tumour size was considered as an absolute measurement there was a significant difference in the under 44 years, and post menopausal groups, and also when the groups were considered as a whole.

When the mean tumour size of the AL and EL study groups were compared there was no significant difference for any of the age groupings considered, where the tumour size was taken as an absolute measurement. However when cases with tumours above and below 2cm were considered there was a significant difference in the pre menopausal and over 44 years old age groupings, and also when the group was considered as a whole.

Node status

Table 3.10 **Node status for the three study groups**

Group	% LN +ve cases all ages	% LN +ve cases pre menopausal	% LN +ve cases post menopausal	% LN +ve cases 44 years and under	% LN +ve over 44 years
AA	71.5 (108/151)	71 (63/89)	73 (45/62)	79 (52/66)	66 (56/85)
AL	59.5 (22/37)	82 (14/17)	41 (8/20)	91 (10/11)	46 (12/26)
EL	49.0 (37/75)	51 (21/41)	47 (16/34)	56 (14/25)	46 (23/50)

Table 3.10 shows AA to have the highest percentage of lymph node positive cases and EL the lowest, with AL being intermediate. All study groups had more pre menopausal lymph node positive cases than post menopausal, and more cases under 44 years of age than over.

Table 3.11 compares node status within groups and shows that in the EL study group there was no significant difference in node status between pre or post menopausal patients, nor between patients under or over 44 years of age. In the AL study group a significant difference in node status was found when comparing the under and over 44 years age groupings and the pre and post menopausal groupings.

Table 3.11 **Comparison of node status within study groups**

Study group	Age grouping (total number of cases)	calculated χ^2	calculated χ^2 value compared to critical value of $\chi^2 = 3.8$
AA	pre(89) and post menopausal(62)	0.06	< same
	under(66) and over 44 years(85)	3.04	< same
AL	pre(17) and post menopausal(20)	6.8	> different*
	under(11) and over 44 years(26)	6.4	> different*
EL	pre(41) and post menopausal(34)	0.12	< same
	under(25) and over 44 years(50)	0.67	< same

NB *: At least one cell contained less than 5 entries and a total less than 40

No significant difference was found in either the pre and post menopausal or the under and over 44 years comparisons in study group AA.

Table 3.12 **Comparison of node status in the three study groups**

Study groups (total number of cases)	age ranges (years)	calculated χ^2	calculated χ^2 value compared to critical value of $\chi^2 = 3.8$
AA(151)EL(75)	all ages	10.73	> different
AA(89)EL(41)	pre menopausal	4.7	> different
AA(62)EL(34)	post menopausal	6.17	>different
AA(66)EL(25)	under 44	4.19	> different
AA(85)EL(50)	over 44	5.13	> different
AA(151)AL(37)	all ages	2.03	< same
AA(89)AL(17)	pre menopausal	0.96	< same
AA(62)AL(20)	post menopausal	7.02	>different
AA(66)AL(11)	under 44	0.88	< same
AA(85)AL(26)	over 44	3.26	< same
AL(37)EL(75)	all ages	1.02	< same
AL(17)EL(41)	pre menopausal	4.87	>different
AL(20)EL(34)	post menopausal	1.07	< same
AL(11)EL(25)	under 44	4.19	>different
AL(26)EL(50)	over 44	0.0002	< same

Table 3.12 shows that there was a significant difference in node status in all age groupings considered between the AA and EL populations.

A significant difference between AL and AA was only found in the post menopausal age group, although the difference in the over 44 years age group approached significance. Neither of the younger age groupings, under 44 or pre menopausal, showed a significant difference. For AL and EL both of the two younger age groupings were significantly different, whereas all other age groupings showed no significant differences.

Grade

χ^2 analysis of grade for all groups at all ages produces a calculated chi value of 24.6, the critical chi value at 4 degrees of freedom being 9.5, indicating that there is significant variation in grade between the three study populations.

The grade ratio published by Elston (1987) (1:2.06:2.5) was used as a standard, the population being geographically close to Leicestershire (Nottingham) and both centres use the same criteria for grade classification; a χ^2 analysis for goodness of fit was made.

Table3.13 **Number of cases in each grade category for the three study groups.**

	Grade 1	Grade 2	Grade 3	calculated χ^2 value	calculated χ^2 value compared to critical value of $\chi^2 = 5.99$ (2df)
AA	3	54	124	51.69	>5.99 different
AL	4	16	22	2.28	<5.99 same
EL	12	31	35	0.47	<5.99 same

Table 3.13 shows a significant difference in the ratio of grades present in AA compared to the standard, whereas neither of the Leicester populations show a significant difference.

The results of t-tests between different age groupings of the same study group are presented in table 3.14.

Table 3.14 **Comparison of grade III with grades I+II within study groups**

Study group	Age grouping (% of grade 3,total number of cases)	χ^2	calculated χ^2 value compared to critical value of $\chi^2 = 3.8$
AA	pre(66,111)and post(71,70) menopausal	0.45	< same
	under(71,85) and over(65,96) 44 years	0.78	< same
AL	pre(68,23) and post(39,19) menopausal	3.58	< same
	under(63,11) and over(47, 31) 44 years	0.97	< same
EL	pre(55,42) and post(34,36) menopausal	3.60	< same
	under (58,26) and over(38,52) 44 years	2.59	< same

Because of the limited numbers of grade 1 cases in AA and AL, it was not possible to analyse grades separately; grades 1 and 2 were combined and compared with grade 3, using χ^2 analysis. None of the study groups showed any significant difference in grade between the age groupings studied, AA having the lowest χ^2 values, and EL the highest.

Table 3.15 **Comparison of grade III with grades I+II in the three study groups**

Study groups (total number of cases)	age ranges (years)	χ^2	calculated χ^2 value compared to critical value of $\chi^2 = 3.8$
AA(181)EL(78)	all ages	12.85	> different
AA(111)EL(42)	pre menopausal	1.8	<same
AA(70)EL(36)	post menopausal	14.21	> different
AA(85)EL(26)	under 44	1.8	< same
AA(96)EL(52)	over 44	10.11	> different
AA(181)AL(42)	all ages	3.92	>different
AA(111)AL(19)	pre menopausal	0.02	< same
AA(70)AL(23)	post menopausal	7.79	>different
AA(85)AL(11)	under 44	0.31	< same
AA(96)AL(31)	over 44	2.94	< same
AL(42)EL(78)	all ages	0.62	< same
AL(19)EL(42)	pre menopausal	1.01	< same
AL(23)EL(36)	post menopausal	0.21	< same
AL(11)EL(26)	under 44	0.11	< same
AL(31)EL(52)	over 44	0.78	< same

Table 3.15 shows that there was a significant difference in the number of patients in the AA and EL populations with grade 3 tumours when all ages were considered, and in the post menopausal and over 44 years old groupings, however there was no difference in the pre menopausal or under 44 groups.

Similarly significant differences were found between AL and AA at all ages and post menopausal groups, with the over 44 year group approaching significance; and again there was no difference the two younger groupings.

No significant difference was found between AL and EL in any of the age groups analysed.

Nottingham Prognostic Index

Table 3.16 **Variation in NPI within the study groups**

	Range	var	SD	mean	coefvar
AA	3.16 - 8.00	1.35	1.16	5.6	20.77
AL	2.30 - 7.40	1.62	1.27	5.0	25.45
EL	2.20 - 7.70	1.68	1.3	4.55	28.47

Table 3.16 shows the NPI score presented as a continuous variable; the higher the score the worse the prognosis. AA had the highest mean score and EL the lowest, EL has the highest coefficient of variation and AA the lowest.

NPI can be placed into discrete categories of good, moderate and poor prognosis according to score. Scores of NPI according to category are presented in table 3.17.

Table 3.17 **NPI prognostic categories**

Prognostic category (NPI score range in brackets)	good (< 3.4)	moderate (3.4 - 5.4)	poor (> 5.4)
AA	1	40	66
AL	3	14	17
EL	14	33	18

All study groups had the least number of cases in the good prognostic group, but AA and AL had more in the poor category whereas most of the EL cases were in the moderate category. A χ^2 analysis of this data resulted in a chi value of 40.01 which is more than the critical value of chi of 9.5 at 4 degrees of freedom, and thus shows that there was a significant difference in NPI categories between the study groups.

Since there were only a few cases in the good prognostic category in AA and AL it was not possible to analyse each category and age group separately. Instead prognostic categories good and moderate were combined and compared to the poor prognostic category.

Comparison of NPI as a continuous variable and prognostic category within study groups are presented in table 3.18.

Table 3.18 **Comparison of NPI within study groups**

Study group	Age grouping (%poor catagory,total number of cases)	t value (NPI cont. var.)	χ^2 (NPI prog. cat.)	calculated t value compared to critical value (degrees of freedom)	calculated χ^2 value compared to critical value of χ^2 =3.8 for 2x2 table
AA	pre(58,64) and post(67,43) menopausal	0.66	1.01	0.66< 1.98 (99) same	same
	under(64,47) and over(60,60) 44 years	1.35	0.16	1.35< 1.98 (97) same	same
AL	pre(63,16) and post(39,18) menopausal	2.81	1.89	2.81> 2.05 (32) different	same
	under(60,10) and over(46,24) 44 years	1.24	0.57	1.24< 2.05 (21) same	same*
EL	pre(30,37) and post(25,28) menopausal	1.05	0.18	1.05 < 2.00 (55) same	same
	under(29,24) and over(27,41) 44 years	1.63	0.04	1.63 < 2.00 (59) same	same

*at least one cell less than 5 with the total less than 40

The results show that in all of the study groups there was no significant difference in NPI, calculated as a continuous variable or as a prognostic category, between pre or post menopausal patients, nor between patients under or over 44 years of age, with the exception of the comparison of pre and post menopausal cases in the AL population when NPI was considered as a continuous variable.

The mean NPI was calculated for each of the three study groups at age intervals of one year and the resulting values plotted, (figure 3.5). This shows that the AA study group tended to have a higher NPI than EL. All three study groups showed a marked degree of variation in NPI. Table 3.19 presents the data divided into seven selected age ranges which is plotted in Fig 3.6.

Table 3.19 **NPI in the three study groups over seven selected age ranges**

AGE	NPI AA	NPI AL	NPI EL
20-29	5.99	6.10	
30-39	5.47	4.98	4.75
40-44	6.24	5.43	5.03
45-49	4.91	5.93	4.39
50-54	5.90	4.77	4.03
55-59	5.38	5.17	4.01
60-69	5.73	4.13	5.07

Fig 3.5   **NPI at age intervals of one year**
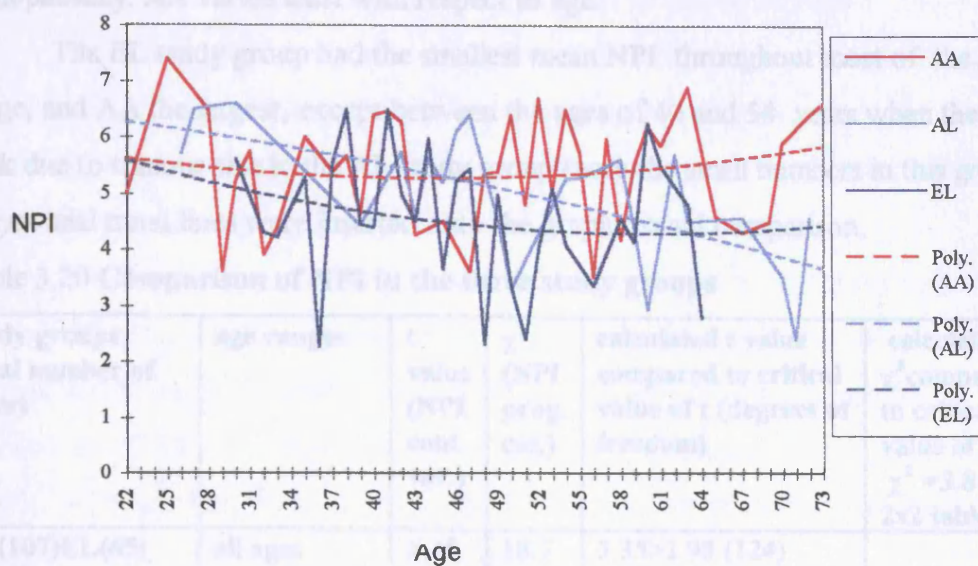
Fig 3.6   **NPI at seven selected age intervals**
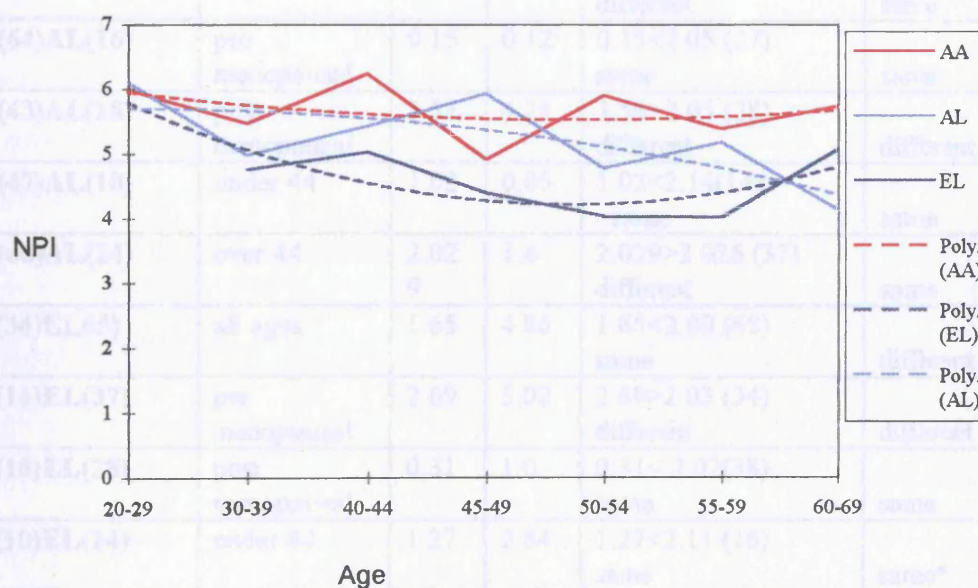
Figure 3.6 shows that NPI was high for all three study groups in the 30 - 39 years age range but then rapidly decreased with age for the EL group. AA and AL remained high and were similar premenopausally, AL tended to converge with EL post menopausally. AA varied least with respect to age.

The EL study group had the smallest mean NPI throughout most of the age range, and AA the largest, except between the ages of 40 and 54 years when there was a peak due to tumour size in the AL study group (note the small numbers in this group). Polynomial trend lines were inserted onto the graphs to aid comparison.

Table 3.20 **Comparison of NPI in the three study groups**

Study groups (total number of cases)	age ranges	t value (NPI cont. var.)	χ^2 (NPI prog. cat.)	calculated t value compared to critical value of t (degrees of freedom)	calculated χ^2 compared to critical value of χ^2 =3.8 for 2x2 table
AA(107)EL(65)	all ages	5.35	18.7	5.35>1.98 (124) different	different
AA(64)EL(37)	pre menopausal	3.28	7.41	3.28>1.99 (75) different	different
AA(43)EL(28)	post menopausal	4.40	12.2 2	4.40>2.01(48) different	different
AA(47)EL(24)	under 44	3.28	7.65	3.28>2.01(51) different	different
AA(60)EL(41)	over 44	4.18	10.7 7	4.18> 1.99 (74) different	different
AA(107)AL(34)	all ages	2.45	1.45	2.45>2.00 (54) different	same
AA(64)AL(16)	pre menopausal	0.15	0.12	0.15<2.05 (27) same	same
AA(43)AL(18)	post menopausal	3.58	4.28	3.58>2.05 (28) different	different
AA(47)AL(10)	under 44	1.02	0.05	1.02<2.14(14) same	same
AA(60)AL(24)	over 44	2.02 9	1.4	2.029>2.026 (37) different	same
AL(34)EL65)	all ages	1.65	4.86	1.65<2.00 (68) same	different
AL(16)EL(37)	pre menopausal	2.69	5.02	2.69>2.03 (34) different	different
AL(18)EL(28)	post menopausal	0.31	1.0	0.31< 2.02(38) same	same
AL(10)EL(24)	under 44	1.27	2.84	1.27<2.11 (16) same	same*
AL(24)EL(41)	over 44	1.36	2.44	1.36<2.01(50) same	same

*at least one cell less than 5 and the total less than 40

A statistical comparison was made of NPI in the three study groups at different age groupings, table 3.20.

There was a significant difference in the NPI, both as a continuous variable and prognostic categories, between AA and EL in all of the age groupings.

When prognostic categories of NPI were considered, a significant difference between AA and AL was only found in the post menopausal group. However when NPI was considered as a continuous variable in addition to the post menopausal group the over 44 years and all age groups were also significantly different.

There was no significant difference between AL and EL for under and over 44 years, nor for the post menopausal group. The pre menopausal group however showed a significant difference, similar results being obtained irrespective of the mode of assessment of NPI. However a significant difference was found when comparing AL and EL (all ages) for NPI (prognostic categories) but not for NPI (continuous variable).

Yorkshire Breast Cancer Group Prognostic Index

Table 3.21 **Variation in YBCGPI within the study groups**

	Range	var	SD	mean	coefvar
AA	1.68-4.10	0.4	0.63	2.85	22.17
AL	1.25-3.50	0.46	0.68	2.60	26.18
EL	1.20-4.15	0.57	0.75	2.40	31.42

Table 3.21 shows YBCGPI as a constant variable. The AA study group had the largest mean YBCGPI and the EL group the smallest. The coefficient of variation was the least in AA and highest in EL. Table 3.22 presents the YBCGPI scores as discrete prognostic categories.

Table 3.22 **Prognostic categories of YBCGPI**

Prognostic category (YBCGPI score range)	good (<1.21)	moderate (1.21<>1.82)	poor (>1.82)
AA	0	10	100
AL	0	5	29
EL	1	22	46

It was not possible to perform a χ^2 analysis of all three categories separately because some of the cells lacked cases. Although all study groups had most cases in the poor prognostic category it can be seen that there were differences in the ratio of moderate to poor: EL was approximately 1:2, AL 1:6, and AA 1:10.

Comparison of YBCGPI as a continuous variable and prognostic categories within study groups is presented in table 3.23.

Table 3.23 **Comparison of YBCGPI within study groups**

Study group	Age grouping (% poor catagory, total number of cases)	t value (YBCG PI cont. var)	χ^2 (YBCG PI cont. var)	Calculated t value compared to critical value (degrees of freedom)	Calculated χ^2 value compared to critical value of χ^2=3.8 for 2x2 table
AA	pre(88, 64)and post(96,46) menopausal	0.76	2.15	0.76< 1.98 (103) same	same
	under (94,47) and over (89,63) 44 years	1.24	0.73	1.24< 1.98 (101) same	same
AL	pre(100,16) and post(39, 18) menopausal	3.41	*	3.41> 2.04 (31) different	*
	under (100,10) and over(26,24) 44 years	2.76	*	2.76> 2.04 (30) different	*
EL	pre(68,38) and post(65,31) menopausal	0.33	0.12	0.33< 2.0 (65) same	same
	under (72, 25) and over (64,44) 44 years	0.98	0.5	0.98< 2.0 (56) same	same

*χ^2 analysis could not be performed in where cells with zero cases were present

Table 3.23 shows that for the AA and EL study groups there was no significant difference between the age groups considered regardless of how YBCGPI was assessed. However when YBCGPI was considered as a continuous variable there was a significant difference between both pre and post menopausal and between under and over 44 year old age groups for AL.

The mean YBCGPI was calculated for each of the three study groups at age intervals of one year and the resulting values plotted, polynomial trend lines were inserted to aid comparison (figure 3.7). The AA study group tended to have a higher

YBCGPI than EL, and for AL it tended to be higher in the younger cases and lower in the older.

The data was tabulated into seven selected age ranges, table 3.24 and figure 3.8 plotted.

Table 3.24 **YBCGPI in the three study groups over seven selected age ranges**

AGE	AA	AL	EL
20-29	2.78	3.10	
30-39	2.84	3.04	2.48
40-44	3.19	2.84	2.54
45-49	2.47	2.95	2.26
50-54	3.03	2.46	2.20
55-59	2.73	2.61	2.26
60-69	3.05	2.00	2.69

The figures showed that the AA study group had the largest mean YBCGPI except between the ages of 40 and 54 years, which was due to a peak in tumour size in the AL study group. The EL study group had the smallest YBCGPI throughout most of the age range.

Table 3.25 compares YBCGPI in the three study groups. A significant difference was found between all the age groupings when AA was compared with EL, both when YBCGPI was taken as a continuous variable and as a prognostic category.

Fig 3.7   **YBCGPI at age intervals of one year.**
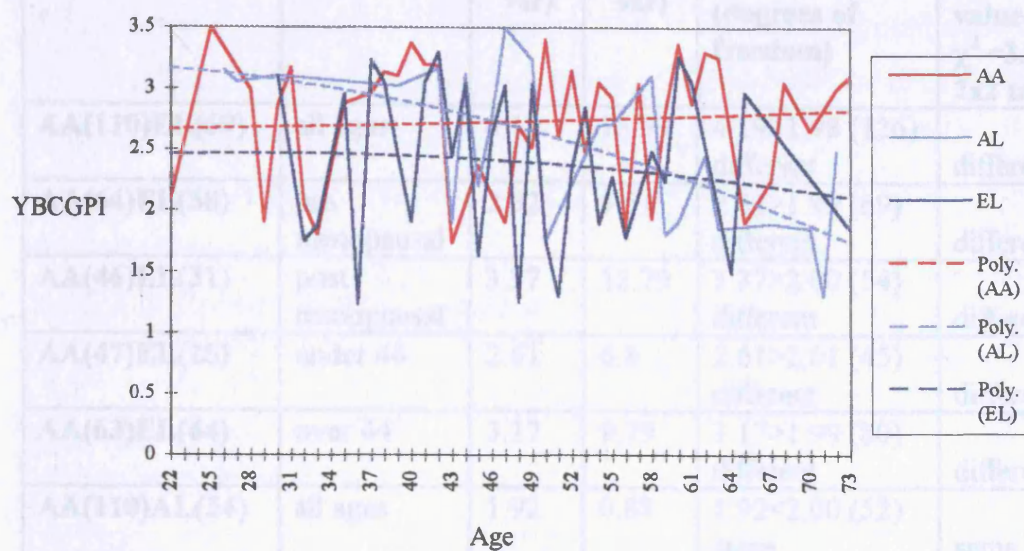
Fig 3.8   **YBCGPI at seven selected age groups**

Table 3.25 **Comparison of YBCGPI in the three study groups**

Study groups (total number of cases)	age ranges (years)	t value (YBC GPI cont. var)	χ² (YBC GPI cont. var)	calculated t value compared to critical value (degrees of freedom)	calculated χ² compared to critical value of χ²=3.8 for 2x2 table
AA(110)EL(69)	all ages	4.19	16.59	4.19>1.98 (126) different	different
AA(64)EL(38)	pre menopausal	2.62	5.51	2.62>1.99 (69) different	different
AA(46)EL(31)	post menopausal	3.37	12.79	3.37>2.00 (54) different	different
AA(47)EL(25)	under 44	2.61	6.8	2.61>2.01 (45) different	different
AA(63)EL(44)	over 44	3.17	9.79	3.17>1.99 (80) different	different
AA(110)AL(34)	all ages	1.92	0.88	1.92<2.00 (52) same	same
AA(64)AL(16)	pre menopausal	1.01	*	1.01<2.04 (30) same	*
AA(46)AL(18)	post menopausal	3.45	7.29	3.45>2.05 (28) different	different
AA(47)AL(10)	under 44	0.22	*	0.22<2.08 (21) same	*
AA(63)AL(24)	over 44	2.03	1.38	2.03> 2.02 (38) different	same
AL(34)EL(69)	all ages	1.38	3.99	1.38< 1.99 (72) same	different
AL(16)EL(38)	pre menopausal	3.10	*	3.10> 2.02 (43) different	*
AL(18)EL(31)	post menopausal	0.41	0.31	0.41< 2.02 (39) same	same
AL(10)EL(25)	under 44	2.53	*	2.53> 2.05 (29) different	*
AL(24)EL(44)	over 44	0.6	1.76	0.6< 2.01 (51) same	same

* some cells with zero cases therefore χ² analysis not possible

When AA and AL YBCGPI (continuous variable) were compared there was no significant difference in either of the two younger age groups (pre menopausal and under 44 years) but there was a significant difference in the two older age groupings, although this was not seen in the over 44 year group when YBCGPI was considered as a prognostic category. There was no significant difference between AA and AL, by either assessment method when all ages were analysed.

In the comparison between AL and EL no significant difference was found, by either assessment method in either of the two older age groups. When YBCGPI was taken as a continuous variable a significant difference was found in both of the two younger groups. When all ages were considered there was a significant difference when YBCGPI was a continuous variable but not when prognostic categories were analysed.

Table 3.47 (page 167) gives a summary of the results of the clinic-biological section with reference to study group and age group.

3.5.5 CELLULARITY AND MITOTIC INDEX

Reproducibility study

Table 3.26 **Paired t-Test for two Samples Cellularity Vv**

	INITIAL CALCULATION OF Vv	REPEAT CALCULATION OF Vv
Case one	7.69	8.08
Case two	8.28	8.51
Case three	7.09	7.23
Pearson Correlation	0.98	
t Stat	3.47	
t Critical two-tail	4.30	

Comparison of the initial calculation and the repeat calculation for Vv shows that there is no significant difference (t<4.30). The result is supported by the high value of 0.98 for the Pearson correlation coefficient.

Table 3.27 **Paired t-Test for two Samples VMI**

	INITIAL CALCULATION OF VMI	REPEAT CALCULATION OF VMI
Case one	21.29	31.42
Case two	14.36	15.26
Case three	11.16	13.21
Pearson Correlation	0.98	
t Stat	1.50	
t Critical two-tail	4.30	

There is no significant difference between the initial calculations of VMI and the repeat (t<4.30, Pearsons coef.=0.98).

Cellularity

The volume fraction (Vv) assessed as part of the calculation of VMI is a measurement of cellularity.

Table 3.28 **Comparison of Vv within the study populations**

Study group	Range	Mean	Var	SD	Coeff Var
AA	3.7-9.49	7.77	1.15	1.07	13.79
AL	3.55-9.36	6.94	1.47	2.16	21.2
EL	3.78-9.12	6.82	1.74	1.32	19.32

AA has the highest mean value of Vv and EL the lowest. AA shows the lowest coefficient of variation for Vv, values for AL and EL are very similar AL being slightly higher.

Table 3.29 **Comparison of Vv within study groups**

Study group	Age grouping	t value	critical value of t (degrees of freedom in brackets)
AA	pre (45) and post menopausal (32)	0.53	< 2.00(64) Same
	under (33) and over 44 years(44)	1.88	<1.99 (69) Same
AL	pre (19) and post menopausal (20)	0.03	<2.03 (37) Same
	under (11) and over 44 years (28)	1.10	<2.12(16) Same
EL	pre (41) and post menopausal (32)	0.56	<2.00 (57) Same
	under (26) and over 44 years (47)	0.08	<2.00 (60) Same

No significant difference was found in Vv within any of the three study groups for any of the age groupings considered in table 3.29. Data was compiled at age intervals of one year, and at seven selected age ranges (table 3.30), figs 3.9 and 3.10 were plotted from this data.

Table3.30 **Vv in seven selected age groups**

Age	AA	AL	EL
20-29	7.44	4.24	
30-39	8.16	5.92	6.93
40-44	8.18	8.32	6.66
45-49	7.26	7.54	7.06
50-54	7.50	6.19	5.95
55-59	7.87	6.72	7.09
60-69	7.40	7.42	6.57

Both figs 3.9 and 3.10 show AA and EL having similar trends with respect to age, although the level of cellularity is higher in AA. The trend for AL showed more variation with age. AL was as closer to EL for most of the age ranges apart from the peri menopausal period (40-49 years) when it was closer to AA.

Values for Vv in table 3.31 showed no significant difference between AL and EL for any of the age groupings, conversely all age groupings of AA, when compared to EL, showed a significant difference except... there was a significant difference for all age groupings of AA compared to EL, except the last remaining over 44 or post menopausal groups, suggesting the difference in cellularity between AA and AL occurred primarily in the younger age groupings. Since AL showed no difference between either the AA or EL study groups in the older age groupings, suggests that AL...

Table 3.31 Comparison of Vv in the three study groups.

Fig 3.9   Vv at age intervals of one year
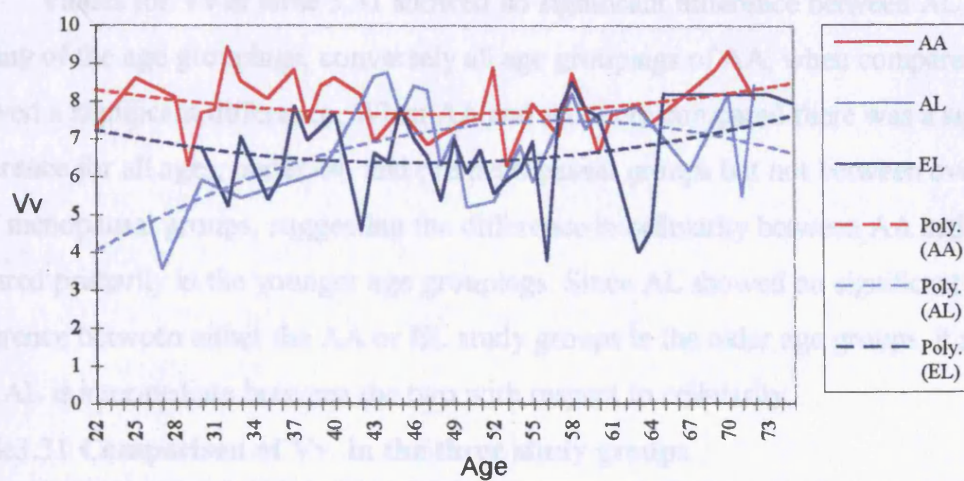
Fig 3.10   Vv at seven selected age intervals

Both figs 3.9 and 3.10 show AA and EL having similar trends with respect to age, although the level of cellularity is higher in AA. The trend for AL showed more variation with age, AL was closer to EL for most of the age ranges apart from the peri menopausal period (40-49 years) when it was closer to AA.

Values for Vv in table 3.31 showed no significant difference between AL and EL for any of the age groupings, conversely all age groupings of AA, when compared to EL showed a significant difference. When AA and AL were compared there was a significant difference for all ages, under 44, and premeno pausal groups but not between over 44 or post menopausal groups, suggesting the difference in cellularity between AA and AL occured primarily in the younger age groupings. Since AL showed no significant difference between either the AA or EL study groups in the older age groups, it suggests that AL is intermediate between the two with respect to cellularity.

Table3.31 **Comparison of Vv in the three study groups**

Study groups	Age	t value	critical value of t (degrees of freedom in brackets)	
AA(77)EL(33)	all ages	4.86	>1.98 (139)	Different
AA(45)EL(41)	pre menopausal	3.88	>1.99 (81)	Different
AA(32)EL(32)	post menopausal	2.96	>2.00 (57)	Different
AA(33)EL(26)	under 44	4.18	>2.01 (51)	Different
AA(44)EL(47)	over 44	2.90	>1.99 (85)	Different
AA(77)AL(39)	all ages	3.16	>2.00 (59)	Different
AA(45)AL(19)	pre menopausal	2.40	>2.06 (26)	Different
AA(32)AL(20)	post menopausal	1.95	<2.04 (32)	Same
AA(33)AL(11)	under 44	2.88	>2.16 (13)	Different
AA(45)AL(28)	over 44	1.53	<2.01 (47)	Same
AL(39)EL(73)	all ages	0.42	<1.99 (71)	Same
AL(19)EL(41)	pre menopausal	0.08	<2.05 (29)	Same
AL(20)EL(32)	post menopausal	0.53	<2.02 (41)	Same
AL(11)EL(26)	under 44	0.57	<2.14 (14)	Same
AL(28)EL(47)	over 44	0.87	<2.00 (58)	Same

VOLUME CORRECTED MITOTIC INDEX

Table 3.32 **Comparison of VMI within the study populations**

Study group	Range	mean	Var	SD	Coeff Var
AA	0.91-51.12	13.31	83.72	9.15	68.76
AL	1.22-56.59	15.16	169.89	13.03	85.95
EL	0.69-80.25 (0.69-53.15)	13.82 (12.90)	206.89 (146.78)	14.38 (12.12)	104.07 (93.93)

Numbers in brackets represent EL data after a single case with very high VMI (80)was removed.

AL had the highest VMI with both EL and AA having similar values. EL had the highest coefficient of variation and AA the lowest.

Table 3.33 **Comparison of VMI within study groups**

Study group	Age grouping (number of cases in brackets)	calculated t value	critical value of t (degrees of freedom in brackets)	
AA	pre(45) and post menopausal (32)	0.46	<2.00 (54)	Same
	under (33) and over 44 years (44)	0.70	<1.99 (74)	Same
AL	pre(19) and post menopausal (20)	0.42	<2.03 (37)	Same
	under (11) and over 44 years (28)	0.04	<2.09 (19)	Same
EL	pre(44) and post menopausal 29)	1.85	<2.00 (62)	Same
	under (26) and over 44 years (47)	2.46	>2.01 (50)	Different

Table 3.33 only shows a significant difference in VMI between the under and over 44 old year age groups in EL, the difference however between the pre and post menopausal groupings in EL did not reach significance. When t was recalculated for EL under and over 44 years with out the high VMI case the new t value (3.2) was even larger, and therefore the difference more significant. AA and AL showed no significant difference between any of their age groupings.

Fig 3.11 shows VMI with respect to age, plotted at yearly intervals. The data was also grouped into seven selected age ranges, table3.34, and plotted in fig 3.12.

Table 3.34 **VMI in seven selected age groups**

Age	AA	AL	EL
20-29	14.33	33.96	
30-39	16.49	7.09	17.81
40-44	10.03	16.25	20.96
45-49	9.42	17.14	11.90
50-54	14.38	16.51	6.82
55-59	7.92	17.85	10.27
60-69	17.30	14.18	15.48

Figs 3.11 and 3.12 show much variation in all three study groups EL exhibited the most difference with age and AA the least. The high degree of variation may help explain the difference in the pattern of trend lines observed between figs 3.11 and 3.12.

Fig 3.11   VMI at age intervals of one year
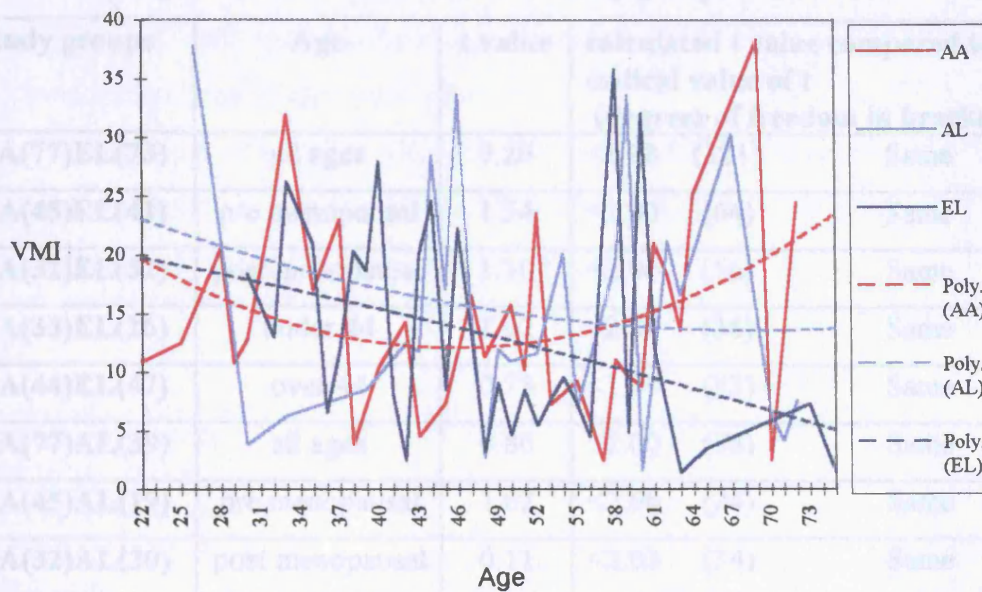
Fig 3.12   VMI at seven selected age groups
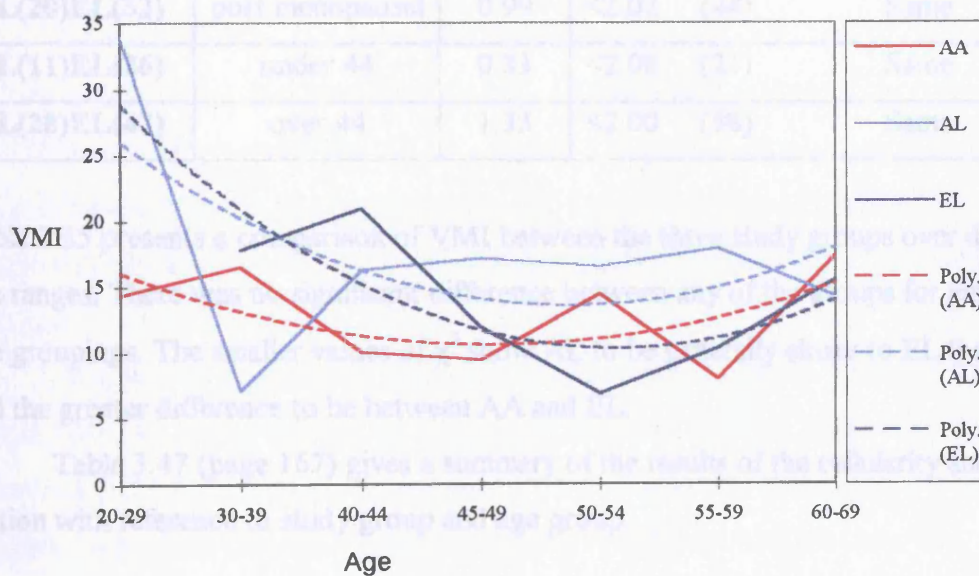
Figs 3.11 and 3.12 show much variation in all three study groups EL exhibited the most difference with age and AA the least. The high degree of variation may help explain the difference in the pattern of trend lines observed between figs 3.11 and 3.12.

Table3.35 **Comparison of VMI in the three study groups**

Study groups	Age	t value	calculated t value compared to critical value of t (degrees of freedom in brackets)		
AA(77)EL(73)	all ages	0.26	<1.98	(121)	Same
AA(45)EL(41)	pre menopausal	1.54	<2.00	(64)	Same
AA(32)EL(32)	post menopausal	1.10	<2.00	(56)	Same
AA(33)EL(26)	under 44	1.62	<2.02	(38)	Same
AA(44)EL(47)	over 44	0.75	<1.99	(83)	Same
AA(77)AL(39)	all ages	0.80	<2.00	(58)	Same
AA(45)AL(19)	pre menopausal	1.02	<2.06	(24)	Same
AA(32)AL(20)	post menopausal	0.11	<2.03	(34)	Same
AA(33)AL(11)	under 44	0.29	<2.16	(13)	Same
AA(44)AL(28)	over 44	0.83	<2.01	(45)	Same
AL(39)EL(73)	all ages	0.50	<1.99	(85)	Same
AL(19)EL(41)	pre menopausal	0.14	<2.03	(37)	Same
AL(20)EL(32)	post menopausal	0.99	<2.02	(44)	Same
AL(11)EL(26)	under 44	0.83	<2.08	(21)	Same
AL(28)EL(47)	over 44	1.33	<2.00	(58)	Same

Table 3.35 presents a comparison of VMI between the three study groups over different age ranges. There was no significant difference between any of the groups for any of the age groupings. The smaller values of χ^2 show AL to be generally closer to EL than AA, and the greater difference to be between AA and EL.

Table 3.47 (page 167) gives a summary of the results of the cellularity and VMI section with reference to study group and age group.

3.5.6 NUCLEAR MORPHOMETRY

Specificity and reproducibility study

The results shown in table 3.36 demonstrate that the morphometric measurements of area were of sufficient sensitivity to be able to distinguish between two different cases selected at random and selected according to contrasting appearance, and also identify that there were no significant differences in nuclear area, within the same case, thus testing intra tumour variability.

The same set of microphotographs measured at different times were also not significantly different providing evidence for the reproducibility of the technique.

Table 3.36 **Specificity and Reproducibility T-Tests**

Slides	Mean NA	VNA	T test statistic	Comments
Two different cases selected at random	34.87 40.51	150.6 183.7	3.68	3.68>1.97 different
Two different cases contrasting in appearance	22.1 77.44	50.08 1103.9	17.41	17.4 > 1.98 different
Same case Two Sets of Micro Photographs	30.49 31.98	107.29 109.63	1.55	1.55< 1.97 same
Same case Same set of Micro Photographs Measured at different times	34.87 36.75	150.6 120.7	1.52	1.51< 1.97 same

Analysis of morphometric measurements at age interval of one year

Figures 3.13, 3.14, 3.15 for each of the study groups show sets of curves for the nuclear morphometric characteristics studied. All study groups showed marked variability for all the parameters. AL was slightly less variable at ages less than 42 but this may be attributed to low numbers of cases. Trend lines varied between the study groups AA appeared to vary least with age and EL the most showing a trend downwards with age.

Within each study group there was notable similarity between most of the curves, however shape and ratio of major and minor axis (D1:D2) appeared to be somewhat different in form. The morphometric measurements considered can be divided into two main categories, those which are primarily a function of size and others which are a function of shape. Parameters with size function are VNA, meanNA10, SDNA and SDNP (the latter is also influenced by the nuclear shape). The remaining two measurements, Shape and D1:D2, represent shape of the nucleus.

Fig 3.13 Nuclear morphometric measurements in AAat age intervals of one year
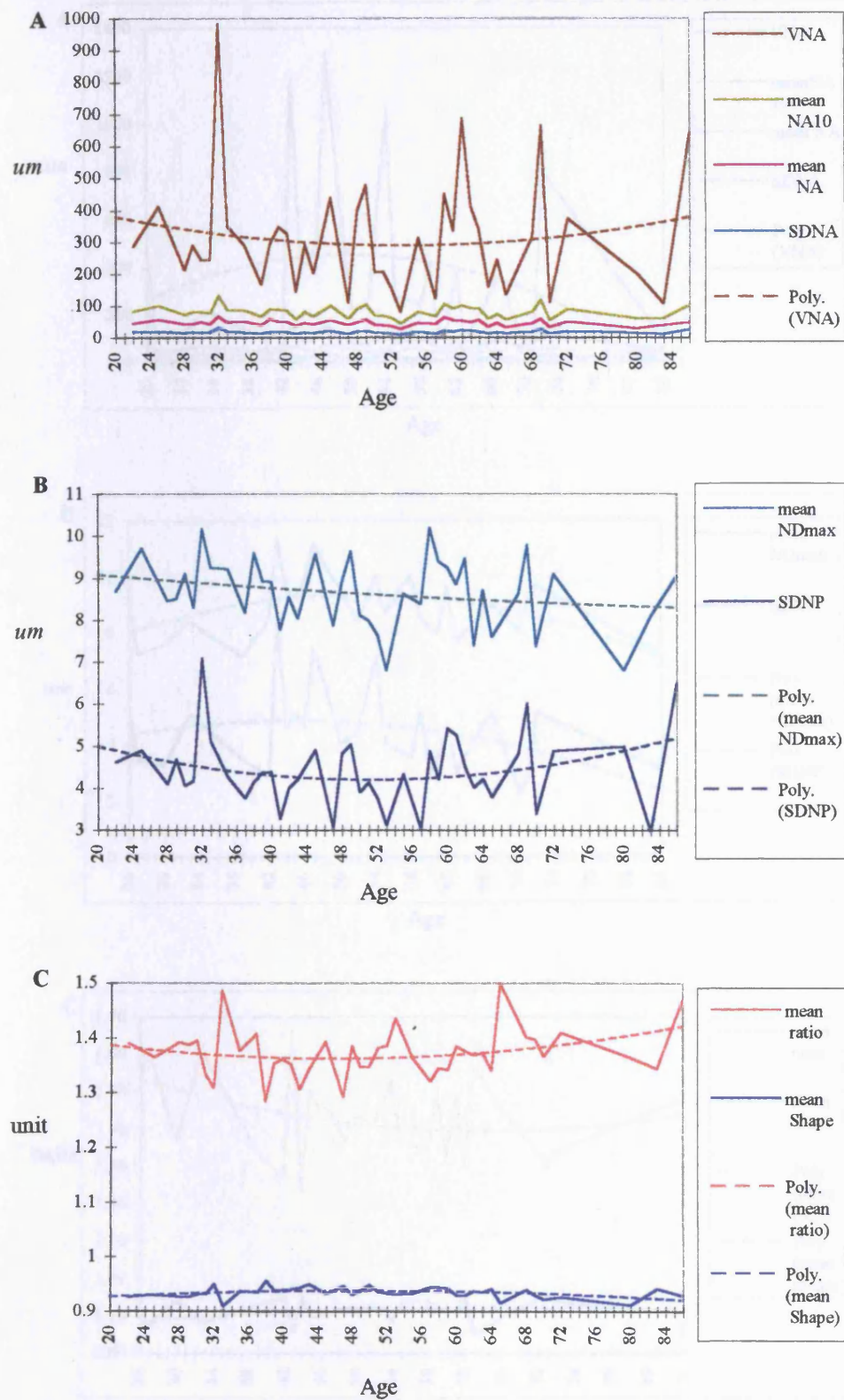
Fig 3.14 Nuclear morphometric measurements in AL at age intervals of one year
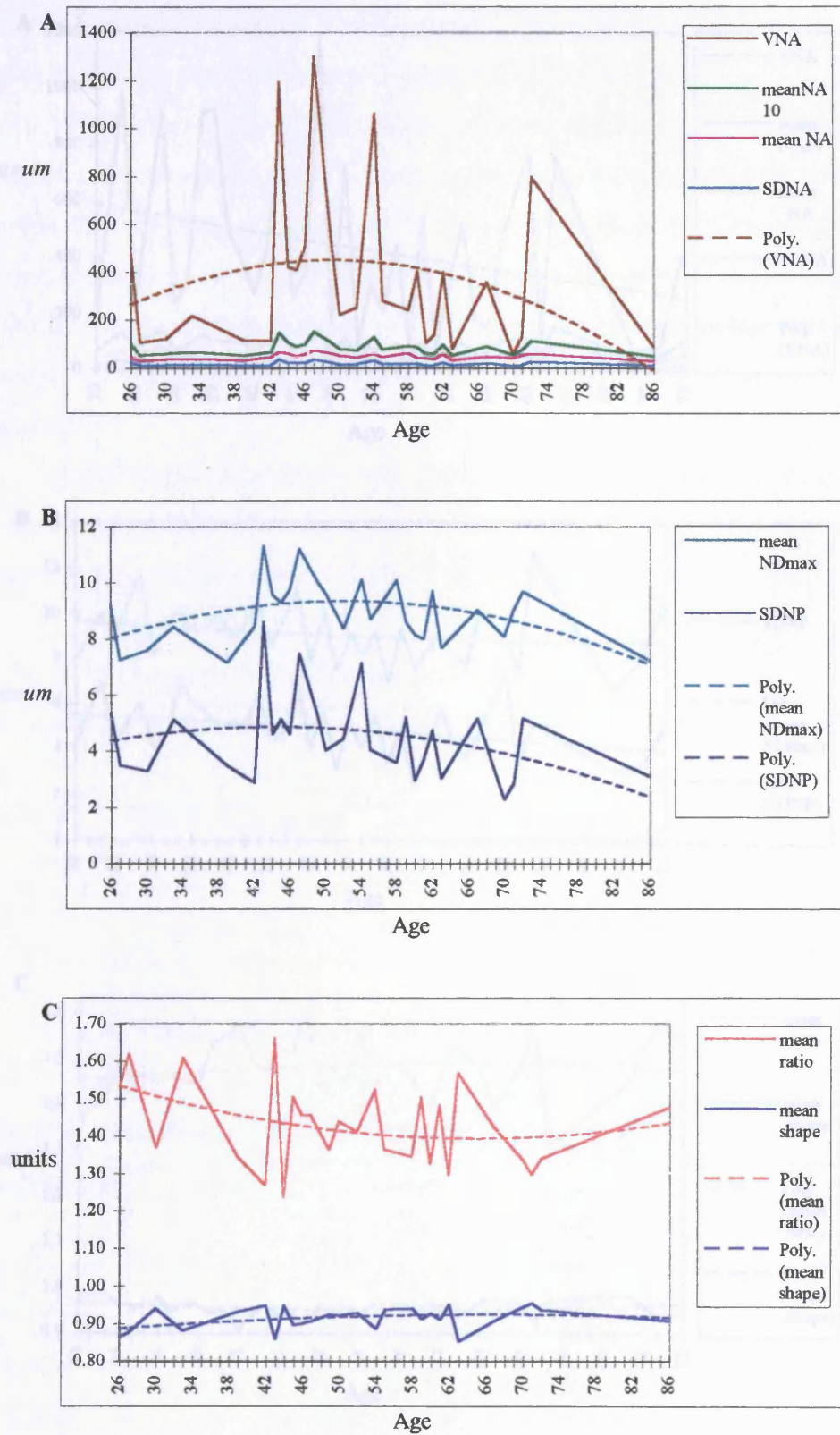
Fig 3.15 Nuclear morphometric measurements in EL at age intervals of one year

A

um

Age

Legend: VNA; mean NA10; mean NA; SDNA; Poly. (VNA)

B

um

Age

Legend: mean NDmax; SDNP; Poly. (mean NDmax); Poly. (SDNP)

C

units

Age

Legend: mean ratio; mean Shape; Poly. (mean ratio); Poly. (mean Shape)
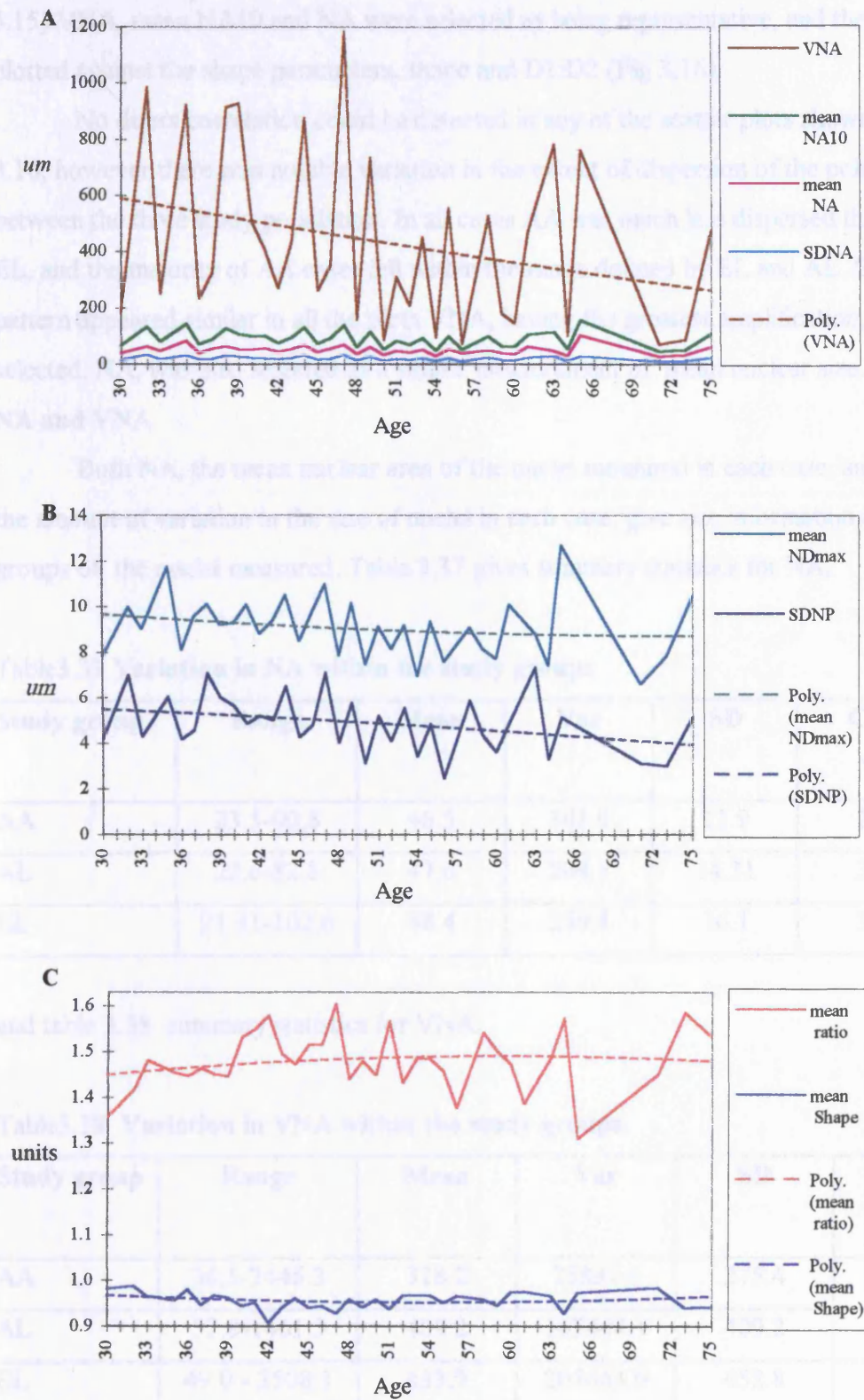
Comparison of nuclear shape with respect to size

To assess correlation between shape and size parameters, scatter plots were made. The three size parameters showing the most amplification (figs 3.13, 3.14, and 3.15) VNA, mean NA10 and NA were selected as being representative, and these were plotted against the shape parameters, shape and D1:D2 (Fig 3.16).

No direct correlation could be detected in any of the scatter plots shown in fig 3.16, however there was notable variation in the extent of dispersion of the points between the three study population. In all cases AA was much less dispersed than AL or EL, and the majority of AA cases fell within the range defined by EL and AL. Since the pattern appeared similar in all the plots VNA, having the greatest amplification, was selected. NA, was also selected as a simple measurement of mean nuclear size.

NA and VNA

Both NA, the mean nuclear area of the nuclei measured in each case, and VNA, the amount of variation in the size of nuclei in each case, give size information about the groups of the nuclei measured. Table 3.37 gives summary statistics for NA,

Table3.37 **Variation in NA within the study groups**

Study group	Range	Mean	Var	SD	Coeff Var
AA	23.5-90.8	46.5	141.0	11.9	25.5
AL	22.6-82.8	47.6	204.7	14.31	30.1
EL	21.31-102.6	48.4	259.4	16.1	33.2

and table 3.38 summary statistics for VNA.

Table3.38 **Variation in VNA within the study groups.**

Study group	Range	Mean	Var	SD	Coeff Var
AA	36.5-2445.3	318.2	75837.6	275.4	86.5
AL	57.8-1461.5	409.2	167444.1	409.2	99.9
EL	49.0 - 2508.1	433.7	205063.0	452.8	104.4

Fig 3.16   Scatter plots of size factors against shape factors
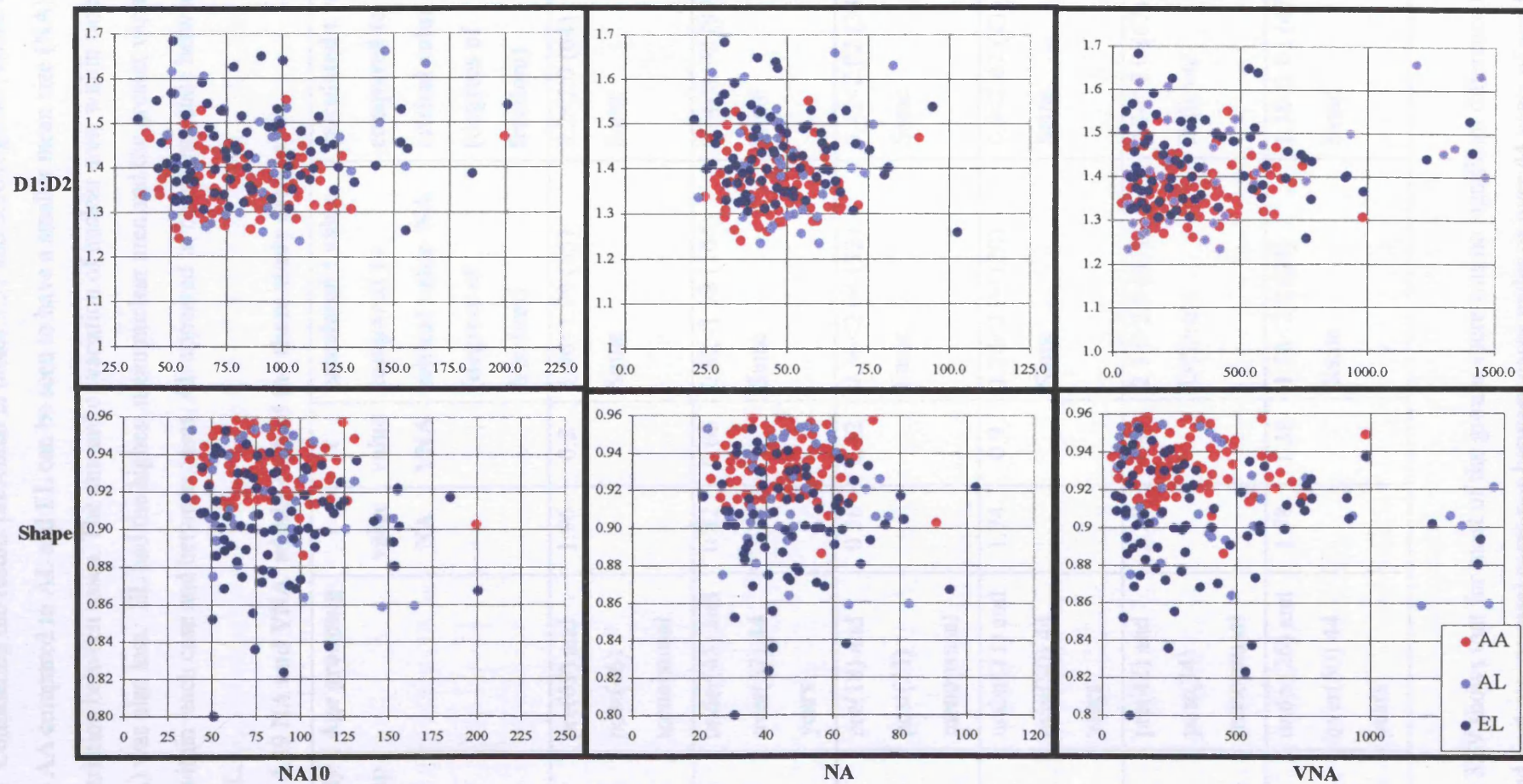
Considering the results presented in tables 3.37 and 3.38 together, nuclei in group AA compared to AL and EL can be seen to have a smaller mean size (NA) with less variation between cases. The amount of variation of nuclear area within cases (VNA) was also less. EL had the highest mean nuclear area and the greater variation both within each case and between cases. AL appeared to be intermediate between AA and EL.

Table 3.39 **NA and VNA within each of the three study groups**

Study group	Age grouping	t value NA	t value VNA	calculated t value compared to critical value NA (degrees of freedom)	calculated t value compared to critical value VNA (degrees of freedom)
AA	pre(61) and post(46) menopausal	1.96	0.2	1.96<1.99 (85) Same	0.2<2.0 (64) Same
	under(45) and over(62) 44 years	0.82	0.66	0.82<1.98 (104) Same	0.66<1.98 (99) Same
AL	pre(18) and post(23) menopausal	0.36	0.42	0.36<2.04 (32) Same	0.42<2.02 (38) Same
	under(11) and over(30) 44 years	1.74	0.9	1.74<2.09 (20) Same	0.9<2.07 (23) Same
EL	pre(42) and post(34) menopausal	2.38	2.34	2.38>2.0 (67) Different	2.34>2.0 (67) Different
	under(26) and over(50) 44 years	1.78	1.38	1.78<2.0 (64) Same	1.38<2.01 (47) Same

Table 3.39 shows that for most of the groups there was no significant difference between pre and post menopausal cases nor between cases under or over 44 years of age for either NA or VNA. Nuclear size therefore does not change significantly with age for these groups. The exception was the pre and post menopausal cases in study group EL

where a significant difference was found in both NA and VNA. It is of interest to note that there was no significant difference when under and over 44 years cases for EL were compared. Values for under 44 cases and pre menopausal cases were very similar (meanVNA under 44=534.97 meanVNA pre menopausal=534.52) the difference being observed when older age groups were considered (meanVNA over 44=381.05, meanVNA post menopausal=309.16), similar pattern to that for NA. These observations indicate an apparent significant change in nuclear size between cases over and under the age of 50 years (menopause) in the EL cases studied.

Table 3.40 **Mean NA and VNA in seven selected age groups**

AGE	NA AA	NA AL	NA EL	VNA AA	VNA AL	VNA EL
20-29	47.90	36.89		315.05	294.50	
30-39	48.93	33.14	52.81	308.22	136.46	552.81
40-44	44.97	54.57	52.31	273.27	574.74	514.14
45-49	51.12	59.42	51.91	390.81	620.28	533.81
50-54	36.85	51.73	38.67	181.89	652.51	231.97
55-59	51.40	46.71	44.93	327.51	315.2	387.64
60-69	48.27	42.27	49.74	464.28	197.94	359.71

Figs 3.17 and 3.18 prepared from the data in table 3.40 show the similarity of changes in NA and VNA with age in all the study groups.

NA in study group AA varied less with age than either AL or EL, and was the smallest for most of the age ranges. NA showed a greater difference between study groups pre menopausally, values for all groups converging to be closer by the age of 55 years; a similar pattern was seen for VNA.

AL was closer to AA under 40 years after which mean NA increased with values larger than those for EL, until at 55 years all groups tended to converge; and results for VNA were again similar. However small sample size in AL must be considered before generalising from these results.

Comparison of NA and VNA between the study groups in various age groupings

Table 3.41 shows that there was very little significant difference in NA between the study groups at any of the age ranges considered, only a comparison of AL and EL under 44 years showing a significant difference. Similarly there was very little difference in VNA, only AA and EL in the younger age groupings showing a significant difference.

Fig 3.17   NA at seven selected age intervals
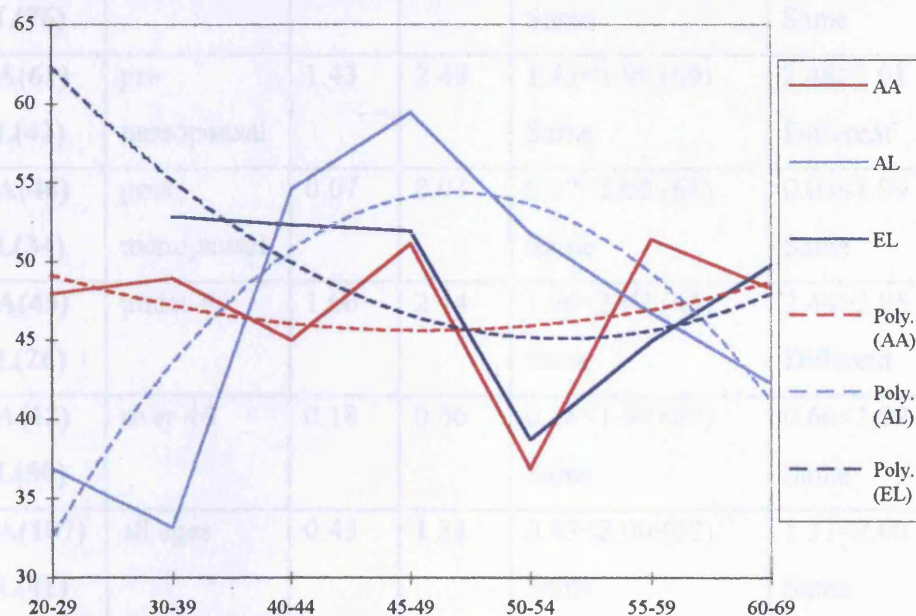
Fig 3.18   VNA at seven selected age intervals
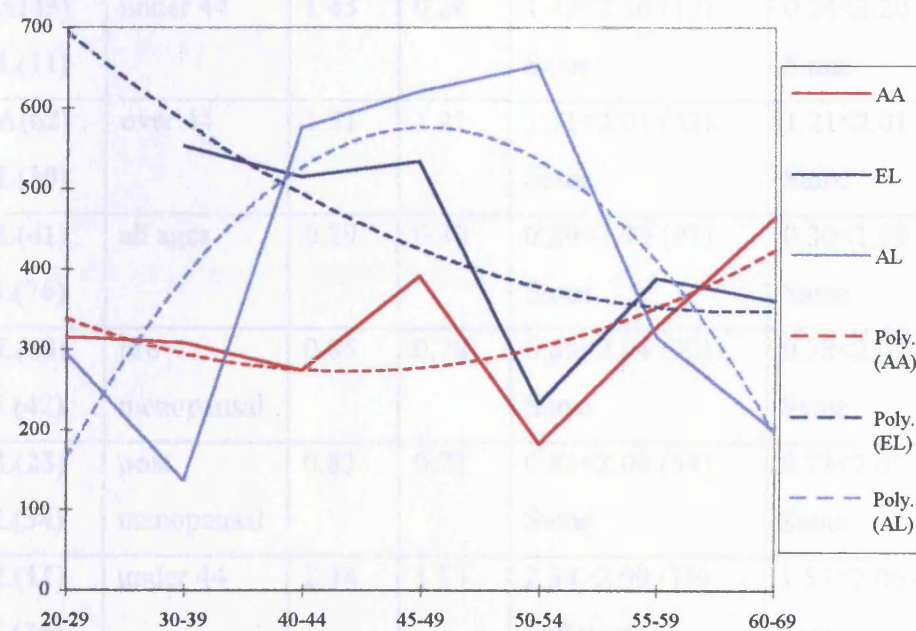
Table 3.41 Comparison of NA and VNA in the three study groups

Study groups	age ranges (years)	t value NA	t value VNA	calculated t value compared to critical value NA (degrees of freedom)	calculated t value compared to critical value VNA (degrees of freedom)
AA(107) EL(76)	all ages	0.88	1.978	0.88<1.98 (130) Same	1.978<1.981(114) Same
AA(61) EL(42)	pre menopausal	1.43	2.48	1.43<1.99 (69) Same	2.48>2.01 (48) Different
AA(46) EL(34)	post menopausal	0.07	0.03	0.07<2.00 (61) Same	0.03<1.99 (77) Same
AA(45) EL(26)	under 44	1.66	2.44	1.66<2.02 (43) Same	2.44>2.05 (29) Different
AA(62) EL(50)	over 44	0.18	0.66	0.18<1.99 (89) Same	0.66<1.99 (89) Same
AA(107) AL(41)	all ages	0.43	1.31	0.43<2.00 (62) Same	1.31<2.00 (54) Same
AA(61) AL(18)	pre menopausal	0.004	1.24	0.004<2.08 (21) Same	1.24<2.09 (20) Same
AA(46) AL(23)	post menopausal	0.9	0.70	0.9<2.01 (45) Same	0.70<2.03 (37) Same
AA(45) AL(11)	under 44	1.43	0.24	1.43<2.16 (13) Same	0.24<2.20 (11) Same
AA(62) AL(30)	over 44	1.31	1.21	1.31<2.01 (52) Same	1.21<2.01 (46) Same
AL(41) EL(76)	all ages	0.29	0.30	0.29<1.99 (91) Same	0.30<1.99 (89) Same
AL(18) EL(42)	pre menopausal	0.85	0.78	0.85<2.04 (30) Same	0.78<2.02 (44) Same
AL(23) EL(34)	post menopausal	0.83	0.73	0.83<2.00 (54) Same	0.73<2.03 (36) Same
AL(11) EL(26)	under 44	2.34	1.53	2.34>2.09 (19) Different	1.53<2.06 (26) Same
AL(30) EL(50)	over 44	0.98	0.59	0.98<1.99 (70) Same	0.59<2.00 (62) Same

Mean ratio of the maximum and minimum nuclear diameter and shape coefficient

Maximum and minimum nuclear diameter, D1:D2, and the shape coefficient (4π area/ (perimeter^2)) shape, are both ratios which describe the shape of the nucleus, a perfect circle having a score of 1 for both D1:D2 and shape.

Table 3.42 **Variation in maximum and minimum nuclear diameter within the study groups**

	Range	Mean	Var	SD	Coeff Var
AA	1.24-1.55	1.37	<0.01	0.06	4.54
AL	1.24-1.66	1.42	0.01	0.11	7.99
EL	1.26-1.68	1.44	0.01	0.09	6.2

Table 3.43 **Variation in mean shape within the study groups**

	Range	Mean	Var	SD	Coeff Var
AA	0.89-0.96	0.93	<0.01	0.01	1.54
AL	0.85-0.95	0.92	<0.01	0.03	3.18
EL	0.8-0.95	0.9	<0.01	0.03	3.27

None of the study groups showed much variation in either shape or D1:D2, AA having the lowest. AA was also closest to the value of one for both D1:D2 and shape and therefore had more regularly shaped nuclei. EL had the least regular nuclei and again AL had an intermediate value.

Table 3.44 presents the results of t-tests between different age groupings within the same study group. No significant intra group variation was found in any of the study groups for the two shape parameters, irrespective of age groupings.

Table 3.44 Comparison of mean ratio of the maximum and minimum nuclear
diameter and shape coefficient within study groups

| Study group	Age grouping	t value D1:D2	t value shape	calculated t value compared to critical value D1:D2 (degrees of freedom)	calculated t value compared to critical value VNA (degrees of freedom)
AA	pre(61) and post(46) menopausal	0.45	0.02	0.45<1.98 (100) Same	0.02<1.99 (95) Same
	under(45) and over(62) 44 years	0.41	0.13	0.41<1.99 (95) Same	0.13<1.99 (90) Same
AL	pre(18) and post(23) menopausal	0.42	1.08	0.42<2.03 (34) Same	1.08<2.03 (35) Same
	under(11) and over(30) 44 years	0.33	0.66	0.33<2.16 Same	0.66<2.14 (14) Same
EL	pre(42) and post(34) menopausal	1.00	1.53	1.00<1.99 (73) Same	1.53<1.99 (74) Same
	under(26) and over(50) 44 years	0.46	0.2	0.46<2.01 (53) Same	0.2<2.01 (50) Same

**Comparison of mean ratio and shape between the study groups in various age groupings**

Table 3.45 Mean ratio and Shape coefficient at seven selected age groups

AGE	D1:D2 AA	D1:D2 AL	D1:D2 EL	Shape AA	Shape AL	Shape EL
20-29	1.38	1.58		0.93	0.87	
30-39	1.38	1.40	1.40	0.93	0.92	0.91
40-44	1.35	1.39	1.46	0.94	0.91	0.89
45-49	1.37	1.42	1.47	0.93	0.91	0.89
50-54	1.38	1.47	1.42	0.94	0.91	0.91
55-59	1.35	1.41	1.43	0.94	0.93	0.90
60-69	1.39	1.40	1.40	0.93	0.91	0.91

Fig 3.19 and 3.20 were prepared from the data in table 3.45. Both figures show AA had the least amount of variation with respect to age. AA shows least shape deviation.

There was more difference between the study groups premenopausally, all three groups tended to converge during the post menopausal period. Throughout the age ranges AL was closer to EL than AA.

Fig 3.19   D1:D2 at seven selected age intervals
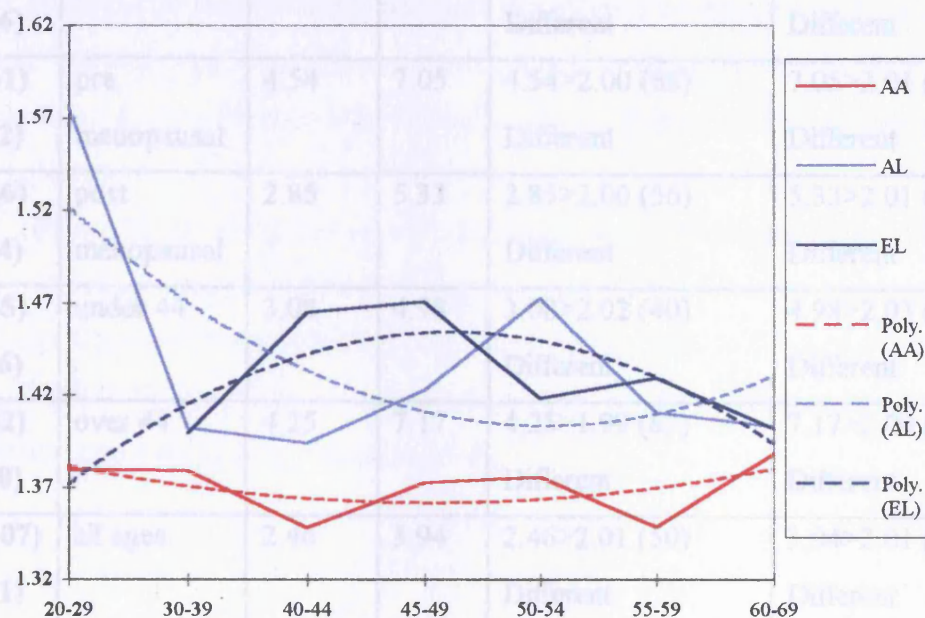
Fig 3.20   Shape coefficient at seven selected age intervals
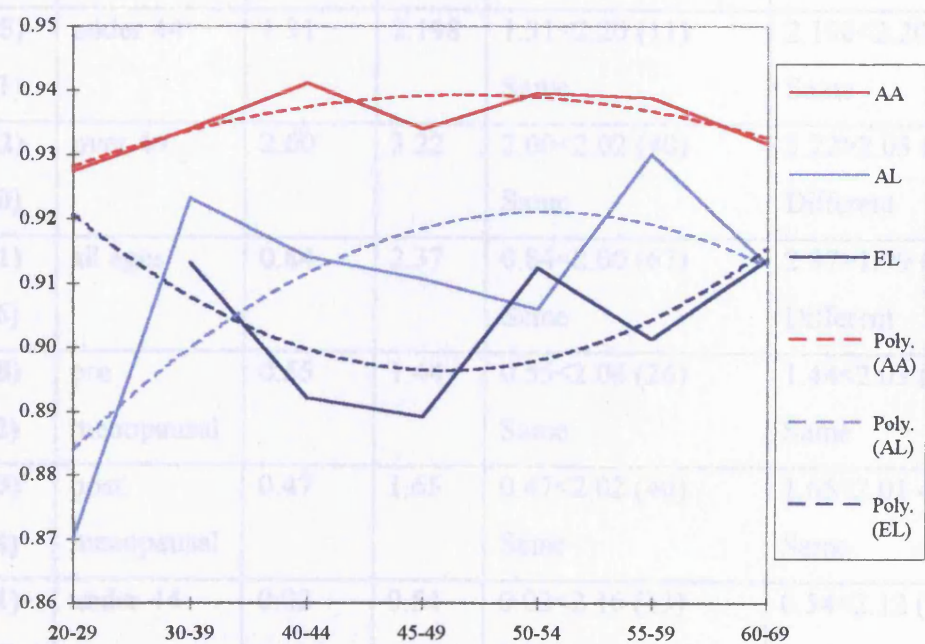
Table 3.46 **Comparison of D1:D2 and Shape in the three study groups**

Study groups	age ranges (years)	t value D1:D2	t value Shape	calculated t value compared to critical value D1:D2 (degrees of freedom)	calculated t value compared to critical value Shape (degrees of freedom)
AA(107) EL(76)	all ages	5.32	8.78	5.32>1.98 (125) Different	8.78>1.98 (100) Different
AA(61) EL(42)	pre menopausal	4.54	7.05	4.54>2.00 (68) Different	7.05>2.01 (53) Different
AA(46) EL(34)	post menopausal	2.85	5.33	2.85>2.00 (56) Different	5.33>2.01 (48) Different
AA(45) EL(26)	under 44	3.08	4.98	3.08>2.02 (40) Different	4.98>2.03 (33) Different
AA(62) EL(50)	over 44	4.25	7.17	4.25>1.99 (83) Different	7.17>2.00 (66) Different
AA(107) AL(41)	all ages	2.46	3.94	2.46>2.01 (50) Different	3.94>2.01 (48) Different
AA(61) AL(18)	pre menopausal	1.90	3.31	1.90<2.09 (20) Same	3.31>2.09 (19) Different
AA(46) AL(23)	post menopausal	1.49	2.30	1.49<2.05 (29) Same	2.30>2.05 (28) Different
AA(45) AL(11)	under 44	1.31	2.198	1.31<2.20 (11) Same	2.198<2.201 (11) Same
AA(62) AL(30)	over 44	2.00	3.22	2.00<2.02 (40) Same	3.22>2.03 (37) Different
AL(41) EL(76)	all ages	0.84	2.37	0.84<2.00 (67) Same	2.37>1.99 (83) Different
AL(18) EL(42)	pre menopausal	0.55	1.44	0.55<2.06 (26) Same	1.44<2.03 (34) Same
AL(23) EL(34)	post menopausal	0.47	1.65	0.47<2.02 (40) Same	1.65<2.01 45) Same
AL(11) EL(26)	under 44	0.02	0.54	0.02<2.16 (13) Same	0.54<2.12 (16) Same
AL(30) EL(50)	over 44	1.12	2.52	1.12<2.00 (57) Same	2.52>2.00 (67) Different

Table 3.46 presents a comparison of D1:D2 and shape in the three study groups at different age groupings.

AA and EL varied the most, being significantly different for both shape parameters at all of the age groupings considered.

AL was also significantly different from AA for shape for all age groupings except the under 44 years of age. There was less difference between the two groups when D1:D2 was considered. Although there was a significant difference when all ages were compared, the difference was no longer significant when specific age groupings were compared.

When EL was compared with AL no significant difference was found for D1:D2 for any of the age groupings. When all ages were considered for shape however there was a significant difference, which appeared to be attributable to the over 44 years age group. Table 3.47 (page 167) gives a summary of the results of the morphometry section with reference to study group and age group.

3.6 OVERALL SUMMARY

This summarises the points raised in sections 3.5.4, 3.5.5 and 3.5.6. Abbreviations used in figures and tables are as before except (c) indicates a continuous variable and (dc) a discontinuous variable.

3.6.1 INTRA GROUP VARIATION

- AA was the only one of the three study groups that showed no significant intra group variation for any of the parameters.

- AL was significantly different in the number of LN+ve cases when both pre and post menopausal groups, and under and over 44 years old groups were compared. The related measurement of YBCGPI was also found to be significantly different for AL.

- EL varied significantly for mitotic index, and both morphometric size parameters (NA and VNA).

3.6.2 RANK ORDER

Each of the mean values for the tumour characteristics was given a rank order (1 for smallest- 3 for largest) and plotted for each of the study groups (fig 3.21) as was Coefficient of variation (fig 3.22). These two graphs demonstrate the following characteristics:

- AA has the largest mean tumour size, greatest percentage of lymph node positive cases, the highest percentage of grade three tumours, and correspondingly highest NPI and YBCGPI. AA also had the highest cellularity. EL had the lowest rank position for all these characteristics with AL being intermediate.

- AA however has the lowest morphometric size parameters and shape parameters closest to spherical. Conversely EL has the largest nuclear size parameters and shape parameters furthest from spherical.

- AA is the most homogeneous of the study groups having the lowest coefficient of variation for all the tumour characteristics assessed, with the exception of tumour size. AA showed the greatest variation in tumour size.

- For all characteristics, except T (c) and D1:D2, EL was the most heterogeneous of the three study groups.

Fig 3.21   **Rank order of tumour characteristics in the three study groups**
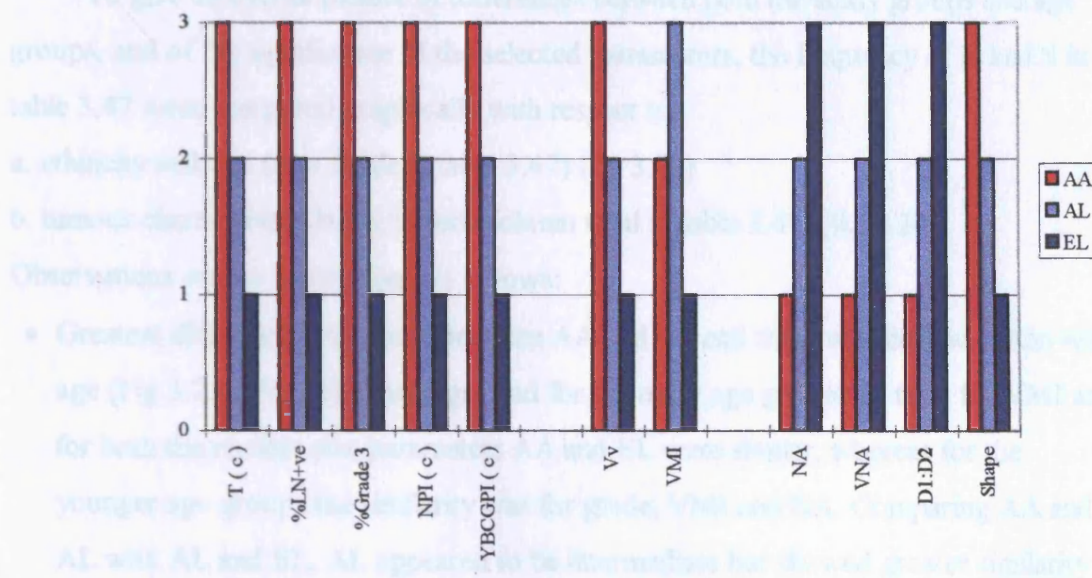
NB.   Graph was plotted after the exclusion of a single high VMI value in the EL study group.
           If included the rank order would read AL,EL,AA not AL,AA,EL

Fig 3.22   **Rank order of coefficient of variation in the tumour characteristics**
              for the three study groups
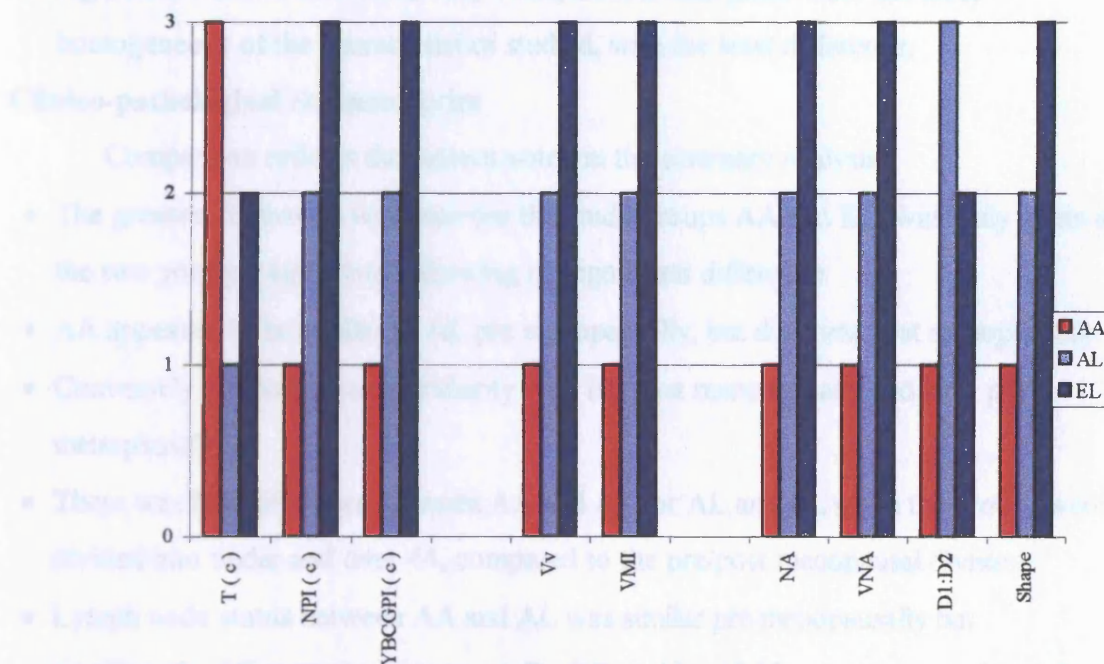
NB.   Inclusion of ommitted VMI value for EL does not effect rank order

3.6.3 INTER GROUP COMPARISON OF TUMOUR CHARACTERISTICS

Table 3.47 is a summary of the comparison of all the tumour characteristics analysed in chapter 3, between the three study groups.

Summary analysis

To give an overall picture of differences between both the study groups and age groups, and of the significance of the selected parameters, the frequency of D and S in table 3.47 were compared graphically with respect to:

a. ethnicity and age (row totals in table 3.47) (fig 3.23)

b. tumour characteristic being tested (column total in table 3.47) (fig 3.24)

Observations can be summarised as follows:

- Greatest difference was found between AA and EL and there was little variation with age (Fig 3.23). For combined ages and for the older age groupings tests for VMI and for both the nuclear size parameters AA and EL were similar, whereas for the younger age groups the similarity was for grade, VMI and NA. Comparing AA and AL with AL and EL, AL appeared to be intermediate but showed greater similarity to EL. When age groupings for AA and AL were examined there was greater similarity pre menopausally compared to post menopausally, and also greater similarity under 44 compared to over 44 years of age. However when age groupings for AL and EL were compared the similarity was greater in the older age groupings.

- Figure 3.24 shows that VMI, NA, VNA, D1:D2 and grade were the most homogeneous of the characteristics studied, with the least difference.

Clinico-pathological characteristics

Comparison reflects the pattern noted in the summary analysis:

- The greatest difference was between the study groups AA and EL, with only grade in the two younger age groups showing no significant difference.

- AA appeared to be similar to AL pre menopausally, but different post menopausally.

- Conversely AL had greater similarity with EL post menopausally and least pre menopausally.

- There was less difference between AA and AL, or AL and EL when the groups were divided into under and over 44, compared to the pre/post menopausal division.

- Lymph node status between AA and AL was similar pre menopausally but significantly different post menopausally. When AL and EL were compared lymph node status for both pre menopausal and under 44 years cases were significantly different, but not post menopausally or over 44 years.

Table3.47 **Summary of tumour characteristics** (Key to symbols in text)

Study groups	age ranges (years)	CLINICO-PATHOLOGICAL CHARACTERISTICS								CELLULARITY AND MITOTIC INDEX		NUCLEAR MORPHOMETRY			
		T (c)	T (dc)	LN +ve	grade	NPI (c)	NPI (dc)	YBGPI (c)	YBGPI (dc)	Vv	VMI	NA	VNA	D1:D2	SHAPE
AA&EL	pre menopausal	D	D	D	S	D	D	D	D	D	S	S	D	D	D
	post menopausal	D	D	D	D	D	D	D	D	D	S	S	S	D	D
	all ages	D	D	D	D	D	D	D	D	D	S	S	S	D	D
	under 44	D	D	D	S	D	D	D	D	D	S	S	D	D	D
	over 44	D	D	D	D	D	D	D	D	D	S	S	S	D	D
AA&AL	pre menopausal	S	S	S	S	S	S	S	**	D	S	S	S	S	D
	post menopausal	D	S	D	D	D	D	D	D	S	S	S	S	S	D
	all ages	D	S	S	D	D	S	S	S	D	S	S	S	D	D
	under 44	D	S	S	S	S	S	S	**	D	S	S	S	S	S
	over 44	S	S	S	S	D	S	D	S	S	S	S	S	S	D
AL&EL	pre menopausal	S	D	D	S	D	D	D	**	S	S	S	S	S	S
	post menopausal	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	all ages	S	D	S	S	S	D	S	D	S	S	S	S	S	D
	under 44	S	S*	D	S	S	S	D	**	S	S	D	S	S	S
	over 44	S	D	S	S	S	S	S	S	S	S	S	S	S	D

Fig 3.23 **Comparison of degree of difference between study groups**

Number of tests

S

D

AA&EL pre, AA&EL post, AA&EL all, AA&EL under, AA&EL over, AA&AL pre, AA&AL post, AA&AL all, AA&AL under, AA&AL over, EL&AL pre, EL&AL post, EL&AL all, EL&AL under, EL&AL over
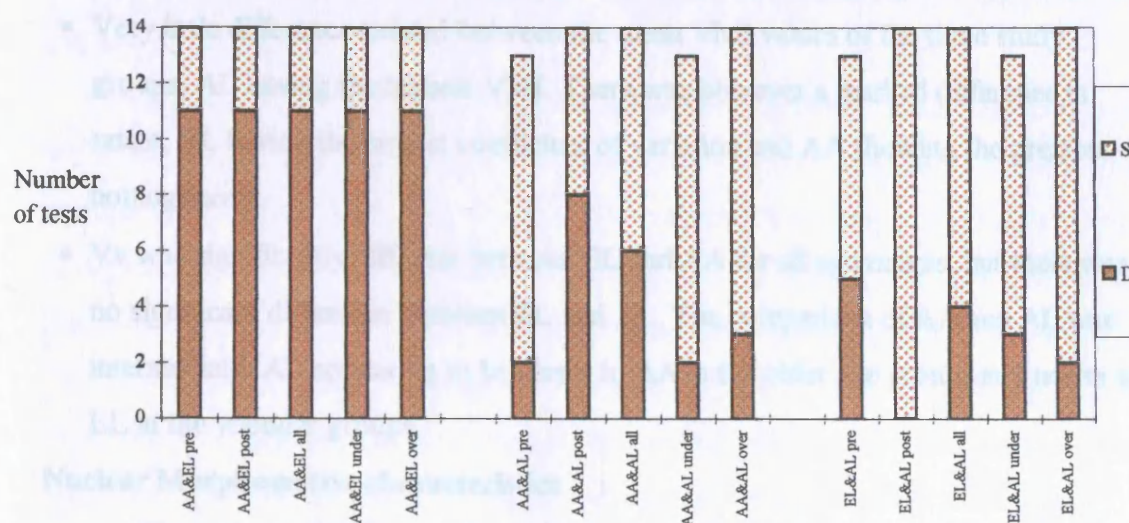
Fig 3.24 **Comparison of degree of difference between tumour characteristics**

Number of tests

S

D

T (c), T (dc), LN (+ve), grade, NPI (c), NPI (dc), YBCGPI (c), YBCGPI (dc), Vv, VMI, NA, VNA, D1:D2, Shape
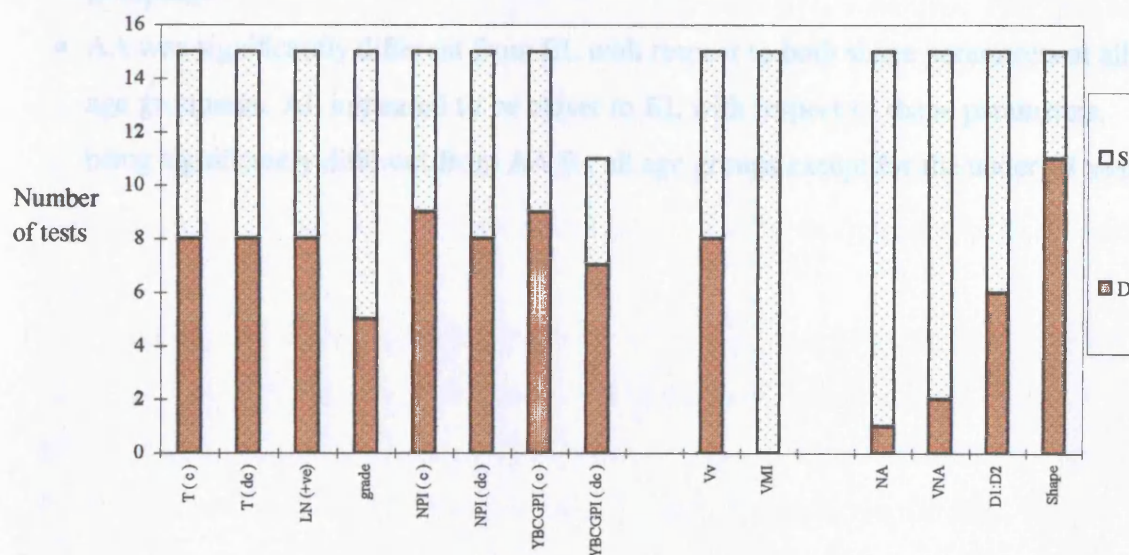
KEY

Number of tests showing significant difference (D)

Number of tests showing no significant difference (S)

Cellularity and VMI

These do not follow the pattern observed for clinico-pathological characteristics:

- The range of cellularity was similar for all three study groups, but AA, having a lower coefficient of variation, appeared to be more homogeneous than AL or EL.

- Very little difference existed between the mean VMI values of the three study groups, AL having the highest VMI. There was however a marked difference in range, EL having the largest coefficient of variation and AA showing the greatest homogeneity.

- Vv was significantly different between EL and AA for all age ranges, but there was no significant difference between EL and AL. The comparison of AA and AL was intermediate, AL appearing to be closer to AA in the older age groups and nearer to EL in the younger groups.

Nuclear Morphometric characteristics

The pattern of difference found in the size parameters was somewhat different from that between shape parameters:

- There was little significant difference in size parameters, and it is notable that the three tests in which a significant difference were detected are all in younger age groupings.

- AA was significantly different from EL with respect to both shape parameters at all age groupings. AL appeared to be closer to EL with respect to shape parameters, being significantly different from AA for all age groups except for the under 44 years.

3.7 DISCUSSION

3.7.1 SELECTION OF CASES

The aim of this chapter was to analyse and compare surrogate markers of breast cancer occurring in the three study groups, taking into consideration their ethnic origin and age of patients.

The first step was to obtain material for study from breast cancer cases occurring within the Western Region. Since it was not possible to discriminate between Saudis and non-Saudis from the information available it was necessary to establish that there were no significant differences between the two breast cancer populations with respect to age. The majority of non-Saudis in the Western Region are contract workers. The population therefore comprises mainly individuals between the ages of 20 to 65 years of age, and fewer older or younger individuals would be expected. The ages of Saudi and non Saudi patients in the registry from the crude frequency data was examined up to the age of 65years (retirement age). Less cases would be expected in the older age group since the majority of contract workers or spouses of workers return home at retirement age. The t-test performed on the data from the Saudi and non-Saudi breast cancer populations shows that there is a greater than 95% probability of them belonging to the same population. The results of the Z test also shows that there is no significant difference in the mean age of onset of the two data sets and they can be considered as coming from the same population. In view of this and that the non-Saudi population are of similar ethnic make up to the Saudi population, all non-Saudis from low incidence countries in the study group of the Western Region were included in the study group for the Western Region.

Ideally the study population should be selected at random from within the NCR data base for the Western Region; the referral hospital and patient medical record number recorded, and the required samples and patient and tumour information obtained from the referral hospital. However the cases available for study were limited, both by access to the data and material and the quality of the samples. Permission for access to data and samples had to be obtained on a hospital to hospital basis; ethical committee clearance, permission from the senior administration and the individual laboratories had to be obtained, and this was not always possible. In addition to these limitations the quality of the samples varied, some not having the quality of fixation required for morphometry. Others had insufficient material remaining (this was particularly true in hospitals having an active research program). Within these limitations data was collected from five major hospitals in Jeddah, samples being available from three of them. All

available cases of invasive carcinomas were examined and material collected so within each hospital there was no selection bias. In addition the five hospitals are located in different areas of Jeddah, serving a wide range of socio-economic groups. The raw data from these hospitals was compared with the raw data from the NCR Western Region and it was concluded that the Jeddah data base can be considered as a statistically representative sub group of the NCR Western Region data base, with respect to age. To ensure a suitable standard quality, all samples were examined by Dr Rosemary Walker (who was also responsible for selecting the breast cases from Leicestershire), before inclusion in the study. From hospitals where many blocks were found to have limited material due to past research, only data was collected since it was felt that use only of blocks not previously utilised would introduce possible bias.

The majority of cases for which sections were available were from the period 1991 to 1997. In order to be comparable Leicester European cases were selected from between 1993 and 1995 and were age matched. They were not consecutive cases since they came from a research bank collected by Dr Walker. These were cases for which tissue had been frozen and a block for fixation and processing taken, which was separate from the routine diagnostic material. This meant that the tumours had to be of a sufficient size to allow this, therefore precluding tumours 10-15mm in maximum diameter. Cases which had received therapy prior to surgery and screen-detected cases were excluded.

The number of cases of breast cancers occurring in Leicester Asians was limited and so the computer files of Leicestershire Pathology Service were searched for invasive carcinomas in that population for the period 1982 (start of computerisation) to 1998. Again pre-operative chemotherapy/ radiotherapy and mammographic detection were exclusion criteria.

3.7.2 METHODOLOGY

Once the cases for the study groups had been identified, care was taken at all stages to, as far as possible, avoid selection bias and yet at the same time avoid the introduction of additional variables which might act as confounding factors. One of the biggest variables affecting nuclear morphometry and mitotic index is fixation of tissues. The majority of cases from Leicester were optimally fixed prior to processing (see methods). Because the Leicester Asian cases were from a wider time frame the fixation schedule was more variable, but blocks were selected that gave the best preservation. The situation in Jeddah is more complex because several different institutions were involved. Within these institutions different companies are employed to operate the

hospitals with the Saudi staff, and these companies come from various parts of Europe and North America often bringing with them their own standard protocols.

All of the cases from Jeddah were reviewed by Dr Walker and only blocks with good preservation with regards to fixation and processing were selected. There were five cases where only tissue that had been used for frozen section analysis was available but in which the nuclear features were still sufficiently well preserved. Freezing and thawing of tissues can lead to nuclear collapse (Baak and Ort, 1991; Kronqvist et al, 1995), and such sections were only used when there was no evidence of nuclear collapse and when no other material was available.

Another important variable is section thickness. All sections from Leicester were cut on the same microtome by one person (Mrs S.J.Dearing). The Jeddah cases were from three different laboratories each with a different microtome and a different person cutting. However greater variations were seen in the EL group than the AA group suggesting that these technical differences did not contribute.

The stringent guidelines suggested by Baak et al (1982) were followed for both the nuclear morphometry and the mitotic index measurement, and preliminary experiments confirmed the reproducibility of the measurements and their sensitivity to distinguish between measurements from within the same tumour and between two tumours selected at random. Baak et al (1982) found 25-50 nuclei sufficient for reproducible assessments but others have used more, e.g. Pienta and Coffey (1991) used 150 nuclei. In this project between 100-150 nuclei were measured since once measurement was started on a microphotograph image then all tumour nuclei on that image were measured, to avoid selection bias (see Methods). Cases were identified by slide number until after measurements were completed when the code indicating age and ethnic origin was allocated. Because of labelling it was possible to distinguish Leicester slides from Jeddah slides, but the ageof the patient or whether they were AL or EL was not known until after the measurements were completed.

3.7.3 ANALYSIS

The age groupings for statistical analysis were formulated so that the prognostic indicators could be compared in pre and post menopausal patients. In chapter 2 I demonstrated that there were differences in the relative risk of breast cancer between the populations of the Western Region, and the Leicester Asian and European populations when cases were grouped above or below the age of 44 years, so these age groupings were also investigated.

For the continuous variables (absolute tumour size, NPI, YBCGPI, Vv, mitotic index, and the selected morphometric measurements), the data was divided into seven selected age groups, 5 year intervals over the age range 40 - 59 years, 10 year intervals at 20-40 years and 60-69 years. It was not possible to use 5 year intervals through out because of insufficient cases at the two extremes. A graph at yearly age intervals was plotted, to illustrate the amount and distribution of heterogeneity with respect to age, and to demonstrate if trends found in summarised data could be applied in the individual data. Both of these graphs had polynomic trend lines inserted to aid comparison.

The coefficient of variation, the standard deviation expressed as a percentage of the mean, was used because it allows comparison of the variation within a data set independent of scale and unit (Daniel, 1991). Using this approach it was possible to demonstrate the amount of variation and thus the extent of heterogeneity in the continuous variables within each study group.

T-tests were performed between pre and post menopausal, and under and over 44 year old within each study group to assess if there were significant differences within the groups with respect to age. To compare differences between groups t-tests were performed between AA and EL, AA and AL, and AL and EL, for all ages, pre and post menopausal, and under and over 44 years of age. By using different age splits it was possible to assess not only if significant differences existed but also to help identify the age range with the greatest difference.

For the non continuous variables (tumour size under or over 2cm, node status, grade, NPI and YBCGPI categories), χ^2 analysis was performed to compare values between pre and post menopausal, and under and over 44 year old within each study group. This was to assess if there were significant differences within the groups with respect to age, and to compare differences between groups using the same age splits as for the continuous data.

3.7.4 FINDINGS

In chapter 2 I demonstrated that there are differences in the incidence of breast cancer for the age range 20-69 years between the Western Region, Leicester European and Leicester Asian populations. Significant differences were identified for the under and over 44 year old group between the Leicester Europeans and the Western Region, whilst the Leicester Asian population in the 20-44 age range were similar to the Western Region, and the 45 and over range closer to the Leicester Europeans. In this chapter I have examined a range of biological features to determine whether there are other differences present apart from incidence.

Comparison of the basic statistics of each surrogate marker for each study group results in the following general profile of tumours (figs 3.21, 3.22).

Ethnicity

When clinico-pathological parameters are considered AA appears to have a worse prognostic profile than EL (fig 3.21), and with the exception of the younger age groupings, for grade these differences are statistically significant (table 3.47). Similar findings of large tumour size (Krieger et al, 1997; Ijaduola and Smith, 1998), and high percentage of lymph node involvement (Aziz et al, 1999; Boyer-Chammard et al, 1999) and high grade tumours (Mohla et al, 1982), have been reported for patients in other low breast cancer incidence populations.

Most tumour morphometry studies have examined the relative prognostic value of the morphometric parameters (Theissig et al, 1996; Kronqvist et al, 1998) rather than differences attributable to age or ethnic origin. In general these studies found larger NA, higher degree of pleomorphism, and higher VMI to be poor prognostic factors. With the exception of Vv and VMI, EL has a worse prognostic profile than AA for the morphometric parameters.

Baak et al (1992) studying two populations in different areas of Denmark found that although age, lymph node status, and tumour size were similar, morphometric parameters and 10 year survival rate varied; the population with the worse morphometric profile having the poorer survival rate. Although the two populations in Baak's study were fairly close, the morphometric profile appeared to be independent of the clinico-pathological profile and could have a significant input on prognosis.

Several studies report patients from low incidence populations as having poorer survival (Vernon et al, 1985; Wojcik et al, 1998). Although follow up data was not available for the cases studied, the poor prognostic profile of AA is reflected in the values for NPI and YBCGPI which are significantly worse than those for EL. Despite the poor prognostic profile from clinico-pathological parameters results from follow up studies on Saudi breast cancer cases suggest that survival and disease free interval (dfi) are not significantly different from those found in the West (Ibrahim et al, 1998; Vijay and Raadi pers com). A comparison of British and Japanese patients (Merchant et al, 1999) found premenopausal Japanese patients to have larger tumours than their British counterparts and yet, despite both groups having similar profiles for c-erbB2 and p53 expression, a better dfi and overall survival. Differences in nuclear morphometric characteristics, such as those described in the AA population, could balance out the

clinico-pathological parameters and so provide a possible explanation for these apparently contradictory results.

Age differences

Comparison of grade between AA and EL shows it to be influenced by age (table 3.47). Although the overall occurrence of grade 3 tumours was significantly greater in AA, there was no significant difference between AA and AL, or AL and EL for the younger age groups (pre menopausal, and under 44). Table 3.14 shows that the younger groups generally have a higher percentage of grade three tumours than the older groups, although none of these reached statistical significance. These findings are in agreement with earlier studies that have found a higher proportion of grade three tumours in younger patients (Walker et al, 1996; Gillette et al,. 1997; Fisher et al, 1997). There is a slightly higher percentage of grade 3 in AA post than AA pre but both are high; this probably reflects the degree of homogeneity in the AA tumour population discussed previously.

Edwards et al (1998) found the ratio of LN+ : LN- decreased with age for AmW but remains relatively constant for AmB, the low incidence population. However the results presented here are somewhat different. In the AL study group LN status was found to vary significantly with age in a similar manner to AmW in Edwards et al study. However in the other groups there was no significant difference with age although there was generally proportionally more LN+ve cases in the younger than in the older groups. Differences may reflect the intrinsic heterogeneity of breast cancer or be artifactual due to the small numbers in some of the age sub groupings, especially AL.

Three parameters showed no significant difference when combined age groups, "all ages", were compared for AA and EL: VMI, NA, and VNA. However VNA showed significant differences when both of the younger age groupings (under 44 and pre menopausal) for AA and EL were analysed. Tests for VNA between AA and AL, and between AL and EL all show no significant difference for any of the age groupings. This suggests that there may be ethnic differences in VNA in young breast cancer.

Comparing AA and EL, VMI and NA showed no significant differences regardless of age group. This suggests that within the populations studied these factors are generally independent of age and ethnic origin. An exception is the finding of a significant difference between AL and EL for NA in the under 44 age group, which, assuming that it not an artefact due to the small size of the AL group, might reflect age related differences between AL and EL.

3.7.5 Conclusions

Grade and lymph node status, in particular, appear to vary with age, although AA shows greater homogeneity with age for the parameters studied compared to EL. An interesting observation from figs 3.21 and 3.22 is that AA appears to have a poor prognostic profile compared to EL if clinico-biological parameters are considered, but morphometrically appears to be better than EL. EL conversely shows the worst, but more variable morphometric profile but has a better clinico-biological prognostic profile than AA. Furthermore table 3.47 shows most of these differences to be statistically significant.

The observed differences in the parameters studied may therefore reflect underlying different genetic alterations, and these could vary between ethnic/age groups.

CHAPTER FOUR

COMPARISON OF SELECTED GENETIC ALTERATIONS WITH REFERENCE TO AGE AND ETHINICITY IN THE THREE STUDY POPULATIONS

4.1 INTRODUCTION

The three study populations have been shown to vary in terms of the pattern of incidence of breast cancer (2.6), and prognostic indicators (3.6), which raises the possibility that breast cancer arising in the three populations may vary genetically with respect to age and/or ethnic origin. The general trend of the results appears to show age specific ethnic differences, with the Leicester Asian (AL) group tending to be intermediate between the Saudi (AA) group and the Leicester European (EL)group. Similarities between AA and AL breast cancers are more pronounced pre menopausally with AL breast cancers tending to have a closer relationship with those in the EL population in the older age range. The menopause, and the pre menopausal period appears to be a pivotal point in the relative nature of breast cancer in the three study groups. Also, from the study of the prognostic indicators, both Asian study groups appear to be more homogeneous than the European group, in addition to having larger tumours of a higher grade and more lymph node involvement at an earlier age than the European group.

It was therefore decided to compare cancers from the three study groups genetically, using determination of loss of heterozygosity / allelic imbalance as the investigatory tool.

4.1.1 ALLELIC IMBALENCE AND LOSS OF HETEROZYGOSITY

Loss of heterozygosity (LOH) of an allele in a tumour when compared to the patients constitutive DNA may not always present as complete loss but as a change in the relative strength of signal, when most copies of one allele have been lost or if copies of the second allele have been gained. Although this situation, where gels only show a partial loss of a band, has improved due to techniques such as microdissection it may be present due to intra tumour heterogeneity.

Gain of an allele is not recorded as frequently in the literature for solid tumours. Devilee et al (1991) reported allelic gain in a breast tumour associated with 1q, in the same paper Devilee introduced the term allelic imbalance, AI, it being more comprehensive and inclusive of cases of LOH. Trisomy, which has been recorded in several chromosomes in breast cancer (Gebhart et al, 1986; Cavalli et al, 1997; Adeyinka et al, 1997), would result in a gain in intensity of one allelic band. Localised amplification, as occurs in homogeneously staining regions which have been recorded in breast cancer (Dutrillaux et al, 1990; Muleris et al, 1995; Bernardino et al, 1998), would also have the same effect.

4.1.2 LOSS OF HETEROZYGOSITY IN BREAST CANCER

Sites identified as exhibiting LOH in breast cancer

There have been many studies analysing LOH in breast cancers, and specific sites of AI have been identified on many chromosomes, reviewed by Devilee and Cornelisse (1994).

Table 4.1 **Allelic Imbalance in Breast Cancer**

Chromosome arm	% informative cases showing AI	Range (%) of AI at different sites along the arm
1p	26.5	3 - 47
1q	33.0	0 - 50
3p	33.6	9 - 47
6q	36.4	9 - 52
7q	23.7	0 - 41
8p	38.7	27 - 50
9q	24.0	9 - 36
11p	27.1	8 - 41
11q	53.0	42 - 66
13q	27.7	0 - 33
16q	52.3	40 - 62
17p	57.0	37 - 75
17q	38.6	5 - 75
18q	20	3 - 36

Most of the chromosomes exhibit more than one possible site of AI, the frequency varying between sites. Changes in 6q, 7p, 16q, 17p, and 17q appear to be early events in the process of tumourigenesis, since they have been detected in between 25 - 50% of in situ ductal carcinoma (Stratton et al, 1995; Walker et al, 1997). A recent study, Buerger et al (1999b), using comparative genomic hybridisation (CGH), a technique which allows the entire genome to be screened for loss or amplification of loci, have reported frequent occurrence of AI at additional loci including losses at 11q and gains at 1q.

CGH has also been used to compare lymph node positive and lymph node negative tumours. Studying lymph node negative tumours Hermsen et al (1998) found gains in 8q, 1q, Xq, 5q,4q,and 3q, and losses at 19p, 1p, 17p, 22q, 4q and 8p, whereas Adeyinka et al

(1999) reports a higher frequency of losses in 16q, 6q, and chromosome 10, and gains at chromosome 18. A possible explanation for the apparent disparity between these studies may be due to intra tumoural heterogeneity, Aubele et al (1999) demonstrated such heterogeneity; using micro dissection and CGH. Even normal tissue associated with a tumour may show genetic heterogeneity; including LOH (Lakhani et al, 1999).

Significance of LOH in tumour progression

McGuire and Naylor (1989) discussed the question as to whether LOH in cancer was cause or effect of tumour progression. Vogelstein et al (1989) proposed two explanations for the observed LOH in tumour cells; either that it is caused by a deletion closely linked to a tumour suppressor, and so has a role in neoplastic progression, or that the observed AI, including LOH, are random events being the consequence of neoplastic progression. Chen et al (1992) investigated this problem by examining 98 cases of breast cancer for LOH in 12 different chromosomal regions. Five of these regions were randomly selected from areas not known to be prone to LOH, to act as a baseline against which to assess non specific LOH. Seven regions were selected at known sites of LOH in breast and other cancers. The randomly selected areas resulted in a baseline level of LOH at 4% (5/124 tests) whereas 6 of the 7 LOH associated sites had LOH in excess of 21%. From these experiments Chen et al (1992) concluded that LOH in breast cancer is not a random event.

Association of loss of heterozygosity with clinico-pathological parameters

Tumour Size

Studies have found a correlation between total LOH and tumour size; Nagayama and Watatani (1993) comparing tumours under and over 2cm, and Kerangueven et al (1997) in a study of 75 tumours tested with 185 microsatellite markers.

Lymph node involvement

Diverse results have been found comparing LOH with LN status. LOH at 16q and p53 have both been associated with LN+ tumours (Sato et al, 1990; Takita et al, 1992), although other studies on 16q have found no association at all (Cleton-Jansen et al, 1994) or an association with LN-ve tumours (Caligo et al, 1998).

Grade

A high total incidence of LOH in a tumour is associated with a high grade and a poor prognosis (Iwase et al,1998). Although LOH at some loci has been associated with low grade, in particular 16q (Hansen et al, 1998; Roylance et al, 1999) others have failed to find

this correlation (Tsuda et al, 1994; Tsuda et al, 1999) (see section 4.1.3).

Somatic Mutations and Loss of Heterozygosity

Loss of heterozygosity at a site on a chromosome is taken as being indicative of the possible proximity of a tumour suppressor gene (Lasko et al, 1991). LOH studies utilising microsatellites have been frequently used to locate potential tumour suppressor genes (Sato et al, 1990; Tsuda et al, 1994; Orphanos et al, 1995). According to Knudsens two hit hypothesis the remaining allele should have a mutation and the protein product of the gene either absent or non functional (section 1.3.2 fig 1.7). However there are many other cases where no such correlation can be found and where the remaining allele appears to be wild type with no mutations (Thompson et al, 1992; Deng et al, 1994). However mutations may be more frequent in tumours which exhibit LOH (Mazars et al, 1992).

Assuming that the LOH is greater than 4% and therefore non random there are several alternative scenarios which could account for this apparent paradox:

1.Loss of one copy of the tumour suppressor without a mutation in the second, could result in a growth advantage due to dosage effect (Deng et al, 1994).

2.The undeleted allele may be silenced by aberrant methylation of the gene promoter region or of a controlling gene (Ottaviano et al, 1994; Petrangeli et al, 1995; Bilanges et al, 1999).

3.LOH may occur at several sites in the same tumour and combined hemizygosity at two or more specific loci may contribute to the process of tumourigenesis (Di Cristofano et al, 1998).

4.Aberrant post transcriptional or post translational modification of the product of the undeleted allele.

5.The LOH may be affecting an alternative tumour suppressor, rather than the candidate gene (Kashiwaba et al, 1995).

4.1.3 SELECTION OF CHROMOSOMAL REGIONS FOR STUDY

Three chromosomal regions were chosen which had previously been investigated in breast cancer and been shown to have high incidence of AI (Devilee et al, 1991). The aim was to determine whether there is any difference in the incidence of allelic imbalance within cancers from the three study groups.

Chromosome Arm 6q

Possible relevance to breast cancer

Deletion involving chromosome arm 6q is the second (Devilee et al, 1991) most frequently recorded changes in breast tumours (Dutrillaux et al, 1990; Mars and Saunders, 1990; Takita et al, 1992). Studies of microsatellite polymorphisms confirm the deletion on 6q seen in the cytogenetic studies (Mars and Saunders, 1990).

AI has been described at 6q13 (Devilee et al, 1991; Orphanos et al, 1995), 6q21 (Noviello et al, 1996) and 6q23.3-25 (Theile et al, 1996). Sheng et al (1996) identified at least three regions 6q14-q16.2, 6q16.3-q23, and 6q22.3-23.1. AI has also been observed at 6q24 - 27 (Orphanos et al, 1995) and deletions in the region 6q24-25 have been correlated with advanced tumours and an aggressive phenotype (Noviello et al, 1996). Noviello et al (1996) found four regions of LOH 6q13, 6q27, 6q21, 6q24-25. Iwase et al (1995) correlated total LOH on 6q with increased grade and age, patients over the age of 50 years having a significantly higher incidence of LOH.

These studies indicate the presence of at least three or four potential tumour suppressor genes on chromosome arm 6q. Region 6q24-27 is of particular interest because it includes the ER locus.

Candidate tumour suppressors in the region 6q24-27

The oestrogen receptor gene is located on the long arm of chromosome 6 at 6q25.1 (Orphanos et al, 1994). The function of the ER is as a transcription factor and it is activated by oestrogen. With the pivotal role oestrogen has in breast development, menarche and menopause, the oestrogen receptor gene is an obvious candidate when considering a condition of the breast with possible different pre- and post- menopausal manifestations. Oestrogen receptor expression is lost in 30-40% of sporadic breast tumours (Clark and McGuire, 1988) and these oestrogen receptor negative (ER-ve) tumours tend to be poorly differentiated and have a poorer prognosis than receptor positive tumours. However deletions within the ER gene region do not appear to correlate with the loss of the oestrogen receptor, since several studies have found the oestrogen receptor to be present even in the presence of these deletions (Iwase et al, 1995; Noviello et al, 1996; Chappell et al, 1997). LOH was found in 19% of informative tumours (Iwase et al, 1995).

Another gene within this region has also been proposed as a tumour suppressor; Bilanges et al (1999) described a gene ZAC located at 6q24-25, close to the ER gene which shows loss of expression in primary breast cancer, although none of the 45 tumours selected

for LOH in this region had any detectable mutation in the remaining allele. The ZAC gene is thought to be involved in the induction of apoptosis and control of the cell cycle (Bilanges et al, 1999).

Chromosome Arm 16q

Possible relevance to breast cancer

AI has frequently been reported in the long arm of chromosome 16 (Sato et al, 1990; Takita et al, 1992; Tsuda et al, 1994). Comparative genomic analysis has also confirmed these losses (Schwendel et al, 1997). Locus 16q24.2 has the highest incidence of AI (Cleton-Jansen et al, 1994) in breast tumours independant of stage (I-III) (Tsuda et al, 1994) suggesting that it is an early event in tumourigenesis. However LOH at 16q24 has also been correlated to the occurrence of distant metasises (stage IV) (Lindblom et al, 1993). Sato et al (1990) found a correlation between LOH at 16q24 and lymph node metastases, however this correlation failed to reach statistical significance and in a later larger study by the same group (Takita et al, 1992) could not be demonstrated. Conversely Caligo et al (1998) found LOH at 16q correlated to lymph node negative tumours. Cleton-Jansen et al (1994) found no correlation with lymph node status, histology, stage or grade, but did find a correlation between LOH at 16q with ER positive tumours. LOH at 16q was however associated with low grade and to be a marker of good prognosis independent of menopausal status (Hansen et al, 1998). Fujii et al also quotes LOH at 16q as being independent of age.

At least two other regions along this arm appear to be hot spots for AI, 16q22.1 (Cleton-Jansen et al, 1994; Dorion-Bonnet et al, 1995), and 16q22-23 (Sato et al, 1991).

Known genes of interest in the region 16q24

There have been several candidate tumour suppressor genes proposed within this region. Chromosome arm 16q is the site of seven of the cadherin family of genes (Kremmidiotis et al, 1998) some of which are expressed in breast tissue (1.1.3).

H-Cadherin is located at 16q24 and is also a potential tumour suppressor (Kremmidiotis et al, 1998). It is occasionally found to be mutated in breast carcinomas (Miki et al, 1997) and has diminished expression in breast cancer (Lee, 1996), whereas expression of the wild type gene inhibits tumour formation (Lee et al, 1998).

A second region of LOH at 16q 22.1 is also the location of a cadherin genes, E-cadherin and P-cadherin (Kremmidiotis et al, 1998).

In microcell hybrid experiments a gene SEN16 located at 16q24.3, just proximal to D16S413, was found to restore cellular senescence to selected immortalised breast cell lines, making it a candidate tumour suppressor (Reddy et al, 1999). Gene 16S4448, BBC-1 (Breast basic conserved gene), also located at 16q24.3, was found to be expressed at higher levels in benign fibroadenomas compared to breast carcinoma, however a function for the gene is unknown (Cleton-Jansen et al, 1995). Another gene located at 16q24.3 is the CAR/CARM (Cell adhesion regulator molecule) described by Pullman and Bodmer et al (1992). A later study (Moerland et al, 1997) excluded the role of tumour suppressor gene for the BBC-1 gene and the CAR/CARM gene. However this decision was done on the basis of observations that no mutations were found in the remaining allele after LOH.

Chromosome Arm 17p

Possible relevance to breast cancer

Early studies using restriction fragment length polymorphism analysis generally found AI at 17p in 32% of cases using the BHP3 probe, and in 47 % with the YNZ22 probe (Sato et al, 1990). The BHP3 probe is closely related to the P53 locus at 17p12 whereas the YNZ22 probe maps to a more distal region at the tip of 17p (Takita et al, 1992), indicating at least two possible tumour suppressor genes on 17p. Microsatellite studies also confirm these results (Kirchweger et al, 1994). Kirchweger et al (1994) found LOH in 45.9% of informative tumours at the P53 locus and 58.8% at locus close to the tip of 17p.

LOH at 17p has been found to be independent of age (Fujii et al, 1998), but associated with high grade (Niederacher et al, 1997), high mitotic index (Callahan et al, 1993) and lymph node involvement (Takita et al, 1992).

Known genes of interest at 17p13.1

P53 is now classed as a tumour suppressor gene (see section 1.2.2) however it was initially studied in its mutated form and was thought to be an oncogene (Matlashewski et al, 1984; Lane and Benchimol, 1990). The P53 gene is located on the short arm of chromosome 17 at 17p13.1. P53 through its ability to detect DNA damage and once detected to activate pathways which inhibit cell division, and allow DNA repair, or apoptosis, helps maintain the integrity of the genome (Lane and Benchimol, 1990). Loss of function of the gene or its regulatory mechanism could lead to an increased accumulation of mutations, genomic instability and a resultant faster rate of tumourigenesis (Harris, 1996).

4.1.4 TYPES OF GENETIC MARKERS

A marker can only be considered informative for a given individual if it exists in the heterozygous state in the normal tissue. If the individual is constitutively homozygous LOH/AI is difficult to detect and the marker is considered to be non informative for that individual. Any genetic marker used therefore needs to be sufficiently polymorphic to ensure a reasonable rate of informativeness at the locus being studied. Several types of markers exist and may be used to study LOH/AI.

Restriction Fragment Length Polymorphisms

Use is made of changes close to the locus of interest which result in the variable occurrence of restriction sites. A deletion occurring would result in the loss of the site. After restriction of DNA with the relevant restriction enzyme gel electrophoresis will demonstrate fragments of different sizes, RFLPs. The presence or absence of the locus of interest is then located by Southern blotting (Southern, 1975) and hybridisation with a probe coding for the gene of interest (Lander and Botstein, 1980). However RFLP sites are not very polymorphic, a site is either there or it is absent, and the number and location of RFLP sites are limited.

Variable Number of Tandem Repeats

Variable Number Tandem Repeats (VNTRs) are regions of tandemly repeated DNA with each repeat being 11- 60 bp in length. Since the number of repeats may vary between individuals there are typically several alleles for any given VNTR site; this is a much higher level of polymorphism than found in the RFLPs and results in higher levels of informativity (Nakamura et al, 1987). Detection is usually by Southern blotting and hybridisation, although for the smaller repeats polymerase chain reaction analysis, PCR, is possible.

Microsatellites or Short Tandem Repeats

The polymorphism between short tandem repeats (STR) results from different numbers of repeated di, tri, or tetra nucleotides resulting in different lengths of DNA. Since their discovery STR have been much more widely used than either RFLPs or VNTR (Weber and May, 1989; Litt and Luty, 1989) because they are highly polymorphic and therefore more informative and also are spread widely throughout the genome. STR are analysed by PCR analysis using primers flanking the region of interest. The method is quicker than Southern blotting and hybridisation and requires less DNA.

4.1.5 POLYMERASE CHAIN REACTION

The discovery of the thermo stable DNA polymerases (Innis et al, 1988) and the development of the automated polymerase chain reaction procedure (PCR) (Mullis et al, 1986) has revolutionised research in molecular biology.

Simplified theory of the polymerase chain reaction system

PCR is an in vitro method for DNA synthesis, and enables the amplification of specified pieces of DNA, making analysis of even single copy target sequences possible (Saiki et al, 1988); the process is outlined in table 4.2.

Table 4.2   Summary of PRC process

Components required for the PCR
• A source of template DNA
• Reaction buffer supplying copies of the 4 nucleotides required for the synthesis of DNA in a suitable buffering system
• Pair of oligonucleotide primers designed to hybridise to opposite strands flanking the region of interest on the target DNA.
• Taq polymerase, (thermostable DNA polymerase enzyme)

The basic PCR reaction comprises of three phases:	
Pre PCR	The template DNA is denatured at 95-98°C for at least 5mins
The cycling stage	The temperature cycles between three steps 1.Denaturing, at 95°C ready for DNA synthesis. 2.Annealing; primers anneal to the complementary sequences on the template DNA. The temperature is dependant primarily on the primer sequence, temperatures above the optimal sequence result in more stringent hybridisation, lower temperatures allow less specific matching . 3. Extension; usually at 72°to allow synthesis of new DNA molecules. These new molecules are then denatured at the start of the next cycle and act as templates for the next round of amplification. These three steps are repeated as many times as required to achieve sufficient product although too many cycles can result in non specific background products.
Final extension	usually at 72°C, to allow completion of the reaction.

Optimisation of the PCR reaction

Optimisation to maximise both yield and specificity of product may be achieved by altering the proportion of the reagents in the reaction, and or the time and temperature parameters of the reaction. Some of the common optimisation strategies are outlined in table 4.3. and may involve one or all of the components.

Table 4.3 **General optimisation strategies for PCR**

Component	Action	Effect
Reaction Buffer	• increase Mg^{2+} ion • increase nucleotides	Lowers specificity
Taq Polymerase	• increase concentration	Improves yield but excess may result in non specific products
Primer /substrate ratio	• should balance eg dilute substrate/ low primer concentration • "booster PCR"	Improves yield Excess primer may result in substrate independent amplification "primer dimers". Initial concentration of primers is low, but more are added after a few cycles. Improves yields for dilute substrates
PCR cycling parameters	• increase number of cycles and/or increase steptime • Annealing temperature • "Hot start PCR" (Erlich et al, 1991; Ruano et al, 1992). • "Touch down PCR" (Don et al, 1991)	Improves yields but also may increase non specific amplification. Higher temperature, more specificity but lower yield. At least one essential component is omitted from the hybridisation tube, until after the DNA denaturing step. Annealing temperature starts 5-10°C above optimal for the primer pair and then reduces by 0.5-1°C /cycle until the optimal temperature is reached and then continues for the remaining cycles.

Precautions.

It is possible for single copies of a DNA region to be amplified to approximately 6×10^9 molecules/µl (Saiki et al, 1988). With such sensitivity it is obvious that any source of extraneous DNA contamination could result in erroneous results. A particular problem is that of cross contamination between samples before the amplification is complete and care must be taken to avoid it at all stages. Stringent care is necessary especially if the quantity template DNA/reaction tube is small since any contaminant DNA could swamp out the test material.

Table 4.4 **Basic precautionary measures to limit the risk of contamination**

Potential sources of contamination	Methods of avoiding contamination
Extraneous DNA	• Disposable latex gloves should be worn at all stages of sample preparation, processing, and analysis and changed regularly.
Cross contamination between sample batches	• Physically separating the laboratory areas for all procedures up to and including PCR (Orrego, 1990). • Each work area should have its own set of micropipettes • Effect of any contamination can be limited by aliquoting all reagents used for sample preparation and PCR reaction
Cross contamination between samples in the same batch.	• Care in preparing samples to avoid cross contamination (precautions varying according to the source and nature of the template). • Avoiding the creation of aerosols containing template while handling and opening eppendorfs during the PCR set up and pippetting. • Use of fresh disposable micro pippette tips for each solution and each tube once the tube contains sample or PCR product

Monitoring for contamination

Use of a negative control is necessary to ensure that no contamination has occurred during the processing of a particular batch. The control should contain all the components

necessary for the PCR reaction but should lack template DNA, this tube should be the last of the batch to be set up to maximise the risk of contamination.

Use of paraffin embedded tissues as a source of DNA template

Despite the extra care that must be taken to avoid cross contamination during extensive manual manipulation of the samples when cutting, dewaxing etc routinely fixed sections are excellent PCR substrates (Shibata, 1994). PCR amplification from formalin fixed, paraffin embedded tissue has advantages. Amplification is possible from a single 10μm section or less and so is much more conservative than DNA extraction followed by Southern blot analysis which requires much more starting material. Use of routine fixed, embedded tissue makes possible retrospective studies, which is particularly important when studying a disease with a low incidence rate.

There are some disadvantages in using DNA from such material as a template (Wright and Manos, 1990). Formaldehyde fixation results in breakage of the DNA so that sequences longer than 650 bp are rarely amplifiable and there may be more non specific amplification than with DNA from unfixed material. Shibata (1994) suggests 80-170bp as being the optimal size for PCR targets. The amplification process may be less efficient due to traces of chemicals remaining from fixation and extraction, for example NP40 a non-ionic detergent used during extraction has an inhibitory effect on Taq polymerase even at 0.1% (Kawaski, 1990). Cell debris may also inhibit amplification, but this can be limited by centrifugation after extraction, with dilution of the resulting supernatant.

Microdissection

By using tissue sections stained with haematoxylin and eosin as a guide it is possible to selectively remove areas of interest and to use this material for amplification, rather than the whole section. This technique may then either be used to investigate specific or multiple areas of the tumour, or to enrich the sample.

4.1.6 GEL ELECTROPHORESIS

When considering the separation of a double stranded DNA product, as is the case in LOH analysis, the choice of agarose or polyacrylamide as the matrix on which the product is separated is mainly dependant on the resolution required between the expected bands.

Electrophoresis and gel visualisation

Love et al (1990) working with microsatellite polymorphisms in mice stated that approximately 50% of alleles were resolvable by agarose gel electrophoresis, when the size difference was 8-base pairs or greater. The same group (Todd et al,1991) used 4% agarose

gel electrophoresis and only if no size variation was found did they repeat the sample on 6-10% non denaturing acrylamide gels, which can resolve size variation of 2 base pairs or greater. The gels were visualised using ethidium bromide or silver staining, both having the advantage of being non isotopic.

Although the majority of papers studying microsatellite markers in humans have used denaturing PAGE with isotopic labelling, others report the use of non denaturing PAGE (Bethwaite et al, 1995; Koreth et al, 1995; Martinelli et al, 1996; Lahiri et al, 1997), all of whom used ethidium bromide as the means of band visualisation. The avoidance of isotopically labelled nucleotides enables non denaturing gels to be used. The incorporation of the radioactive nucleotide in the PCR product induces partial strand separation (Weber and May, 1989), and it is preferable therefore that the strands are made to separate completely by denaturing and loaded on a denaturing gel in order to give consistent and comparable results.

Interpretation of results

Non denaturing PAGE may also produce extra bands, but use of a molecular size DNA ladder on all gels allows identification of the bands of interest. Another problem common to all LOH studies, regardless of the type of gel chosen, is when alleles are apparently only partially lost. This is usually due to the tumour sample containing a significant proportion of normal cells and has been greatly helped by the introduction of microdissection techniques (section 4.4.3). It is not however possible to resolve this problem completely in all cases and the decision of LOH or retention has to be done on the basis of relative signal strength..

Densitometry

Densitometry provides an independent quantitative method for assessing LOH and is useful in cases of incomplete loss particularly when the relative strengths of the normal and tumour sample vary (Orphanos et al, 1995; Stratton et al, 1995).

Calculation of relative ratios was basically the same in all reports.

T:N

where T= t1/t2 and N= n1/n2

t1, t2, n1, and n2 being the intensities of alleles 1 and 2 in tumour and normal tissue samples respectively. A ratio less than 0.5 or more than 2.0 was taken as evidence for AI (Bethwaite et al, 1995; Koreth et al, 1995; Orphanos et al, 1995; Munn et al, 1996; Noviello et al, 1996). Bonsing et al (1993) took 1.4 as the cut off to allow for cases where there had been

allelic gain of a copy and up to 40% normal cells in the tumour sample. In addition Bonsing et al (1993) commented that when scored by eye 1.4 is usually perceived as retention whereas greater than 1.4 would be considered AI.

Some studies utilising microdissection techniques have found that densitometry is no longer necessary due to the improved selection of tumour and normal samples (Fujii et al, 1996; Chappell et al, 1997).

4.2 AIMS

The aims of this chapter are to

- Establish in the Jeddah laboratory a non isotopic technique to screen for loss of heterozygosity in the three study populations at the selected chromosomal locations.

- To test whether there are significant differences between the three study groups for the selected genetic markers.

- To test whether there are significant differences between the different age groups for the selected genetic markers.

4.3 MATERIALS

4.3.1 Tissues

For each case, selected using criterion as in 3.4.1, sections of tumour plus a section including normal tissue for use as a source of constitutional DNA were identified. For the majority of cases the source of normal tissue comprised lymph nodes with no evidence of metastasis, selected by Dr Walker. When such lymph nodes were not available skin from the nipple was used as the source of normal cells. In two cases tissue from earlier surgery was utilised as the source of normal cells, namely cervix, and fallopian tube. In one case non involved breast tissue had to be used as the source of normal cells.

Samples from the archival blocks could not be taken from all the identified patients either because of unavailability, or if only a limited amount of tissue remained. Tonsil fixed and processed in a similar manner, was used for technique development.

4.3.2Primers

Requirements were that the primers studied should be highly polymorphic loci mapping to the regions of interest (4.1.4). Also, because the study involved comparison of LOH in the three groups at different ages rather than a search for new candidate tumour suppressors, the primers had to map to regions already established as sites of known LOH in breast cancer from previous studies.

Five primer pairs were selected, (table 4.5) being situated at the three sites described in section 4.1.1.

Table 4.5 Selected primers

location	primer	sequence	size of product in base pairs
6q24-27	ER(TA)ₙ [a]	ER1:GACGCATGATATACTTCACC 3' ER2:GCAGAATCAAATATCCAGATG3'	193
6q26	D6S186[b]	F:TTACCCACTACCTACCCAGAG3' R:GTCCCTTGGAAAATTCTCCCT3'	235
6q27	D6S193[b]	F:AGAGCAGGCTCTGCATGGTTA3' R:CTGACAAAAGAACATATTGTTTCCC3'	190
16q24.4	D16S413[c]	F:GGTCACAGGTGGGTTC3' R:ACTCCAGCCCGAGTAA3'	140
17p13.1	P53 (AAAAT)ₙ[d]	F:AAACAGCTCCTTTAATGGCAG3' R:ATCATTTGAATCCGGGAGGA3'	170
house-keeping gene	GAPDH	F:AGA ACA TCA TCC CTG CCT CC R:GCC AAA TTC GTT GTC ATA CC	360

a Del Senno et al, 1992 b Saito et al, 1992 c Weissenbach et al, 1992 d Futreal et al, 1992

4.3.3 Chemicals

The recipes for the buffers and chemicals used, with suppliers of the individual components, are listed in appendices B and C.

4.4 METHODS

Although PCR analysis of DNA purified from blood haemoglobinopathy studies had been performed previously in the Jeddah laboratories no LOH studies using fixed embedded tissue as the source of DNA had previously been undertaken. It was therefore necessary to establish and validate the techniques.

4.4.1 LABORATORY SETUP

A heated top PCR machine was chosen (Perkin Elmer 2400) to avoid the use of oil and for its faster ramp time and versatile programming capabilities. The work area was set up using the precautions outlined in table 4.4 to limit sources of possible contamination. Gel preparation and electrophoresis was performed using the Bio Rad Protean II apparatus which has a coolant core enabling the gel to be kept at a uniform temperature. After experimentation it was found that a 25 well 0.5mm comb was the most suitable allowing adequate space for up to 60µl of product and enabling up to 12 pairs of patient samples to be analysed on a gel.

Before processing material from the study groups the following preliminary experiments were performed.

All procedures were optimised initially on tonsil before repeating, and reoptimising where necessary, on breast tissue.

Validation of technique was achieved by

- successfully repeating results previously obtained by myself during a training session in the laboratories in Leicester.

- repeating results, previously obtained using purified DNA, when using a crude extract as a PCR template.

Since it was not always possible to process samples immediately reproducibility over time was tested by

- repeating results previously obtained with freshly cut material and with the same material after storage at -4°C for several months.

4.4.2 SAMPLE COLLECTION

Sections from Leicester were selected by Dr Walker, cut and packed in tubes or placed on slides by Sheila Dearing. Sections from Jeddah were located from the records by myself, checked by Dr Walker, and cut by the histology technician at each of the hospitals (named individually in acknowledgements), while I assisted in keeping the area cleaned with

xylene, decontamination of knives and packing sections into tubes or collecting them onto slides. Tonsil sections for the set up procedure were routinely fixed and then cut and packed into tubes or onto slides by myself.

Sections were cut from the previously selected blocks following stringent isolation precautions to avoid cross contamination between blocks or from external sources.

1. Gloves were worn through out the procedure, and changed frequently.

2. The microtome blade, the microtome, and surrounding work bench area were cleaned between blocks with xylene.

3. The water bath, used for floating sections onto slides, was swept clean of all debris between blocks.

From each block one 4μm section and two 10μm sections were cut serially onto glass microscope slides. In addition two 10μm sections were cut into screw top sterile eppendorf tubes.

4.4.3 SECTION STAINING AND CRUDE MICRODISSECTION

This was used to obtain normal tissue and to remove normal tissue adjacent to a tumour. After being floated onto glass slides the sections were allowed to drain and then were incubated at 37°C for 3 hours. Slides were then processed as follows:

- Sections were then dewaxed in xylene (1x5mins, 1x2mins)
- rehydrated by passing through a series of graded alcohols (2x2min 99%, 2x2mins 95%)
- rinsed in running tap water (1x5mins)

The 4μm slide sections were then

- stained with haematoxylin (1x1min)
- rinsed in running water (1x5mins)
- stained in eosin (1x15secs), to complete the H&E stain.

The 10μm slide sections were

- stained with eosin (1x1sec).

Both types of section were then

- dehydrated back through the alcohol series

4μm H&E sections were taken back through xylene prior to mounting in resinous mountant (XAM), 10μm sections were left unmounted.

The H&E sections were examined by Dr Walker, and areas for crude microdissection identified.

The microdissection was as described by Koreth et al (1995) and as modified by Munn et al (1996). Using the H&E section as a guide the required region was located on the eosin stained section by means x40 magnification with a dissection microscope (James Swift Prior dissection microscope). Once located the required region was microdissected out using a drawn glass capillary tube. Some trouble was found on some days with static electricity making dissected product hard to handle, when this occurred it was advantageous to introduce a drop of alcohol into the bottom of the capillary tube. The tube together with the microdissected material were broken off into a sterile eppendorf tube. Using this method it was possible to enrich tumour samples when the section contained large amounts of normal breast cells and or stroma. Also when no unaffected lymph node was available, normal skin or normal breast cells could be microdissected, care being taken to select skin or cells as far away as possible from the tumour.

4.4.4 DNA EXTRACTION

Procedures used were those practised at the Glenfield Hospital Breast Cancer Research Unit in Leicester, where I under went 6 weeks training.

The initial step in preparing the whole tissue sections for PCR amplification is removal of the wax. Sections were

1.incubated at room temperature in 1ml of xylene for 10mins.

2.centrifuged for 5 mins at 13,000 rpm and the supernatant discarded.

3. steps 1 and 2 were repeated was repeated with xylene, and then twice with absolute ethanol.

4.The tissue pellets were then allowed to air dry.

 Microdissected tissue was already dewaxed and did not require any treatment prior to digestion.

Experiments were performed to compare varying amounts of digestion buffer, overnight (18hr) or over the week end (48hr) digestion, and with or without oil. The protocol adopted was as follows:

- 400μl of rapid digestion buffer was added to the whole sections in eppendorfs
- 200μl of microdissection rapid digestion buffer to the microdissected samples
- incubated over night at a temperature of 56°C to yield crude DNA extracts.

- heated the following morning to 95°C for 10mins to inactivate the proteinase K

- microcentrifuged to pellet any undigested tissue debris

- aliquots were taken for PCR amplification.

Typically 10μl was taken from microdissected samples. Whole section digests were diluted 1:10 with distilled water and 10μl used for each PCR reaction. Unused digests were stored at -20°C.

4.4.5 DNA AMPLIFICATION

The quality of each digest was tested using GAPDH, a housekeeping gene readily amplified. This was necessary since it was not possible to process the sections immediately after cutting and some of the sections had been stored several months prior to extraction.

Amplification conditions and optimisation

Each primer pair were amplified generally in accordance to the optimum conditions previously established at the Leicester laboratory. Initially test breast DNA was amplified using these conditions, 15μl of the product run on a 2% Nu sieve agarose gel in TBE buffer at 80V for 1.5 - 2hr hrs, and visualised by staining with ethidium bromide. The set up and PCR conditions were modified if necessary until a sharp band was obtained.

The typical PCR reaction was as follows

10μl DNA extract

6μl reaction buffer

1μl forward primer (10pm/μl)

1μl reverse primer (10pm/μl)

1.5μl Taq (1unit/μl)

water to total reaction volume of 60μl

The amplification conditions are outlined in table 4.6

Table 4.6 **Optimised cycling stage parameters**

Stage	Primer	Conditions
Denaturing	all primers	96°C for 7 mins
Cycling conditions	ERTA, D6S186	(94°C x 1min + 56°C x 1 min + 72°C x 1.20 min) 35 cycles
	D6S193	(94°C x 1min + 57°C x 1 min + 72°C x 1.20 min) 35 cycles
	D16S413	(94°C x 1min + 65°C x 1 min(minus 0.5°C each cycle) + 72°C x 1.20 min) 10 cycles +(94°C x 1min + 60°C x 1 min + 72°C x 1.20 min) 25 cycles
	P53(AAA AAT)	(94°C x 1min + 64°C x 1 min + 72°C x 1.20 min) 35 cycles
	GAPDH	(94°C x 1min + 60°C x 1 min + 72°C x 1.50 min) 35 cycles
Elongation	all primers	72°C for 7 mins followed by a hold at 4°C

Testing for LOH

Once conditions for each primer had been optimised and sample quality had been verified crude extracts from the samples were amplified with each of the 5 primer pairs. Five sample pairs, comprising normal and tumour DNA extract from each individual and a negative control (with no DNA template added) were amplified in each PCR run and the products run on 2% Nuseive agarose gels, TBE buffer at 80v for 2hrs, to check for evidence of amplification.

The amplified sample product was then run on 12% non denaturing 19:1 polyacrylamide gel at 4°C and 8Watts in 1xTBE buffer, using the BioRad Protean II apparatus. Approximately 60μl of sample and loading buffer (2:1) was loaded into each well, and a molecular size ladder was included on each gel.

4.4.6 DNA VISUALISATION

The position of the amplified DNA was visualised on the gel by staining with ethidium bromide (0.5µg/ml) for 30-35 mins. Photographs were then taken by AbduRahiman Pattani using the a Polaroid 545 Land Camera and black and white polaroid films (Polaroid Positive-Negative film 55). The negatives were treated with 18% sodium sulphite solution and rinsed thoroughly with distilled water for five minutes.

4.4.7 ANALYSIS

Interpretation of gels

The position of the bands in each sample pair was examined and scored either as
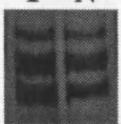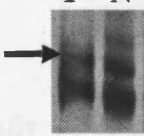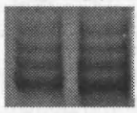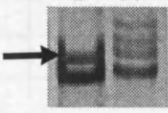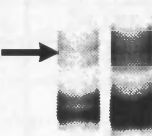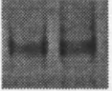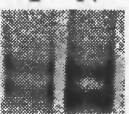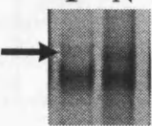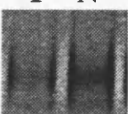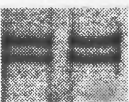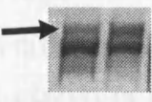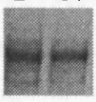
1. Non informative (NI) where the normal DNA was homozygous for the marker.

2. Retained (R) where neither bands were lost or reduced.

3. Heterozygosity (LOH) where a band was lost or markedly reduced in intensity.

Examples, for each of the primers tested is presented in fig 4.1. Scoring of LOH is prone to a high degree of inter observer variability and it is therefore advisable to have at least two persons scoring the gels independently to confirm results. However since no one was available in Jeddah to act as a second scorer the following procedure was adopted to help increase the reliability of the results. Scoring was done up to four times, on the gel, the print, and the negative, and finally with a densitometric reading of the negative. Due to the signal strength and or quality of the negative it was not possible to perform densitometric analysis on all samples. However those samples which did not produce clear results, at least on the gel, were repeated.

Analysis

• Summary tables of loss of heterozygosity, and informativeness were compiled, and overall LOH with respect to informativeness was compared using χ^2 analysis.

• Cases were assessed by the number of primers showing LOH in relationship to the number of informative markers. The distribution of LOH with respect to number of informative sites was compared between the three groups using Pearsons correlation coefficient using Minitab 11.

• Pearsons correlation coefficient was calculated between the three markers tested on 6q for each of the study groups and for pooled data from all three groups.

• Age, grade, lymph node status, and size which have been found in some earlier studies to vary significantly with LOH at various loci were analysed with respect to age and ethnic group. Pooled data from 6q, data from 16q and 17p were all tested.

Fig 4.1 **Examples of LOH and informativity for each of the loci analysed**

Primer	Retained	LOH	Non-informative
D6S186	T N	T N	**non present**
D6S193	T N ratio = 1.02	T N	**non present**
ERTA	T N ratio = 1.2	T N ratio = 2.7	T N
D16S413	T N	T N ratio = 3.8	T N
p53	T N	T N ratio = 2.0	T N

→ LOH

4.5 RESULTS

4.5.1 SUMMARY DATA

Table 4.7 presents the summary data for the molecular study. Percentage informativity varied from primer to primer and population to population. The percentage of LOH is lowest for all the markers in the AL group. AA has the highest LOH for markers D6S186 and D16S413, with EL having the highest percentage LOH for the other markers.

Table 4.7 **Summary data for molecular study**

Group	Primer	(n)	loh/inf	%LOH	%inf	Total LOH /Total inf
AA	erta	(95)	33/80	41.25	84.21	0.36
	D6S193	(93)	24/69	26.00	100.00	
	D6S186	(95)	19/76	20.00	100.00	
	P53	(95)	28/44	63.64	46.32	
	D16S413	(94)	36/76	47.37	80.85	
AL	erta	(35)	10/28	35.71	80.00	0.27
	D6S193	(34)	8/26	24.00	100.00	
	D6S186	(34)	6/28	18.00	100.00	
	P53	(35)	7/16	43.75	45.71	
	D16S413	(35)	4/20	20.00	57.14	
EL	erta	(47)	19/39	48.72	82.98	0.36
	D6S193	(45)	12/33	26.67	100.00	
	D6S186	(46)	7/39	15.22	100.00	
	P53	(47)	21/29	72.41	61.70	
	D16S413	(47)	15/45	33.33	95.74	

A χ^2 test of the total LOH/total informative for each group resulted in a calculated χ^2 value of 2.27 at 2 degrees of freedom which is less than the critical value of 5.99, indicating that there is no significant difference between the three groups.

4.5.2 ASSESSMENT OF THE NUMBERS SHOWING LOH IN RELATIONSHIP TO THE NUMBER OF INFORMATIVE MARKERS.

No cases were totally uninformative (0/0) and few had total LOH and were totally informative (5/5) (table 4.8). More cases of AA (38.95%) and AL(48.57%) had LOH at one site rather than two (AA 25.6%, AL 17.14%), whereas EL had 38.3% of cases with LOH at two sites compared with 23.4% with LOH at only one site.

Table 4.8   Primers showing LOH in relationship to the number of informative markers

	Primers showing LOH in relationship to the number of informative markers (loh/informative markers)																			
Group	0/0	0/1	0/2	0/3	0/4	0/5	1/1	1/2	1/3	1/4	1/5	2/2	2/3	2/4	2/5	3/3	3/4	3/5	4/4	4/5	5/5
AA	0	0	2	5	9	1	0	2	7	17	11	0	2	16	6	0	5	9	0	2	1
AL	0	0	3	2	4	1	0	1	6	7	3	0	0	3	3	0	1	1	0	0	0
EL	0	0	1	3	4	2	0	0	2	5	4	0	1	6	11	0	1	4	0	3	0

Pearsons correlation analysis on this data results in:

The critical value is 0.44 at 18 df, correlation of AA and AL equals 0.6 (significant), AA and EL 0.8 (significant), and AL and EL 0.38, failed to reach statistical significance.

4.5.3 ANALYSIS OF SELECTED CLINICO-BIOLOGICAL PARAMETERS WITH MARKER SPECIFIC LOH

Age, grade, lymph node status and tumour size <2cm>, were further analysed within each of the study groups for the markers on 6q, 16q, and 17p

6q

Correlation between LOH and the three markers on 6q

Table 4.9 presents the results of Pearsons correlation between LOH or no LOH in each of the three markers tested on 6q for each of the study groups and for the pooled data of all three study groups. No significant correlation was found between the three markers tested on chromosome 6.

Table 4.9   **Pearsons correlation between LOH or no LOH in each of the three markers tested on 6q**

Group	ERTA+D6S193	ERTA+D6S186	D6S193+D6S186	Comparison with tabulated critical value
AA	-0.19	0.01	-0.08	< 0.35 at 26 df not significant
AL	0.1	-0.09	-0.39	< 0.58 at 10 df not significant
EL	-0.42	0.17	0.42	< 0.81 at4 df not significant
pooled data	-0.13	0.02	-0.09	< 0.35 at 46 df not significant

In the assessment of LOH with clinico-biological parameters all three markers were considered together as presence or absence of LOH on chromosome arm 6q.

LOH with respect to age was also assesed for each marker individually.

Table 4.10 **LOH at 6q in different age groups**

Group	Age group	LOH	No LOH	% LOH	χ^2 analysis compared to critical value of 3.8	
AA	All	57	34	62.6		
	pre menopausal post menopausal	38 19	15 19	71.7 50.7	4.5	Different
	under 44 years over 44 years	26 31	13 21	66.7 59.6	0.5	Same
AL	All	20	13	60.6		
	pre menopausal post menopausal	10 10	7 6	58.8 62.5	0.05	Same
	under 44 years over 44 years	6 14	4 9	60 60.8	0.002	Same
EL	All	29	16	64.4		
	pre menopausal post menopausal	14 15	9 7	60.8 68.2	0.26	Same
	under 44 years over 44 years	11 18	7 9	61 66.6	0.15	Same

Table 4.11 **LOH at individual markers on 6q in different age groups**

Marker	Group	Age group	LOH	No LOH	% LOH	χ^2 analysis compared to critical value of 3.8	
ERTA	AA	pre menopausal post menopausal	23 10	22 25	51 28	2.75	same
		under 44 years over 44 years	17 16	17 30	50 34.8	1.86	same
	EL	pre menopausal post menopausal	8 12	10 10	44.4 54.5	0.4	same
		under 44 years over 44 years	4 16	10 10	28.6 61.5	3.96	different
D6S193	AA	pre menopausal post menopausal	19 5	20 22	48.7 18.5	6.28	different
		under 44 years over 44 years	15 9	14 28	51.7 24.3	5.27	different
	EL	pre menopausal post menopausal	8 4	8 9	50 30.7	1.09	same*
		under 44 years over 44 years	7 5	5 12	58.3 29.4	2.43	same*
D6S186	AA	pre menopausal post menopausal	11 8	15 12	42.3 40	0.03	same
		under 44 years over 44 years	6 13	11 16	35.3 44.8	0.4	same
	EL	pre menopausal post menopausal	4 4	6 5	40 44	0.04	same
		under 44 years over 44 years	4 4	6 5	40 44	0.04	same

* small number of cases

Table 4.12LOH at 6q in different age groups with reference to selected clinico-pathological parameters

	AA			AL			EL		
	loh	no loh	% loh	loh	no loh	% loh	loh	no loh	% loh
all									
ln+	28	19	59.6	10	8	55.5	13	9	59
ln-	11	9	55	8	4	66.6	15	7	68
pre-menopausal									
ln+	20	7	74	6	7	46	5	5	54.5
ln-	8	5	61.5	3	0	100	8	4	66.6
post-menopausal									
ln+	8	12	40	4	1	80	8	4	66.6
ln-	3	4	42.8	5	4	55.5	7	3	70
under 44									
ln+	13	7	65	5	4	55.5	3	5	37.5
ln-	5	3	62.5	1	0	100	7	2	77.8
over 44									
ln+	15	12	55.5	5	4	55.5	10	4	71.4
ln-	6	6	50	7	4	63.6	8	5	61.5
all									
<2cm	9	4	69	3	1	75	14	8	63.6
>2cm	35	22	61.4	16	10	61.5	14	8	63.6
pre									
<2cm	5	2	71	1	0	100	6	5	54.5
>2cm	24	9	72.7	9	7	56	7	4	63.6
post									
<2cm	4	2	66.7	2	1	66	8	3	72.7
>2cm	11	13	45.8	7	3	70	7	4	63.6
under 44									
<2cm	2	2	50	1	0	100	5	4	55.6
>2cm	17	8	68	5	4	55.5	5	3	62.5
over 44									
<2cm	7	2	77.8	2	1	66.7	9	4	69.2
>2cm	18	14	56	11	6	64.7	9	5	64.3
all									
grade 1&2	15	5	75	10	5	66.7	11	11	50
grade 3	31	25	55.4	10	8	55.6	18	5	78
pre									
grade 1&2	12	4	75	4	1	80	4	5	44.4
grade 3	21	10	67.7	6	6	50	10	4	71
post									
grade 1&2	3	1	75	6	4	60	7	6	53.8
grade 3	10	15	40	4	2	66.7	8	1	88.8
under 44									
grade 1&2	6	3	66.6	3	1	75	3	4	42.8
grade 3	17	9	65.4	3	3	50	8	3	72.7
over 44									
grade 1&2	9	2	81.8	7	4	63.6	8	7	53.3
grade 3	14	16	46.6	7	5	58.7	10	2	83.3

Age

- Comparing combined markers

  - There was a significant difference in LOH 6q between pre and post menopausal cases in the AA study group

  - Generally AA had a higher percentage of LOH in the younger groups. In AL and EL there was higher LOH in the older groups.

  - There was no significant difference when the three study groups were compared for any of the age groupings

- Comparing individual markers

  - ERTA showed more LOH in the older groups of EL and significantly less in the under 44 years, whereas AA had more LOH in the younger groups.

  - Both AA and EL had more LOH at D6S193 in the younger groups. The difference was significant in AA.

  - D6S186 showed little difference with age in either AA or EL.

Lymph node status

- There was no significant difference in LOH with node status for any of the study groups for combined ages.

- In AA there was a significant difference between the frequency of LOH found in LN+ tumours in the pre menopausal cases compared to post menopausal. (χ^2 =5.5, d.f.=1, p<0.025) pre menopausal cases had more LOH.

- LN+ tumours in the AL and EL tended to have more LOH in the older groups.

Size

- For combined age groups there was no significant difference with size for any of the study groups.

- A significant difference was found for LOH in tumours over 2cm between the pre and post menopausal groups in AA, (χ^2 =4.2, d.f.=1, p<0.05), pre menopausal cases exhibiting more LOH. Whereas in EL LOH for both pre and post menopausal, over 2cm, were the same, although it should be noted that the number of cases was small.

Grade

- For combined ages, EL showed significantly more LOH in the grade 3 cases than the grades 1&2 (χ^2 =3.9, d.f.=1, p<0.05). AA and AL had more LOH in grades

1&2 than in grade 3 cases, although the differences were not statistically significant.

- In grade 3 cases in AA there was significantly more LOH in the pre menopausal age group than the post menopausal group (χ^2=4.3, d.f.=1, p<0.05).

16q

Table 4.13 **LOH at 16q in different age groups**

Group	Age group	LOH	No LOH	% LOH	χ^2 analysis compared to critical value of 3.8	
AA	All	36	40	47.4		
	pre menopausal	18	27	40	2.4	Same
	post menopausal	18	13	58.1		
	under 44 years	12	20	37.5	2.16	Same
	over 44 years	24	20	54.5		
AL	All	4	16	20		
	pre menopausal	2	8	20	0	Same
	post menopausal	2	8	20	(numbers small)	
	under 44 years	0	5	0	contains cell with zero cases	
	over 44 years	4	11	26.7		
EL	All	15	30	33.3		
	pre menopausal	6	17	26.1	1.11	Same
	post menopausal	9	13	40.9		
	under 44 years	4	14	22.2	1.67	Same
	over 44 years	11	16	68.8		

Age

- AA, AL and EL all showed greater LOH at 16q in the older age groups but differences were not significant.

- There was a significant difference between AA and AL overall (χ^2=4.87, d.f.=1, p<0.05), and in the post menopausal (χ^2=4.4, d.f.=1, p<0.05) and over 44 years age groups.

Table 4.14 LOH at 16q in different age groups with reference to selected clinico-pathological parameters

	AA			AL			EL		
	loh	no loh	% loh	loh	no loh	% loh	loh	no loh	% loh
all									
ln+	17	24	41	3	10	23	11	12	47.8
ln-	8	10	44	1	4	20	3	18	14.3
pre-menopausal									
ln+	7	18	28	1	7	12.5	4	7	36.4
ln-	5	6	45.5	1	0	100	1	10	9
post-menopausal									
ln+	10	6	62.5	2	3	40	7	5	58
ln-	3	4	42.9	0	4	0	2	8	20
under 44									
ln+	3	14	17.6	0	5	0	3	6	33.9
ln-	3	4	42.9	0	0	-	0	8	0
over 44									
ln+	14	10	58.3	3	5	37.5	8	6	57
ln-	5	6	45.5	1	4	20	3	0	23.1
all									
<2cm	5	3	62.5	0	2	0	2	19	9.5
>2cm	23	28	45.1	4	12	25	12	10	54.5
pre									
<2cm	3	1	75	0	1	0	0	5	0
>2cm	9	20	31.3	2	7	22	10	7	58.8
post									
<2cm	2	2	50	0	1	0	2	9	18.2
>2cm	14	8	63.6	2	5	28.6	7	4	63.6
under 44									
<2cm	2	1	66.6	0	1	0	0	8	0
>2cm	4	16	20	0	4	0	3	6	33
over 44									
<2cm	3	2	60	0	1	0	2	11	15.4
>2cm	19	12	61.3	4	8	33.3	9	5	64.3
all									
grade 1&2	6	10	37.5	1	10	9	6	17	26
grade 3	25	26	49	3	6	33	9	13	41
pre									
grade 1&2	5	9	35.7	0	5	0	3	7	30
grade 3	11	16	68.7	2	3	40	3	10	23
post									
grade 1&2	1	1	50	1	5	16.7	3	10	23
grade 3	14	10	58.3	1	3	33.3	6	3	66.7
under 44									
grade 1&2	3	6	33.3	0	4	0	3	5	37.5
grade 3	8	13	38.1	0	1	0	1	9	10
over 44									
grade 1&2	3	4	75	1	6	14.3	3	12	20
grade 3	17	13	56.7	3	5	37.5	8	4	66.7

Lymph node status

- EL had more cases showing LOH at 16q in lymph node positive cases compared to lymph node negative cases (χ^2=5.69, d.f.=1, p<0.025). Neither AA or AL showed a significant difference.

- AA, AL and EL had a higher percentage of LOH in node negative cases compared to node positive cases for the younger groups. However older groups all showed a higher percentage of LOH in the lymph node positive tumours.

- In AA there was very little difference between the age groupings for the percentage of LOH in lymph node negative tumours. However there were significant differences in the lymph node positive cases between pre and post menopausal cases (χ^2=4.78, d.f.=1, p<0.05), and cases under and over the age of 44 (χ^2= 6.78, d.f.=1, p<0.025).

Size

- EL showed significantly more LOH at 16q in tumours over the size of 2cm (χ^2= 9.2, d.f.=1, p<0.05). AA however had a higher percentage of LOH in tumours less than 2cm, although the difference with size was not significant.

- Both AL and EL had more LOH in the cases with tumours over 2cm (the numbers in AL are very small).

- The older age groups in AA had more frequent LOH in the larger tumours whereas for the younger groups it was in the smaller tumours. There was significantly more frequent LOH in tumours over 2cm in both the post menopausal (χ^2=5.37, d.f.=1, p<0.025) and over 44 years (χ^2=8.37, d.f.=1, p<0.005) age groups compared to the younger groups.

Grade

- There was no significant difference in LOH between grade 1&2 and grade 3 tumours, when all ages were considered together.

- AA and AL showed more LOH in the grade three cases. There was no assessment of AL under 44 years due to the absence of LOH.

- EL had more LOH in grade 3 cases in the older groups but more LOH in the younger groups in grade 1&2 tumours.

17p

Table 4.15 **LOH at 17p in different age groups**

Group	Age group	LOH	No LOH	% LOH	χ^2 analysis compared to critical value of 3.8
AA	All	28	16	63.6	
	pre menopausal	17	8	68	0.13 Same
	post menopausal	11	8	57.9	
	under 44 years	16	2	88.9	8.39 Different
	over 44 years	12	14	46.2	
AL	All	7	9	43.8	
	pre menopausal	1	5	16.7	2.86 Same
	post menopausal	6	4	60	(small numbers)
	under 44 years	1	2	33	0.16 Same
	over 44 years	6	7	46.2	(small numbers)
EL	All	21	8	72.4	
	pre menopausal	10	3	76.9	0.24 Same
	post menopausal	11	5	68.8	(small numbers)
	under 44 years	8	1	88.9	1.77 Same
	over 44 years	13	7	65	(small numbers)

Age

- In AA and EL more frequent LOH at p53 was seen in the younger age groups, and the difference in AA under and over 44 years was statistically significant (χ^2=8.39, d.f.=1, p<0.005).

- AL had more LOH in older age groups than younger groups (numbers of cases small).

- Both AA and EL showed a high percentage of LOH in the under 44 years group (88.9%).

Table 4.16 **LOH at 17p in different age groups with reference to selected clinico-pathological parameters**

	AA			AL			EL		
	loh	no loh	% loh	loh	no loh	% loh	loh	no loh	% loh
all									
ln+	15	10	60	2	6	25	10	5	66.7
ln-	7	0	100	5	1	83	10	3	76.9
pre-menopausal									
ln+	9	6	60	1	5	17	4	2	66.6
ln-	4	0	100	0	0	_	5	1	83.3
post-menopausal									
ln+	6	4	60	1	1	50	6	3	66.6
ln-	3	0	100	5	1	83	5	2	71.4
under 44									
ln+	9	1	90	1	2	33	4	1	80
ln-	3	0	100	0	0	_	3	0	100
over 44									
ln+	6	9	40	1	4	20	6	4	60
ln-	4	0	100	5	1	83	7	3	70
all									
<2cm	1	2	33	1	1	50	7	5	58
>2cm	20	8	71.4	5	6	45.5	13	3	68
pre									
<2cm	1	1	50	0	1	0	2	2	50
>2cm	11	4	73	1	4	20	7	1	87.5
post									
<2cm	0	1	0	1	0	100	5	3	62.5
>2cm	9	4	69.2	4	2	66.7	6	2	75
under 44									
<2cm	0	0	_	0	1	0	1	1	50
>2cm	11	1	91.6	1	1	50	6	0	100
over 44									
<2cm	1	2	33	1	0	100	6	4	60
>2cm	9	7	56.2	4	5	44.4	7	3	70
all									
grade 1&2	4	3	57	4	4	50	12	4	75
grade 3	19	9	67.8	3	5	37.5	9	4	69
pre									
grade 1&2	4	1	80	0	2	0	5	1	83
grade 3	10	6	62.5	1	3	33	5	2	71.4
post									
grade 1&2	0	2	0	4	2	66.7	7	3	70
grade 3	8	3	72.7	2	2	50	4	2	66.6
under 44									
grade 1&2	3	0	100	0	1	0	4	0	100
grade 3	10	2	83.3	1	1	50	4	1	80
over 44									
grade 1&2	1	3	25	4	3	57	8	4	66.6
grade 3	9	7	56.3	2	4	33	5	3	62.5

Lymph node status

- AL had more frequent LOH in lymph node negative tumours when all ages were considered together (numbers too small for statistical analysis). EL also showed more LOH in lymph node negative cases but the difference was not statistically significant. Analysis was not possible for AA since there were no lymph node negative cases without LOH.

- In all age groups in AA, AL and EL lymph node negative tumours had a higher percentage of LOH.

- For AA and EL percentage LOH in lymph node positive cases appeared to be similar in post and pre menopausal cases, but higher in the lymph node positive cases under the age of 44 years compared with those over 44 years (numbers were too small to perform a χ^2 analysis).

Size

- There was no significant difference in LOH at p53 with size when all ages were considered together.

- For all age groupings of AA and EL there was more LOH in tumours over 2 cm. In AL only younger age groups had more LOH in the larger tumours (very small number of cases).

Grade

- For combined age groups, AA had a higher percentage of LOH at p53 in grade 3 tumours whereas both AL and EL had more frequent LOH in grade 1&2 tumours. Numbers were too small to allow χ^2 analysis.

- For all age groupings in EL there was a higher percentage of LOH in grade 1&2 tumours. Younger age groups of AA also had a higher percentage of LOH in grade 1&2 tumours, but older age groupings had more LOH in grade 3 tumours. The opposite was observed in AL, with more LOH in grade 3 tumours in younger age groups, and more LOH in grade 1&2 tumours in the older age groups (number of cases was small).

4.6 DISCUSSION

Differences in clinico-biological markers studied between the three study groups with respect to age and/or ethnicity have been demonstrated in chapter 3. The aims of this chapter were to set-up non isotopic techniques to screen for LOH. In order to enable an investigation of possible genetic variation with respect to age and ethnic origin.

4.6.1 METHODOLOGY

Preparation of DNA template

Purified DNA was used in preliminary experiments, but after testing for reproducibility crude extracts were used in all later experiments (section 4.4.4). The use of crude extracts reduced the preparation time by more than half and therefore reduced the time required to process the samples. Crude extracts appeared to inhibit the PCR reaction slightly causing a lower yield. However this was overcome by increasing the amount of product loaded, and the value of the time saved resulted in crude extracts being the template of choice.

Signal detection

Three main factors influenced the selection of a non-isotopic protocol : Firstly non-isotopic methods carry less risk than those utilising radioactive isotopes. This was particularly relevant since the laboratory where I did my experiments was not at that time using any radio-active sources and consequently the necessary safety and disposal protocols for handling radioactive compounds were not present. The ability to obtain a regular supply of radioactive isotopes in good condition was also a factor. Most molecular grade chemicals need to be imported, and estimated delivery times of 10-24 weeks are the norm for non-isotopic material. Radioactive material would take at least as long. Finally a non- isotopic protocol enables the use of an oil free PCR protocol using small thin walled eppendorf tubes. These tubes allow for shorter ramp times and faster PCR runs, but are unsuitable for use with radiolabelled compounds.

An additional advantage was that avoiding the use of radio labelled nucleotides allowed the use of non-denaturing PAGE (section 4.1.6). It was found that this made it easier to use double gels on the Bio-Rad Protean II apparatus, with a large number of wells, since the need to maintain the sample in the denatured state during running was avoided. Although non-denaturing gels take longer to run, more samples could be processed.

4.6.2 RANDOMISATION OF SAMPLES

Great care was taken to analyse a mixture of cases from Jeddah and Leicester at the same time; starting from the digestion process through to gel interpretation, and to use a labelling method that anonymised them. Therefore during the PCR and gel analysis it was not possible to identify the age or ethnic origin of the samples so reducing the possible bias in gel interpretation.

4.6.3 FINDINGS

Summary data

Percentage informativity for each marker varied between the three study populations, this being a reflection of different patterns of polymorphisms within the populations. Percentage informativity was notably high (100%) for markers D6S186 and D6S193 in all three study populations. Similar differences can be seen in the literature eg. percentage informativeness of marker ESR is quoted as 82% (del Senno et al, 1992), 70.2% (Iwase et al, 1995), and 56% (Chappell et al, 1997).

Although χ^2 analysis showed no significant difference in the overall percentage of LOH between the groups, LOH of individual markers varied between groups.

ERTA

EL had the highest percentage LOH (48.72) and AL the lowest (35.7) with AA having an intermediate value (41.25). The figures are in a similar range to the study of early invasive tumours (40.6%) (Chappell et al, 1997) but are higher than the 19.1% reported by Iwase et al (1995).

D6S193

Percentage LOH ranged from 24% in AL to 27% in EL, AA again being intermediate at 26%. These are similar to the percentages reported in the literature of 27.5 % (Orphanos et al, 1995), 39%, but are lower than those reported by Chappell et al (1997).

D6S186

AA had the highest LOH (20), EL the lowest (15), with AL intermediate (18), which are similar to the 20% described by Orphanos et al (1995) but lower than the 29% recorded by Chappell et al (1997).

D16S413

AL exhibited 20% LOH , EL 33.3% and AA 47%. The latter two compare with 46% LOH at D16S413 reported by Skirnisdottir et al (1995) and 36% for early invasive tumours (T.Walsh Doctoral Thesis, University of Leicester).

p53

AL had a LOH of 43.8%, AA 63.6% and EL the highest at 63.6%. A range of results have been found by others at the p53 locus 68% (Munn et al, 1996), 45.9 % (Kirchweger et al, 1994), and 29.3% (Schmutzler et al, 1997).

Considering that studies in the literature vary from those presented in this thesis, both in terms of age structure and /or ethnic make up, the results from within the three study groups are similar to those published. However this gives no information about the distribution of LOH with respect to the number of informative markers. Pearsons correlation analysis found a correlation between AA and AL, and AA and EL, and between AL and EL. Which might be expected, since if there are more informative cases the chances of having LOH are greater. AA and AL having the closest correlation.

An interesting observation was that EL had more cases with 2 instances of LOH whereas AA and AL had more cases with LOH at only one marker. Increased incidence of LOH has been correlated with greater tumour size (Nagayama and Watatani, 1993; Kerangueven et al, 1997), suggesting a time factor allowing more growth, genetic instability and tumour progression. However I demonstrated in chapter 3 that AA and AL tumours are bigger than those found in EL. This suggests possible genetic differences between the three study groups. The nature of these differences would require further investigation with more cases and a larger panel of markers.

Correlation of LOH for different markers on chromosome arm 6q

Since no correlation was found between LOH for the markers in the primers tested on chromosome arm 6q it was assumed that LOH at these three sites occurs independently. Data from the three loci was pooled to form combined LOH for chromosome arm 6q.

LOH and selected clinico-biological parameters

Age

AL and EL all exhibited a higher frequency of LOH at 16q in the older age groups, although the differences failed to reach significance. Relationship with age varied between markers. For example, at p53 LOH in AA and EL was higher in the younger age groups especially in the under 44 years age group. Apparent ethnic differences also exist, with AL and EL having higher LOH at 6q in the older age groups, whereas AA had more LOH premenopausally , although all fail to reach statistical significance.

Examining the three markers separately LOH at ERTA showed the same pattern as for the combined markers. D6S193 however had more LOH in younger age groups in both AA and EL, suggesting age related differences at a molecular level irrespective of study group. Whereas D6S186 appeared to show little difference in LOH with age for either AA or EL. Findings for EL at the ERTA locus concur with those of Iwase et al (1995). This study, patients from London, found significantly more LOH at ESR in postmenopausal cases compared to premenopausal.

Lymph node status

For LOH at 6q there was no significant difference between LN+ and LN- when all ages were considered together. Other studies also found no significant difference in LOH at 6q when LN+ and LN- tumours were compared (Iwase et al, 1995; Noviello et al, 1996), although Devilee et al (1991) did find an association between the number of involved lymph nodes and LOH at 6q.

There was however a significant difference in LOH at 16q for EL (χ^2=5.69, d.f.=1, p<0.025) with LN+ tumours having more LOH. However AA and AL showed no significant difference, which concurs with Takita et al (1992) Tsuda et al (1994), and Iida et al (1997) who in studies from Japan found no association between LOH at 16q 24 and lymph node metastasis. Although four studies are an insufficient number to draw any definite conclusions the possibility that the relationship between LOH at 16q and lymph node metastasis is different between high and low incidence populations deserves consideration and further investigation.

In all three study groups, at all ages, lymph node negative tumours had more LOH at p53 than node positive tumours; numbers were too small to allow statistical analysis. Sato et al (1990) and Nagai et al (1994) found an association between LOH on 17p and lymph node positive tumours, but both used the marker D17S5 which is distal to the p53 gene locus. Nagayama and Watatani (1993) however using a marker within the p53 locus found lymph node negative tumours to have more LOH than lymph node positive tumours, this finding is not inconsistent with my results.

Considering LN+ tumours only, AA had significantly more frequent LOH at 6q premenopausally than post menopausally (χ^2=4.7, d.f.=1, p<0.05) where as AL and EL had more LOH in the older age groups but this was not significant. All LN+ tumours had more LOH at 16q in the older ages compared to the young, and this difference reached significance for AA (χ^2=4.78, d.f.=1, p<0.05, pre/post menopausal, χ^2=6.68, d.f.=1, p<0.01, under/over 44 years). It was LN+ tumours in the under 44 years of age

group however that showed the highest frequency of LOH at p53 in all the study groups.

Tumour size

No significant differences were found between LOH at 6q in tumours less than 2cm and those over 2cm, similar results were found by Iwase et al (1995) for LOH at ESR. In AA tumours over 2cm significantly more cases of LOH occurred in premenopausal patients than post menopausal ones , but the difference failed to reach statistical significance.

The cases from EL had significantly more LOH at 16q in tumours over 2cm (χ^2 =9.2, d.f.=1, p<0.005), with the same trend being seen in AL, although the numbers were too small to make an analysis. Younger patients in AA had more LOH in smaller tumours, but older patients exhibited significantly more LOH larger tumours (post menopausal χ^2=5.4, d.f.=1, p<0.025, over 44 years χ^2=8.4, d.f.=1, p<0.005). Several groups (Nagayama and Watatani, 1993; Tsuda et al, 1994; Hansen et al, 1998) found no association between LOH at 16q and but although all studied the region 16q24, the marker 16S413 was not assessed. Iida et al (1997) also failed to find an association, however although D16S413 was studied its results were pooled with other 16q markers for analysis.

LOH at p53 appeared to occur more frequently in larger tumours but differences with size were not significant which is consistent with the results described by Nagayama and Watatani (1993) and Nagai et al (1994).

Grade

Examination of LOH at 6q found significantly more in grade 3 tumours (χ^2 =5.2, d.f.=1, p>0.025) for EL cases, although AA and AL tended to have more LOH in grades 1 and 2. Noviello et al (1996) found no correlation with LOH at 6q and grade, however the loci studied, although in the same region, were not those used in this study. Iwase et al (1995) in a study of patients from Guy's hospital also found significantly more LOH at ESR in grade 3 tumours. Unfortunately, my data for LOH 6q is pooled from three loci, there being insufficient cases to consider individually, so a direct comparison with the results of Iwase cannot be made. However the markers studied include ones relating to the ER gene. There is a recognised association between loss of ER expression and grade (Maynard et al, 1978; Winstanley et al, 1991). Chappell et al (1997), in a selected group of predominantly grade 1 and grade 2 carcinomas did not find a relationship between LOH and ER status. Unfortunately, data was not available for ER status for all cases so a correlation was not possible. In

view of the differences between EL, AL, and AA it would be of value to compare ER status in the three ethnic groups.

At p53 AL and EL had a greater frequency of LOH in grade 1 and 2 tumours whereas AA had a higher percentage in grade 3. However, due to the low informativity rate there were too few cases to allow an accurate statistical analysis. The findings for AA varied with age, since the younger age groups had more LOH in grades 1 and 2.

Results from previous studies have varied possibly reflecting tumour heterogeneity. Niederacher et al (1997) described a positive correlation between LOH at p53 and high grade whereas Schmutzler et al (1997), failed to find this correlation. Schmutzler et al (1997) however did report an association between LOH at loci distal to p53 and high grade, but Varley et al (1991) analysing LOH the same region failed to find a correlation with grade.

Recent publications (Hansen et al, 1998; Roylance et al, 1999) have described an association between LOH at 16q and low histological grade, although others in earlier studies have failed to show this relationship (Tsuda et al, 1994). My study also failed to find a relationship between LOH at 16q and low grade, except in the younger age groups of EL. When all age groups were combined there was no significant difference in LOH with respect to grade (grades 1 and 2 compared to grade 3). Generally AA and AL had more LOH in grade 3 cases, and this was also seen in the older cases of EL. Several factors may contribute to the apparent inconsistency in results. These include:

a. the classification of high and low grade. Some studies compared only grade 1 with grade3 (Roylance et al, 1999), others grade 1 with grades 2 and 3 (Hansen et al, 1998), whereas I, due to the low number of grade 1 cases, compared grade 1 and 2 to grade 3.

b.the markers analysed on 16q vary

c.results from CGH studies represent a relatively large region on the chromosome not restricted to a single marker.

d. tumour heterogeneity can not be excluded, and to examine this extensive microdissection of individual tumours would be required.

The occurrence of LOH at 6q, 16q, and 17p in DCIS is indicative of their involvement in early stages of tumourigenesis in breast carcinoma (Fujii et al, 1996; Munn et

al, 1996; Chappell et al, 1997; Buerger et al, 1999). Chappell et al (1997) found no association between LOH at 6q and differentiation in DCIS, and Fujii et al (1996) failed to find any at p53, or 16q. However Buerger et al (1999) did find an association between losses at 16q at well differentiated DCIS, and suggested that such losses might represent a sub type of DCIS. The fact that Buerger et al (1999) used CGH, which analyses all sites rather than selected loci, may account for the variation in results.

4.6.4 Conclusion

In view of the number of cases, and those which are informative for LOH analysis, comparisons between AA and EL are more reliable than for AL. However, the findings indicate possible differences at the molecular level between ethnic groups and possibly even greater differences in relation to age.

In general there was a higher frequency of total LOH at the selected markers for EL cases. Interestingly these carcinomas had a lower frequency of large tumour size, nodal metastasis, and high tumour grade.

Correlation of LOH with the selected clinico-pathological features showed differences between the markers and the different ethnic groups.

For 6q there was a higher frequency of LOH in the older age groups for EL and AL, but premenopausally for AA. LOH at D6S186 appeared to be independant of age or ethnic group. The differences observed between AA and EL in LOH with respect to age appearing to be mainly attributable to differences in LOH at ERTA and D6S193 loci. There was no relationship with node status, no difference with size, more LOH in grade 3 EL tumours but more LOH in grade 1 and 2 in AA and AL cancer. Premenopausal cases in AA also showed significantly more LOH when either LN positive, or grade three tumours were considered whereas EL had more LOH in post menopausal cases. The difference in EL between pre and post menopausal patients however was not statistically significant. These observations illustrate the complexities and points to possible differences between EL and AA in relation to alterations on 6q. Some of this apparent complexity may be due to the fact that data was, in part, pooled from three markers, a study with a larger number of patients would enable each of the markers to be considered individually for all three stuudy groups.

Differences also existed between EL and AA for 16q and 17p. There is a higher frequency of LOH at 16q in the older age groups of EL and AL; differences in LOH at 16q in node positive cases for EL but not AA and AL. However, for 16q age seemed important as well, since only premenopausal EL cancers had an association between LOH and grade, whereas LOH was more frequent in grade 3 cancers for AA, AL and

post menopausal EL. LOH was more frequent in tumours over 2 cms for EL, AL and postmenopausal AA but for premenopausal AA it was related to small size.

LOH at p53 in AA and EL occured in a higher frequency in all younger age groups, particularly in the under 44 years of age. This is of interest in view of the recent evidence of an increased incidence of p53 alterations in familial breast cancer due to changes in BRCA1 and BRCA2, and the interplay of these three genes in DNA repair (Marmorstein et al, 1998; Patel et al, 1998; Gonzalez et al, 1999; Venkitaraman, 2000; Welcsh et al, 2000). In addition LOH at p53, BRCA1 and BRCA2 has also been correlated to ER-ve tumours (Schmutzler et al, 1997).

Therefore, potential differences exist at a molecular level both in respect to ethnic origin and age. Larger studies are required, with other markers such as BRCA1 and BRCA2, to test the robustness of the findings and to elucidate inter-relationships further.

CHAPTER FIVE

CONCLUSION

5.1 PROJECT OVERVIEW

5.1.1 Summary of results

A statistically significant difference was demonstrated in chapter two in the age of onset of breast cancer between those populations with the highest and lowest incidence. The association between ethnic origin and level of incidence was also found to be statistically significant, with the countries with lowest incidence coming from Africa and Asia and those with the highest incidence from the Mediterranean and Europe. This distribution appears not to have changed noticeably over a 20 year period.

The Western Region of Saudi Arabia (AA) and Leicester Asians (AL) were both found to have a younger average age of onset when compared to the Leicester European (EL) population. Use of ASIR and calculation of relative risk allowed the comparison of populations with different age structures over different age ranges. The ASIR for AA appears to be typical of that demonstrated for other low incidence countries with a small excess of very young cases, but a lower overall incidence especially in the age range 30-65 years when compared to EL. ASIR for AA was statistically different from EL in both younger and older age groups, and statistically different from AL in the older age group (45-69 years) but not in the younger age group (20-44 years). ASIR for AL was statistically different from EL in the younger age group but not in the older group.

Chapter three studied variation in selected clinico-biological markers with respect to ethnic origin and age. The function of these parameters was to act as surrogate markers of tumour behaviour. Differences were found between AA, AL and EL, with those between AA and EL frequently reaching a level of statistical significance. However it was not possible to describe either AA or EL as having a more aggressive profile. AA showed more aggressive clinico-pathological characteristics, but had the least aggressive characteristics when nuclear morphometry was considered. The converse was true for EL, which had a more aggressive nuclear morphometric profile, but less aggressive clinico-pathological characteristics. VMI showed no significant difference between any of the groups, the recorded rank order does not follow the pattern seen for nuclear morphometric measurements and for clinico-pathological measurements. Except for VMI AL had an intermediate rank order between AA and EL, with AL tending to be closer to AA in the younger age groups and to EL in the older age groups. AA and EL both possessing different aggressive characteristics may go some way to explaining the findings of some studies (Gillett et al, 1997; Ibrahim et al, 1998; Ezzat et al,1999; Merchant et al, 1999) that despite worse clinico-pathological characteristics patients with young onset sporadic breast

cancer and those from low breast cancer incidence populations do not have a worse survival rate than older patients and those from high incidence populations. A proposition which is supported by observations by Baak et al (1992), that when comparing different populations morphometric characteristics and 10 year survival may be independant of clinico-pathological characteristics.

Data from chapter three also revealed a greater degree of homogeneity in AA compared to EL for all characteristics studied except tumour size. These observations provide support for the proposition that there are two types of breast cancer, and that AA consists predominantly of " young type" breast cancer, without significant numbers of "old type" breast cancer, the presence of which would otherwise add variation and increase the overall incidence.

The molecular markers examined in Chapter Four also exhibited differences between AA, AL and EL. If just AA and EL are considered, since AL numbers were generally small, in relation to the frequency of LOH in older and younger age groups, the following patterns were observed:

• LOH at three of the markers appeared to be related to age rather than ethnic group. AA and EL both exhibited more LOH in the younger age groups for markers D6S193 and p53, and more LOH in the older groups at the marker D16S413.

• LOH at ERTA showed differences between AA and EL (AL for both these was the same as EL). AA showed more LOH at ERTA in the young age groups, whereas EL showed more in the older groups.

• LOH at D6S186 appeared to be independant of age and ethnic group in the populations studied.

The results presented support the proposition made by Manton and Stallard (1992) that genetic factors are important in the aetiology of young breast cancer. With reference to the clinico-pathological characteristics of tumours seen in the AA study group, BRCA2 is a possible candidate gene in this population; however the lack of male breast cancer (Stratton et al, 1994) and young prostate cancer (Gayther et al, 2000) does not support this. Similarly no evidence of multiple incidence of breast and/or ovarian cancer makes BRCA1 an unlikely candidate, as is the recently proposed BRCAx gene(s) (Borg et al, 2000; Stratton, 2000) because of lack of an associated low grade in the series studied.

5.1.2 Fulfilment of aims

The results presented support the hypothesis that breast cancer occurring in

different ethnic groups differs in respect to the age of onset and age specific pattern of incidence.

The Western Region population and Leicester Asian population when compared to the Leicester Europeans were found to have a more aggressive profile with respect to clinico pathological markers, but a less aggressive nuclear morphometry. There was no significant difference in VMI.

A PCR based, non-isotopic protocol was set up to examine molecular differences with respect to age and ethnic group.

At a molecular level differences were found to exist both in relation to age and ethnicity, the nature of the differences varying from marker to marker. There was no significant difference in total extent of LOH between the study groups.

5.1.3 Ethical validity

Although appropriate ethical procedure has been used throughout this study, with regards to getting permission and protecting patient confidentiality, even the use of ethnicity to stratify patients has been criticised as being potentially unethical. Bhopal (1997) condemns much of the research comparing ethnic groups as "black box" epidemiology, which frequently only comes to the conclusion that a difference exists. Within this context my study has shown not only that differences exist between AL and EL but also that there are similarities between AA and AL, and has attempted to analyse these with respect to age, and to investigate them at a molecular level. In addition results are related to international variation in age of onset, overall incidence, and ASIR.

For a study to be epidemiologically sound Bhopal (1997) suggested:

1. Care must be taken to compare like with like: Within this project the majority of all three study populations are urban dwellers with access to comparable medical care. The population size of the study regions is also comparable (Leicestershire having approximately the same population as Jeddah).

2. Ethnic groups must be carefully defined: I chose the use of family name to differentiate between Asian and European patients. This approach has been used in earlier studies (Nicoll et al, 1986; Harland et al, 1997; Winter et al, 1999) and is particularly suitable where there is still limited inter marriage.

5.2 PRACTICAL IMPLICATIONS

Accepting sporadic breast cancer as an important disease in the young brings practical implications:

5.2.1 Education

Awareness of breast cancer and that it not only is a disease of the old, together with knowledge of breast self examination, should be cultivated in the general population. This is especially important in those populations that appear to be predisposed to early onset breast cancer. Unfortunately it is many of these populations, including the Middle East and Asia, where traditional values make it difficult to discuss and demonstrate publicly subjects such as breast self examination. However it is encouraging that in Saudi Arabia now, as well as doctors explaining the procedure to patients on an individual basis, secondary school students are also being given lectures and demonstrations on the subject.

Reminding primary health care clinicians, especially those in the West where the majority of the breast cancer patients would be European and post menopausal, to be vigilant to the possibility of breast cancer in both the younger patient and in patients from ethnic minorities.

Related to breast cancer awareness is the need for regular mammographic screening. Some have reported that uptake of screening among ethnic minorities in the West is less than that in the European populations (Botha et al, 1993; McCarthy et al, 1998; Hedeen et al, 1999). Efforts should be made to ensure that all sections of the community are aware of the benefits of screening and are encouraged to utilise the facility.

5.2.2 Mammographic screening

Tumours can be detected by mammography even before they are palpable and detection of smaller tumours often allows less radical surgery and improved survival (section. 1.4.1). Many have advocated aggressive screening at a young age (McCarthy et al, 1998; El-Tamer et al, 1999) especially for African and Asian patients.

Rosenquist and Lindfors (1998) investigated several screening protocols including screening all women 40 -79 years annually and all were found to be cost effective. Although mammographic screening has been criticised as having a very low degree of sensitivity under the age of 50 years (Peer et al, 1996), advances in accurately imaging the younger breast are being made. Lee et al (1999) proposed the use of MR imaging in the evaluation of problematic mammograms and Okamoto et al (1996) and Kossoff (2000) ultrasonographpy in the initial investigation of young breasts.

On balance I agree with Gail and Rimer (1998) that screening of women should be considered from the age of forty, at least in individuals and communities at higher risk for early onset breast cancer from the age of forty years. Tabar et al (1999) state

that screening interval in women 40-49 years of 12-18 months would achieve a similar reduction in mortality as that seen in post menopausal women. Saudi Arabia, with its high proportion of premenopausal breast cancer would probably be a candidate for such a protocol.

5.2.3 Diet

Though it is still not entirely clear what the exact role of diet is in preventing breast cancer, consensus would appear to advocate a reduction of intake of red meat, animal fats, and alcohol, and probably more importantly an increase in fruit and vegetables (and other sources of phyto oestrogens) (Stephens, 1997). Diet may prove to be only a confounding factor or it may not have the same effect on all populations but modifications such as these would be in any case generally advantageous to health.

5.3 FUTURE WORK

If the breast cancer patients in the Western region consist of, as proposed by this thesis, a relatively homogenous population of young type breast cancer then results of research on this population may be applicable to the minority young type breast cancer populations seen in the UK and other high incidence countries. Possibilities for future work could include:

- A larger scale investigation of the differences described between the cases presented in this project. Use of more cases would allow a more detailed analysis and would help eliminate any findings which were in fact artifactual due to low numbers. This would be particularly applicable to the Leicester Asian population.

- Inclusion of more markers, including those associated with metastatic behaviour e.g. 7q31 and 8p (Bieche et al, 1992; Yaremko et al, 1996; Yokota et al, 1999). Other possible choices of loci to be investigated would be BRCA1 and BRCA2. Both have been described as being associated with distinctive phenotypes in familial breast cancer (Breast Cancer Linkage Consortium, 1997) and it would be of interest to see if LOH at either of these sites correlated to differences described in the populations studied in this thesis. A more speculative choice would be that of the Cowden locus 10q 22-23 (Liaw et al, 1997; Marsh et al, 1997; Singh et al, 1998) associated with both increased risk of breast cancer and thyroid cancer. Both breast cancer and thyroid cancer exhibit a high relative risk in both the Saudi and British Asian populations, neither of which can be readily attributed to infective agents (unlike lymphoma which is also common in both populations and where infective agents may have a role in the aetiology).

- More detailed investigations of the loci studied in this project including possibly the expression of cell adhesion proteins and ER receptor status using immuno histochemistry. Cell adhesion proteins are of interest because the AA population appears to be more poorly differentiated (high cellularity values, and high grade) and have a greater ability to metastasise (higher percentage of lymph node positive cases) which indicates a possible alteration in cell:cell and cell:stromal interactions.

- Lawson et al (1999) suggested that low ER expression in normal Japanese breast is directly related to the low incidence of breast cancer in that country. It would be interesting to see if the same differences could be detected here, and also to compare ER expression in tumour samples.

- P53 could also be profitably investigated by comparing allelotypes as outlined by Sjalander et al (1996), and Weston et al (1997) both of whom described ethnic differences. Weston et al (1997) also found pre menopausal / post menopausal differences within their Caucasian study group.

- The relatively high incidence of tumours under 30 years appears to be a feature of populations which otherwise have a low incidence of breast cancer; a larger study would allow these cases to be considered as a sub group. It would be of interest to compare features of this sub group in the three study populations. A long term prospective study would allow a more detailed evaluation of possible family history in this sub group. None of the 51 cases under 30 years of age in AA were reported as having a family history, but it possible that this may have been been denied (or not revealed) by the patient for social reasons. Careful interviewing, together with genetic counselling if required, should help avoid this potential source of error.

5.4 SUMMARY

In brief, data presented in this thesis adds to the weight of evidence that there are ethnic and age differences in the incidence and presentation of sporadic breast cancer and that these differences are probably best explained by a two disease model of breast cancer of the type suggested by DeWaard and Manton and Stallard. Accepting this to be correct I suggest that it is possible that the pattern of breast cancer seen in the Western Region of Saudi Arabia is due not to an excess of young breast cancer (except possibly in the under 44 years) but a relative absence of the older type of breast cancer. Studying breast cancer in Saudi Arabia, and other low incidence countries may give information which can also be applied to young breast cancer world wide.

APPENDIX A

PART 1

SAUDI ASIANS

(AA)

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA22JDE101	5	0	3	5	2.1	7.3	10.8	4.6	44.5	285.7	16.9	81.5	1.39	8.7	0.93
AA24JDB28	2.8		3												
AA25JDC7			3												
AA25JDE29	7	1	3	7.4	3.5			5.8	66.0	564.9	23.8	117.1	1.37	10.5	0.92
AA25JDE33			3			8.6	12.5	3.9	53.8	297.2	17.2	92.9	1.34	9.4	0.94
AA25JDEmi			3					4.9	49.6	372.2	19.3	91.0	1.38	9.2	0.93
AA26JDC5		1	3												
AA28JDB44	3.5	1	3	6.7	3.15										
AA28JDC25		1	2												
AA28JDC27	8	1	3	7.6	3.6										
AA28JDC35			3												
AA28JDC41			3												
AA28JDE36	4	0	3	4.8	2	7.3	20.9	3.7	35.8	147.4	12.1	60.9	1.36	7.7	0.94
AA28JDE37	4	1	3	6.8	3.2	8.9	20.2	4.4	47.8	275.2	16.6	83.4	1.42	9.1	0.92
AA29JDB32	1.3		2												
AA29JDC36		1	3												
AA29JDE102	3	0	2	3.6	1.9	5.7	4.9	5.0	46.7	364.8	19.1	92.1	1.39	8.9	0.92
AA29JDE105		1	3			6.8	16.6	4.4	39.0	213.0	14.6	68.4	1.39	8.1	0.93
AA30JDD3	8	0				8.8	9.6	4.5	47.5	344.0	18.5	89.7	1.31	8.7	0.95
AA30JDE103	4	0	2	3.8	2	7.3	10.1	4.1	46.2	210.0	14.5	75.2	1.38	8.9	0.93
AA30JDE22	6	1	3	7.2	3.4	6.9	18.7	4.1	44.4	233.3	15.3	76.1	1.45	8.9	0.92
AA30JDE26	3.5	1	2	4.7	3.15			4.1	51.7	228.0	15.1	79.4	1.44	9.6	0.92
AA30JDEMF			3					3.6	52.3	203.7	14.3	78.8	1.39	9.5	0.94
AA31JDE40	1.4	1	2	5.28	2.94	6.5	14.8	3.6	35.0	135.5	11.6	61.1	1.33	7.6	0.93
AA31JDE42	6	1	3	6.2	3.4	9.0	22.5	4.7	49.3	355.6	18.9	92.0	1.34	9.0	0.93
AA32JDA02	1.9					9.4	24.5	7.1	66.3	982.8	31.3	130.9	1.31	10.2	0.95

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA32JDB42	4.5	0	2	3.9	2.05										
AA32JDC34			3												
AA33JDA01		1	2			9.4	39.5	4.8	38.5	247.1	15.7	73.4	1.42	8.2	0.93
AA33JDB41	2	0	3	4.4	1.8										
AA33JDC2		1	3												
AA33JDD47	5		3			7.6	24.4	5.5	56.7	453.1	21.3	101.9	1.55	10.3	0.89
AA35JDB56	2	1	3	6.4	3										
AA35JDC14			1												
AA35JDC23	7	1	3	7.4	3.5										
AA35JDC29		0	3												
AA35JDD16	2.5	1	3	5.5	3.05			3.3	29.2	92.7	9.6	48.6	1.46	7.3	0.92
AA35JDD29	2.5	0	3	4.5	1.85	8.8	17.9	4.0	53.9	252.6	15.9	83.8	1.33	9.4	0.95
AA35JDD61	2.5	0	3	4.5	1.85			4.8	53.4	367.8	19.2	92.2	1.34	9.3	0.96
AA35JDE110						7.6	13.4	5.0	66.8	490.2	22.1	114.5	1.32	10.4	0.94
AA35JDE17	5		3					4.9	58.6	408.0	20.2	99.8	1.48	10.3	0.91
AA35JDE24	6	1	3	7.2	3.4			4.1	49.9	238.8	15.5	81.0	1.45	9.4	0.93
AA35JDE4	1.5	1	3	6.3	2.95	6.8	14.1	3.1	44.9	127.5	11.3	68.7	1.30	8.5	0.96
AA35JDE42	6	1	3	6.2	3.4	9.0	22.5	4.7	49.3	355.6	18.9	92.0	1.34	9.0	0.93
AA36JDC3		1	3												
AA37JDB40	2.5	1	3	6.5	3.05										
AA37JDB5	0.8	0	2	3.16	1.68										
AA37JDB60	3.5	1	3	5.7	3.15										
AA37JDC30	7	1	3	6.4	3.5										
AA37JDC8		1	3												
AA37JDE106	3.5	1	3	5.7	3.15	8.8	23.0	3.7	36.7	130.0	11.4	60.1	1.49	8.2	0.92
AA37JDE43	4.5	1	2	4.9	3.25			3.8	40.5	203.7	14.3	72.6	1.33	8.1	0.95
AA38JDB19	2.5	1	3	6.5	3.05										

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA38JDB26	4	1	2	5.8	3.2										
AA38JDC1		1	2												
AA38JDC4			3												
AA38JDD31	3.5	1	2	4.7	3.15	7.4	3.8	4.1	58.1	295.6	17.2	90.1	1.28	9.6	0.96
AA39JDA06	3	1	2	4.6	3.1	8.3	8.9	4.6	41.8	290.4	17.0	79.3	1.37	8.4	0.93
AA39JDB43	3.3	0	3	4.66	1.93										
AA39JDD34	2		2			8.5	9.9	5.5	62.5	622.7	25.0	116.5	1.40	10.4	0.93
AA39JDE13			3			8.4	2.7	3.0	40.7	128.8	11.3	65.3	1.28	8.1	0.95
AA40JDB45	3.5	1	3	6.7	3.15										
AA40JDB52	8	1	3	7.6	3.6										
AA40JDC16		1	3												
AA40JDC24			1												
AA40JDC33		1	2												
AA40JDC39		1	2												
AA40JDD17	13	1	3	7.6	4.1			6.6	53.6	707.3	26.6	110.2	1.39	9.4	0.92
AA40JDD25	4.5	1	3	6.9	3.25	8.7	18.8	4.5	50.9	327.6	18.1	90.9	1.33	9.1	0.95
AA40JDD72	4.5	1	3	5.9	3.25	9.5	12.5	5.0	56.9	414.6	20.4	96.6	1.36	9.7	0.94
AA40JDD75	3.7	1	2	4.74	3.17	6.5	3.5	3.0	27.8	73.4	8.6	46.1	1.38	6.9	0.94
AA40JDE12		0	3			9.5	6.6	5.0	62.8	463.7	21.5	103.2	1.32	10.1	0.94
AA40JDE20			3			9.1	16.0	3.3	38.5	124.9	11.2	64.2	1.47	8.4	0.92
AA40JDE44	2.5	1	3	5.5	3.05	8.3	5.4	3.3	48.6	171.3	13.1	74.9	1.28	8.8	0.95
AA40JDEME	6	1	2	6.2	3.4										
AA41JDA08		1	3					4.4	49.4	250.4	15.8	78.1	1.36	9.1	0.94
AA41JDB1	3.5	1	3	5.7	3.15										
AA41JDB2	6	1	3	7.2	3.4										
AA41JDB6	2.5	1	3	6.5	3.05										
AA41JDE25			1					2.2	24.7	36.5	6.0	37.9	1.34	6.4	0.94

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA42JDB11	2.5	1	3	6.5	3.05										
AA42JDC26		1	2												
AA42JDE107			3			7.7	21.3	3.0	33.9	93.9	9.7	52.4	1.27	7.3	0.95
AA42JDE7			3			8.6	7.2	5.0	58.2	486.8	22.1	107.3	1.35	9.8	0.93
AA42JDEMC	5.2	1	2	6.04	3.32										
AA43JDB36	1.3	0	3	4.26	1.73										
AA43JDC20	6	1													
AA43JDD50		1	3			6.2	0.9	4.4	42.8	234.1	15.3	69.7	1.35	8.4	0.94
AA43JDD7	5.5		3			7.7	8.1	4.0	36.3	167.8	13.0	64.0	1.33	7.7	0.96
AA45JDB10	2.3	0	2	3.46	1.83										
AA45JDB3			3												
AA45JDB9	2.2	0	2	3.44	1.82										
AA45JDC38			3												
AA45JDD35	2	0	2	3.4	1.8	7.4	16.8	5.3	61.9	546.8	23.4	109.0	1.45	10.4	0.91
AA45JDD37		1	3			8.9	9.5	5.8	59.3	630.2	25.1	112.5	1.37	9.9	0.94
AA45JDD41	2		2			5.5	2.1	3.9	34.1	178.4	13.4	63.8	1.53	8.0	0.92
AA45JDD51	3	1	2	5.6	3.1	7.3	4.6	4.9	64.8	454.4	21.3	106.0	1.35	10.4	0.95
AA45JDD53	2.2	0	3	4.44	1.82	7.6	6.7	5.8	47.1	594.6	24.4	109.1	1.42	9.0	0.94
AA45JDE21		0	2			7.4	4.6	4.6	59.5	420.9	20.5	106.5	1.29	9.7	0.94
AA45JDE27	3	1	3	5.6	3.1	8.0	7.1	4.9	55.4	394.8	19.9	100.2	1.41	9.8	0.92
AA45JDEMK	3	1	2	5.6	3.1			4.0	54.5	275.9	16.6	88.2	1.32	9.4	0.94
AA47JDE32								2.9	35.8	92.4	9.6	53.8	1.24	7.4	0.95
AA47JDE9	3	0	2	3.6	1.9	6.9	16.5	3.2	41.1	125.9	11.2	63.6	1.34	8.3	0.94
AA48JDB12	2	0	3	4.4	1.8										
AA48JDB31	3		3												
AA48JDB50	6	0	3	5.2	2.2										
AA48JDC56		0	2												

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA48JDC73		1	2												
AA48JDC78		1	2												
AA48JDD24	4.5	1	3	5.9	3.25	6.5	17.3	5.4	53.4	468.9	21.7	99.6	1.43	9.6	0.93
AA48JDD39	5	1	3	7	3.3	6.8	16.5	6.7	61.8	747.2	27.3	126.9	1.43	10.3	0.92
AA48JDE11	1	1	2	4.2	2.9	7.7	7.4	2.7	30.8	63.2	8.0	45.3	1.34	7.2	0.94
AA48JDE35	6	0				7.4	3.8	4.5	44.7	303.2	17.4	86.8	1.34	8.6	0.94
AA49JDB25	2	0	3	4.4	1.8										
AA49JDB27	2.3	0	3	4.46	1.83										
AA49JDE108	2.5	1	2	5.5	3.05			4.5	48.9	320.3	17.9	85.9	1.38	9.1	0.94
AA49JDE23	6	1	3	7.2	3.4			5.5	64.9	636.0	25.2	120.9	1.31	10.2	0.94
AA50JDB15	10	1	3	7	3.8										
AA50JDB39	4	1	3	6.8	3.2										
AA50JDC69		1	3												
AA50JDC75		1	3												
AA50JDD28	9	1	3	6.8	3.7	8.1	51.1	4.3	31.3	210.4	14.5	65.2	1.44	7.4	0.93
AA50JDD67	7		3			7.7	12.4	3.7	45.3	168.8	13.0	69.2	1.33	8.6	0.96
AA50JDD73	5	1	3	7	3.3	8.2	5.5	3.5	36.6	149.4	12.2	63.8	1.41	7.9	0.94
AA50JDD77	5.5		3			6.7	4.2	5.0	39.7	403.6	20.1	84.5	1.32	8.0	0.96
AA50JDE28	2	1	2	4.4	3	6.6	5.1	3.7	40.8	150.0	12.2	63.0	1.30	8.1	0.95
AA50JDE45								3.1	47.4	148.4	12.2	73.8	1.28	8.7	0.96
AA51JDA11	3.5		2			7.6	7.4	3.9	31.0	156.2	12.5	60.0	1.31	7.0	0.95
AA51JDD19	2	0	2	3.4	1.8	7.4	12.9	4.4	42.3	258.1	16.1	76.3	1.45	8.6	0.92
AA51JDD5	3.5		3					4.5	40.2	241.6	15.5	70.6	1.42	8.3	0.94
AA51JDE41								4.0	35.0	178.3	13.4	62.6	1.45	7.9	0.91
AA51JDEMH	6	1	2	6.2	3.4			3.8	41.4	203.1	14.3	74.2	1.27	8.1	0.95
AA52JDA03	1.5							3.1	29.6	93.9	9.7	50.9	1.33	7.0	0.95
AA52JDD11	3.5	1	3	6.7	3.15	8.9	23.5	4.4	39.2	207.2	14.4	70.7	1.44	8.3	0.92

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA53JDA04		0				6.3	7.2	3.4	29.4	100.1	10.0	48.4	1.39	7.0	0.94
AA53JDB13	3.5	0	3	4.7	1.95										
AA53JDB23	2	1	3	5.4	3										
AA53JDE14		1	2					2.8	23.5	59.3	7.7	39.3	1.48	6.6	0.93
AA54JDB22	2.5	1	3	6.5	3.05										
AA54JDC65		1	3												
AA55JDA07	1.9	1				8.0	4.2	2.9	26.3	60.2	7.8	40.8	1.47	6.9	0.92
AA55JDA14	4					6.8	5.0	5.5	55.3	554.0	23.5	105.6	1.41	9.7	0.91
AA55JDB29	5	1	3	6	3.3										
AA55JDB34	3.5	1	3	5.7	3.15										
AA55JDB35	4	1	3	5.8	3.2										
AA55JDB48	10	1	3	8	3.8										
AA55JDB58	2.2	0	3	4.44	1.82										
AA55JDC68	3.5	1	2	5.7	3.15										
AA55JDD69	3.5	1	3	5.7	3.15	9.0	18.2	4.7	44.0	317.4	17.8	85.9	1.26	8.3	0.96
AA55JDEMB	2.5	0	3	4.5	1.85			4.2	57.3	315.5	17.8	93.4	1.31	9.6	0.94
AA56JDB55	2.5	0	2	3.5	1.85										
AA57JDB30	1.8	1	3	5.36	2.98										
AA57JDC62			3												
AA57JDE19	3	1	3	6.6	3.1	7.1	2.4	3.0	42.7	123.3	11.1	65.9	1.32	8.4	0.94
AA58JDB16	4	0	2	3.8	2										
AA58JDD57	2.7	0	3	4.54	1.87	8.7	11.0	5.1	67.6	493.7	22.2	107.2	1.33	10.5	0.95
AA58JDEMG								4.7	58.5	405.1	20.1	102.3	1.35	9.9	0.94
AA59JDD65	2	1	3		3	7.9	14.2	5.1	72.0	594.3	24.4	124.4	1.32	10.8	0.96
AA59JDE18	5	1	3	6	3.3	7.7	4.4	3.9	47.4	223.4	14.9	76.9	1.40	9.1	0.93
AA59JDE31	5	0	3	5	2.1	7.7	10.2	3.7	42.9	188.3	13.7	71.7	1.31	8.3	0.94
AA60JDA13		1				7.1	4.0	4.9	51.6	399.6	20.0	93.7	1.39	9.3	0.92

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA60JDB20	4	1	3		3.2										
AA60JDB51	8	1	3	6.6	3.6										
AA60JDC60		1	2												
AA60JDC70		1	3												
AA60JDC76		0	2												
AA60JDC77			2												
AA60JDD13	3.5	1	3	5.7	3.15			4.4	30.9	173.2	13.2	58.1	1.29	7.0	0.95
AA60JDD21	8	1	3	6.6	3.6	8.7	16.8	9.5	90.8	2445.3	49.4	199.7	1.47	12.6	0.90
AA60JDD59	3.5	1	3	6.7	3.15	7.0	8.1	4.3	46.8	226.6	15.1	74.9	1.42	9.0	0.93
AA60JDE46	7	1	2	5.4	3.5	3.7	6.1	3.9	41.7	191.7	13.8	70.7	1.35	8.3	0.94
AA61JDD43	2	1	3	5.4	3	6.8	19.8	5.3	46.9	411.3	20.3	93.5	1.38	8.8	0.93
AA61JDE109	11.5	0	3	6.3	2.75	8.4	22.1								
AA62JDA10		1	3			8.8	16.1	4.3	42.2	252.5	15.9	77.4	1.37	8.4	0.94
AA62JDD63	5	1	3		3.3	6.7	20.3	4.7	66.1	419.3	20.5	105.0	1.36	10.5	0.94
AA63JDC74		1	2												
AA63JDD55	4.5	1	3	6.9	3.25	7.9	13.5	4.0	32.6	159.0	12.6	59.6	1.37	7.4	0.93
AA64JDE34						7.4	25.1	4.2	45.9	244.1	15.6	73.7	1.34	8.7	0.94
AA65JDB7	3	0	3	4.6	1.9										
AA65JDE16		0	2					3.8	31.5	134.2	11.6	56.6	1.50	7.6	0.92
AA67JDB18	6.5	0	2	4.3	2.25										
AA68JDB24	2.5	1	2	4.5	3.05										
AA68JDE15								4.8	43.5	315.0	17.7	80.4	1.40	8.6	0.94
AA69JDA09		1	3			8.8	38.3	6.0	57.0	663.8	25.8	117.4	1.40	9.8	0.93
AA70JDB47	3	1	3	6.6	3.1										
AA70JDC43		0	2												
AA70JDC71			2												
AA70JDC72		1	3												

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AA70JDE104	7							4.2	35.3	162.5	12.7	62.6	1.38	7.7	0.91
AA70JDE39	6	0	3	5.2	2.2	9.3	2.4	2.6	29.4	58.4	7.6	44.7	1.35	7.0	0.93
AA72JDD9	3.5		3			8.1	24.4	6.5	51.8	643.9	25.4	106.9	1.42	9.4	0.92
AA72JDE10	2	1	3	6.4	3			4.1	40.2	199.4	14.1	70.2	1.44	8.4	0.92
AA72JDEMJ	1.5		2					4.0	53.2	274.3	16.6	89.0	1.37	9.5	0.94
AA73JDB46	3	1	3	6.6	3.1										
AA73JDEMD			3												
AA80JDE30	7.5	0	3	5.5	2.35	9.4	16.2	5.0	28.2	199.1	14.1	59.2	1.36	6.8	0.91
AA83JDE8			2					3.0	39.0	106.7	10.3	60.6	1.34	8.1	0.94
AA86JDD1	4	1	3	5.8	3.2	7.7	12.5	6.5	48.1	641.6	25.3	101.9	1.47	9.0	0.93

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade			
AA22JDE101						0	5	3			
AA24JDB28							2.8	3			
AA25JDC7								3			
AA25JDE29					?	1	7	3		KEY	
AA25JDE33								3			
AA25JDEmi								3			LOH
AA26JDC5						1		3			
AA28JDB44						1	3.5	3			Retained
AA28JDC25						1		2			
AA28JDC27						1	8	3			NI
AA28JDC35								3			
AA28JDC41								3		?	failed
AA28JDE36						0	4	3			
AA28JDE37						1	4	3			not tested
AA29JDB32							1.3	2			
AA29JDC36						1		3			
AA29JDE102						0	3	2			
AA29JDE105		?				1		3			
AA30JDD3						0	8				
AA30JDE103						0	4	2			
AA30JDE22						1	6	3			
AA30JDE26						1	3.5	2			
AA30JDEMF								3			
AA31JDE40						1	1.4	2			
AA31JDE42						1	6	3			
AA32JDA02							1.9				

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA32JDB42						0	4.5	2			
AA32JDC34								3			
AA33JDA01						1		2			
AA33JDB41						0	2	3			
AA33JDC2						1		3			
AA33JDD47							5	3			
AA35JDB56						1	2	3			
AA35JDC14								1			
AA35JDC23						1	7	3			
AA35JDC29						0		3			
AA35JDD16						1	2.5	3			
AA35JDD29						0	2.5	3			
AA35JDD61						0	2.5	3			
AA35JDE110											
AA35JDE17							5	3			
AA35JDE24						1	6	3			
AA35JDE4						1	1.5	3			
AA35JDE42						1	6	3			
AA36JDC3						1		3			
AA37JDB40						1	2.5	3			
AA37JDB5						0	0.8	2			
AA37JDB60						1	3.5	3			
AA37JDC30						1	7	3			
AA37JDC8						1		3			
AA37JDE106						1	3.5	3			
AA37JDE43						1	4.5	2			
AA38JDB19						1	2.5	3			

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA38JDB26						1	4	2			
AA38JDC1						1		2			
AA38JDC4								3			
AA38JDD31						1	3.5	2			
AA39JDA06						1	3	2			
AA39JDB43						0	3.3	3			
AA39JDD34							2	2			
AA39JDE13								3			
AA40JDB45						1	3.5	3			
AA40JDB52						1	8	3			
AA40JDC16						1		3			
AA40JDC24								1			
AA40JDC33						1		2			
AA40JDC39						1		2			
AA40JDD17						1	13	3			
AA40JDD25						1	4.5	3			
AA40JDD72						1	4.5	3			
AA40JDD75						1	3.7	2			
AA40JDE12						0		3			
AA40JDE20								3			
AA40JDE44						1	2.5	3			
AA40JDEME						1	6	2			
AA41JDA08						1		3			
AA41JDB1						1	3.5	3			
AA41JDB2						1	6	3			
AA41JDB6						1	2.5	3			
AA41JDE25											

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA42JDB11						1	2.5	3			
AA42JDC26						1		2			
AA42JDE107								3			
AA42JDE7								3			
AA42JDEMC						1	5.2	2			
AA43JDB36						0	1.3	3			
AA43JDC20						1	6				
AA43JDD50						1		3			
AA43JDD7							5.5	3			
AA45JDB10						0	2.3	2			
AA45JDB3								3			
AA45JDB9						0	2.2	2			
AA45JDC38								3			
AA45JDD35						0	2	2			
AA45JDD37						1		3			
AA45JDD41							2	2			
AA45JDD51						1	3	2			
AA45JDD53						0	2.2	3			
AA45JDE21						0		2			
AA45JDE27						1	3	3			
AA45JDEMK						1	3	2			
AA47JDE32											
AA47JDE9						0	3	2			
AA48JDB12						0	2	3			
AA48JDB31							3	3			
AA48JDB50						0	6	3			
AA48JDC56						0		2			

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA48JDC73						1		2			
AA48JDC78						1		2			
AA48JDD24						1	4.5	3			
AA48JDD39						1	5	3			
AA48JDE11						1	1	2			
AA48JDE35						0	6				
AA49JDB25						0	2	3			
AA49JDB27						0	2.3	3			
AA49JDE108						1	2.5	2			
AA49JDE23						1	6	3			
AA50JDB15						1	10	3			
AA50JDB39						1	4	3			
AA50JDC69						1		3			
AA50JDC75						1		3			
AA50JDD28						1	9	3			
AA50JDD67							7	3			
AA50JDD73						1	5	3			
AA50JDD77							5.5	3			
AA50JDE28						1	2	2			
AA50JDE45											
AA51JDA11							3.5	2			
AA51JDD19						0	2	2			
AA51JDD5							3.5	3			
AA51JDE41											
AA51JDEMH						1	6	2			
AA52JDA03							1.5				
AA52JDD11						1	3.5	3			

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA53JDA04						0					
AA53JDB13						0	3.5	3			
AA53JDB23						1	2	3			
AA53JDE14						1		2			
AA54JDB22						1	2.5	3			
AA54JDC65						1		3			
AA55JDA07						1	1.9				
AA55JDA14							4				
AA55JDB29						1	5	3			
AA55JDB34						1	3.5	3			
AA55JDB35						1	4	3			
AA55JDB48						1	10	3			
AA55JDB58						0	2.2	3			
AA55JDC68						1	3.5	2			
AA55JDD69						1	3.5	3			
AA55JDEMB						0	2.5	3			
AA56JDB55						0	2.5	2			
AA57JDB30						1	1.8	3			
AA57JDC62								3			
AA57JDE19		?				1	3	3			
AA58JDB16						0	4	2			
AA58JDD57						0	2.7	3			
AA58JDEMG											
AA59JDD65						1	2	3			
AA59JDE18						1	5	3			
AA59JDE31						0	5	3			
AA60JDA13						1					

242

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA60JDB20						1	4	3			
AA60JDB51						1	8	3			
AA60JDC60						1		2			
AA60JDC70						1		3			
AA60JDC76						0		2			
AA60JDC77								2			
AA60JDD13						1	3.5	3			
AA60JDD21						1	8	3			
AA60JDD59						1	3.5	3			
AA60JDE46						1	7	2			
AA61JDD43						1	2	3			
AA61JDE109						0	11.5	3			
AA62JDA10						1		3			
AA62JDD63						1	5	3			
AA63JDC74						1		2			
AA63JDD55						1	4.5	3			
AA64JDE34											
AA65JDB7						0	3	3			
AA65JDE16						0		2			
AA67JDB18						0	6.5	2			
AA68JDB24						1	2.5	2			
AA68JDE15											
AA69JDA09						1		3			
AA70JDB47						1	3	3			
AA70JDC43						0		2			
AA70JDC71								2			
AA70JDC72						1		3			

Key	ERTA	D6S193	D6S186	p53	D16S413	LN Stat	size cm	grade			
AA70JDE104							7				
AA70JDE39						0	6	3			
AA72JDD9							3.5	3			
AA72JDE10						1	2	3			
AA72JDEMJ							1.5	2			
AA73JDB46						1	3	3			
AA73JDEMD								3			
AA80JDE30						0	7.5	3			
AA83JDE8								2			
AA86JDD1						1	4	3			

APPENDIX A

PART 2

LEICESTER ASIAN

(AL)

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AL26RAW1639	3.5	1	3	5.7	3.2	4.9	37.8	5.3	46.8	482.9	22.0	102.2	1.53	9.3	0.86
AL27RAW1168	2.5	1	3	6.5	3.1	3.6	30.2	3.5	27.0	106.1	10.3	50.7	1.62	7.3	0.88
AL30RAW1889	3	1	3	6.6	3.1	5.9	3.8	3.3	34.3	121.2	11.0	59.4	1.37	7.6	0.94
AL33RAW659		1	3			5.4	6.2	5.1	37.0	218.4	14.8	65.1	1.61	8.4	0.88
AL39RAW1463	2.3	1	2	3.5	3.0	6.8	6.6	4.1	35.4	192.6	13.9	62.8	1.40	7.7	0.90
AL39RAW1843	1.8	1	2	4.4	3.0	6.2	2.8	3.0	36.4	81.0	9.0	51.1	1.29	7.7	0.94
AL39RAWLR3	2.5	1	2	5.5	3.1	5.3	16.0	3.3	22.6	69.2	8.3	39.1	1.34	6.1	0.95
AL42RAW1670	3.8	1	3	6.8	3.2	8.0	23.4	5.5	48.3	565.9	23.8	95.5	1.41	9.1	0.90
AL42RAWGH2	3.5	1	2	4.7	3.2	7.9	1.4	2.9	45.1	116.4	10.8	65.8	1.27	8.5	0.95
AL43RAWLR4	3	0	3	4.6	1.9	8.6	11.8	8.6	64.9	1192.6	34.5	146.3	1.66	11.3	0.86
AL44RAWGH3	3.2	1	3	5.6	3.1	8.7	28.4	4.6	60.0	424.0	20.6	107.9	1.24	9.6	0.95
AL45RAW1688	6	0	3	5.2	2.2	7.6	17.0	5.1	47.8	408.5	20.2	89.9	1.50	9.3	0.90
AL46RAW1728	7	1	3	7.4	3.5	9.4	44.7	2.8	34.5	86.4	9.3	53.0	1.41	7.7	0.89
AL46RAW1760	4	0	3	4.8	2.0	7.4	22.7	6.4	76.7	935.0	30.6	143.9	1.50	11.8	0.91
AL47RAW1170	7	1	3	6.4	3.5	8.3	14.3	7.5	72.8	1298.8	36.0	152.9	1.45	11.2	0.91
AL48RAW1553	4.5		3			6.3	2.7								
AL49RAW0232		2	2			7.7	8.0	5.4	55.0	445.2	21.1	93.7	1.31	9.4	0.95
AL49RAW1654	5	1	2	6.0	3.3	6.9	4.9	5.1	55.2	407.2	20.2	96.4	1.39	9.7	0.90
AL49RAWGH1	3.8	1	3	5.8	3.2	6.7	22.7	5.6	74.0	760.8	27.6	130.9	1.39	11.2	0.93
AL50RAW1821	1.5	0	2	3.3	1.8	5.2	10.9	4.0	51.9	222.6	14.9	79.5	1.44	9.6	0.92
AL52RAW0753		0	1					4.0	30.3	141.3	11.9	52.2	1.57	7.6	0.90
AL52RAW1425		2	2			5.3	11.6	4.9	54.5	365.4	19.1	91.4	1.26	9.2	0.95
AL54RAW1771	3.5	0	3	4.7	2.0	8.7	33.7	8.3	82.8	1445.2	38.0	164.6	1.63	12.7	0.86
AL54RAW1819	3.4	1	2	5.7	3.1	4.3	6.5	5.1	47.1	398.1	20.0	95.6	1.47	9.1	0.90
AL54RAWLR2	2	1	2	5.4	3.0	7.4	19.8	8.1	43.8	1342.5	36.6	126.0	1.47	8.5	0.90
AL55RAW1674	3.7	1	3	6.7	3.2	6.0	2.8	5.8	48.1	557.5	23.6	102.2	1.38	9.0	0.93

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
AL55RAW1943	2.8	1	3	5.6	3.1	6.7	14.4	3.8	52.2	224.8	15.0	81.0	1.30	9.2	0.95
AL55RAWGH4	2.8	0	2	3.6	1.9	4.9	3.4	2.5	36.3	58.5	7.6	50.4	1.41	8.0	0.93
AL58RAW1472	3	1	3	5.6	3.1	8.2	19.3	3.6	61.1	241.5	15.5	91.2	1.34	10.1	0.94
AL59RAW0249		1	2			8.5	10.6	6.4	41.4	591.4	24.3	97.7	1.47	8.5	0.92
AL59RAW1342	2	0	3	4.4	1.8	6.0	56.6	4.0	41.1	217.7	14.8	73.8	1.53	8.7	0.91
AL60RAW1620	2.8	1	1	3.6	2.6	9.3	1.2	3.7	38.6	147.6	12.2	60.7	1.41	8.2	0.91
AL60RAW1887	1.5	0	1	2.3	1.3	6.1	1.9	2.2	42.0	62.8	7.9	57.7	1.24	8.1	0.95
AL61RAW0774		2	2					3.7	35.6	141.0	11.9	60.1	1.48	8.0	0.91
AL62RAWLR1	8.5	0	3	5.7	2.5	7.8	21.1	4.7	59.4	399.4	20.0	96.6	1.29	9.7	0.95
AL63RAW1608	2.5	0	3	4.5	1.9	7.8	16.6	3.0	30.6	78.6	8.9	50.4	1.57	7.7	0.85
AL67RAW1683	2.9	0	3	4.6	1.9	6.2	30.2	5.2	47.4	358.2	18.9	87.8	1.42	9.0	0.91
AL70RAW1924	2.5	0	2	3.5	1.9	8.2	6.2	2.3	38.8	57.8	7.6	54.0	1.34	8.1	0.94
AL71RAW1938	2	0	1	2.4	1.3	5.5	4.1	2.9	50.4	130.8	11.4	74.7	1.30	9.0	0.95
AL72RAW1204	3.5	1	3	5.7	3.2	8.7	13.6	7.1	70.0	1461.4	38.2	155.3	1.40	10.9	0.92
AL72RAWGH5	2.2	0	2	3.4	1.8	8.1	1.4	3.2	45.7	134.3	11.6	68.0	1.27	8.5	0.95
AL86RAW0362		2	2					3.1	28.9	87.7	9.4	48.6	1.48	7.2	0.91

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade				
AL26RAW1639						3	3.5	1				
AL27RAW1168						3	2.5	1				
AL30RAW1889						3	3	1		Key		
AL33RAW659						3		1				
AL39RAW1463						2	2.3	1			LOH	
AL39RAW1843						2	1.8	1				
AL39RAWLR3						2	2.5	1			Retained	
AL42RAW1670						3	3.8	1				
AL42RAWGH2						2	3.5	1			NI	
AL43RAWLR4						3	3	0				
AL44RAWGH3						3	3.2	1		?	Failed	
AL45RAW1688						3	6	0				
AL46RAW1728						3	7	1			Not tested	
AL46RAW1760						3	4	0				
AL47RAW1170						3	7	1				
AL48RAW1553												
AL49RAW0232						2		2				
AL49RAW1654						2	5	1				
AL49RAWGH1						3	3.8	1				
AL50RAW1821						2	1.5	0				
AL52RAW0753						1		0				
AL52RAW1425						2		2				
AL54RAW1771						3	3.5	0				
AL54RAW1819						2	3.4	1				
AL54RAWLR2						2	2	1				
AL55RAW1674						3	3.7	1				

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade			
AL55RAW1943						3	2.8	1			
AL55RAWGH4			?			2	2.8	0			
AL58RAW1472						3	3	1			
AL59RAW0249						2		1			
AL59RAW1342						3	2	0			
AL60RAW1620						1	2.8	1			
AL60RAW1887						1	1.5	0			
AL61RAW0774						2		2			
AL62RAWLR1						3	8.5	0			
AL63RAW1608						3	2.5	0			
AL67RAW1683						3	2.9	0			
AL70RAW1924						2	2.5	0			
AL71RAW1938						1	2	0			
AL72RAW1204						3	3.5	1			
AL72RAWGH5						2	2.2	0			
AL86RAW0362		?				2		2			

APPENDIX A

PART 3

LEICESTER EUROPEAN

(EL)

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
EL30RAW1634	3	1	2	5.6	3.1	6.8	18.3	4.2	37.9	180.7	13.4	64.9	1.31	7.8	0.93
EL32RAW1567	1.5	0	3	4.3	1.8	5.2	13.0	7.0	62.0	984.0	31.4	132.3	1.37	10.0	0.94
EL33RAW1376	1.8	0	2	3.4	1.8	6.4	7.9	4.1	49.0	278.0	16.7	87.0	1.35	9.0	0.93
EL33RAW1496	4	0	3	4.8	2.0	7.0	17.6	4.0	43.2	231.2	15.2	76.1	1.31	8.4	0.94
EL33RAW1606	2.5	0	3	4.5	1.9	7.6	53.2	4.7	47.2	236.4	15.4	78.4	1.63	9.7	0.87
EL35RAW1579	1.5	1	2	5.3	3.0	5.4	20.9	6.0	81.2	920.5	30.3	141.9	1.40	11.8	0.90
EL36RAW1464	1.3	0	1	2.3	1.2	6.0	6.5	4.2	38.6	232.2	15.2	70.0	1.40	8.1	0.93
EL37RAW1228	8	1	2	6.6	3.6	8.2	5.5	4.1	54.9	267.4	16.4	84.9	1.43	9.8	0.90
EL37RAW1328	2.9	1	2	4.6	3.1	7.1	11.1	4.6	49.9	295.0	17.2	84.3	1.48	9.4	0.88
EL37RAW1532	2.1	1	3	5.4	3.0	8.3	19.1	5.1	55.8	417.0	20.4	95.3	1.33	9.5	0.91
EL38RAW1324	2.5	1	3	6.5	3.1	7.0	20.3	7.1	61.7	913.7	30.2	125.1	1.40	10.1	0.92
EL39RAW1457	2.2	0	3	4.4	1.8	8.5	44.7	10.2	80.2	2350.6	48.5	185.2	1.39	11.3	0.92
EL39RAW1500	2	1	2	5.4	3.0	5.3	6.3	5.1	39.1	339.5	18.4	75.3	1.42	8.2	0.92
EL39RAW1530	2.5	1	1	3.5	2.6	8.1	4.9	2.8	38.6	93.3	9.7	57.1	1.37	8.1	0.90
EL40RAW1236	5.2	0	3		2.1	7.8	25.4	6.3	47.8	542.6	23.3	96.4	1.49	9.2	0.90
EL40RAW1345		2	3			8.1	17.4	6.0	61.0	687.7	26.2	116.9	1.46	10.3	0.91
EL40RAW1373	2.5	0	3	4.5	1.9	8.8	47.4	4.2	46.5	252.0	15.9	82.8	1.32	8.7	0.94
EL40RAW1505	1.5	0	3	4.3	1.8	6.0	21.5	6.1	40.9	535.5	23.1	90.8	1.65	8.9	0.82
EL41RAW1255	2.5	1	3	6.5	3.1	6.2	8.2	4.8	56.4	409.1	20.2	99.2	1.50	10.1	0.90
EL42RAW1445	5	1	2	5	3.3	4.6	2.5	5.2	41.9	269.7	16.4	73.3	1.5	8.8	0.86
EL43RAW1221	2.5	1	2	4.5	3.1	6.8	8.3	4.8	43.8	313.8	17.7	84.3	1.40	8.6	0.92
EL43RAW1466	2	0	3	4.4	1.8	5.2	14.1	5.7	64.1	559.6	23.7	116.4	1.51	10.8	0.84

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
EL43RAW1526	4	1	2	4.8	3.2	7.0	47.0	3.0	33.4	93.4	9.7	52.5	1.42	7.6	0.89
EL43RAW1531	1.5	0	3	4.3	1.8	7.5	10.5	6.5	62.3	755.9	27.5	119.2	1.44	10.4	0.91
EL44RAW1242	3	1	3	6.6	3.1	5.5	29.8	7.2	75.6	1232.3	35.1	156.3	1.44	11.4	0.90
EL44RAW1350	1.8	1	3	5.4	3.0	6.4	19.4	5.8	54.1	518.1	22.8	102.8	1.40	9.6	0.92
EL45RAW1201		0	1			7.8	7.3	2.7	37.4	81.9	9.1	55.7	1.33	7.9	0.94
EL45RAW1244	2.5	0	1	2.5	1.4	7.3	1.6	2.9	21.3	49.0	7.0	35.6	1.51	6.3	0.92
EL45RAW1455	3	0	3	4.6	1.9	7.7	13.1	6.0	62.8	681.1	26.1	114.1	1.40	10.3	0.90
EL45RAW1459	2.5	0	3	4.5	1.9	9.1	5.0	4.2	42.2	224.3	15.0	76.6	1.37	8.4	0.91
EL45RAW1478	1.3	0	1	2.3	1.2			4.4	30.7	102.7	10.1	51.2	1.68	8.0	0.80
EL45RAW1574	2	0	3	4.4	1.8	7.2	15.6	5.4	55.1	430.6	20.8	94.1	1.49	10.0	0.87
EL46RAW1264	13.5	1	2	7.7	4.2	5.8	38.2	5.5	65.0	550.6	23.5	112.4	1.37	10.4	0.93
EL46RAW1435	2	0	2	3.4	1.8	6.3	2.7	3.4	40.6	141.0	11.9	67.3	1.49	8.6	0.87
EL46RAW1444	3	0	3	4.6	1.9	8.9	25.9	5.5	57.5	365.0	19.1	97.8	1.53	10.3	0.87
EL47RAW1216		1	2			5.6	11.0	5.1	60.6	475.7	21.8	108.6	1.48	10.5	0.89
EL47RAW1237	2	1	3	5.4	3.0	6.2	23.5	6.4	46.5	570.8	23.9	100.4	1.64	9.5	0.87
EL47RAW1450	2.5	1	3	5.5	3.1	7.6	4.4	9.8	94.9	2508.1	50.1	200.8	1.54	13.0	0.87
EL48RAW1449	1.5	0	1	2.3	1.3	5.4	3.2	4.0	36.3	156.9	12.5	58.9	1.39	7.8	0.91
EL49RAW1436		1	3			8.0	7.0	7.6	71.2	1412.5	37.6	153.1	1.46	11.1	0.89
EL49RAW1479	3	1	3	5.6	3.1	7.0	5.6	5.7	59.2	590.2	24.3	108.4	1.38	9.9	0.90
EL50RAW1213	1.6	0	2	3.3	1.8	6.1	4.6	3.1	32.0	86.5	9.3	51.8	1.40	7.4	0.91
EL51RAW1506	2	0	1	2.4	1.3	6.7	8.4	5.2	45.0	309.9	17.6	81.9	1.52	9.1	0.89
EL52RAW1351	1.4	0	1	2.3	1.2	5.8	8.7	4.7	46.9	349.2	18.7	85.5	1.36	8.8	0.93

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
EL52RAW1441	1.7	1	2	5.3	3.0	5.3	4.3	4.3	39.7	205.4	14.3	69.5	1.47	8.4	0.89
EL52RAW1465	6	1	2	5.2	3.4	5.5	1.0	3.7	34.8	142.5	11.9	60.8	1.37	7.6	0.89
EL52RAW1535	2	0	2	3.4	1.8			3.7	35.7	135.5	11.6	57.9	1.33	7.7	0.95
EL53RAW1347	3	1	1	4.6	2.6	4.4	7.6	5.1	45.8	297.7	17.3	79.9	1.44	9.0	0.90
EL53RAW1546	3	1	3	5.6	3.1	7.4	7.6	6.0	51.9	605.1	24.6	103.9	1.43	9.3	0.93
EL54RAW1240	1.8	0	2	3.4	1.8	5.2	11.9	3.2	27.9	87.4	9.4	46.1	1.46	7.1	0.91
EL54RAW1524	4	0	3	4.8	2.0	7.1	7.2	3.6	26.9	100.5	10.0	48.3	1.41	6.8	0.92
EL55RAW1209		1	3			5.2	6.5								
EL55RAW1232	2.5	1	3		3.1	8.5	15.9	7.8	68.3	1402.2	37.4	153.4	1.53	11.1	0.88
EL55RAW1469	3	1	3	5.6	3.1	6.3	10.7	5.5	51.9	586.5	24.2	104.5	1.35	9.2	0.90
EL55RAW1492	1.3	0	1	2.3	1.2	6.8	1.7	3.4	34.1	113.4	10.7	57.3	1.41	7.7	0.89
EL55RAW1509	1.4	0	2	3.3	1.7	7.9	1.4	2.6	52.2	113.7	10.7	73.7	1.34	9.3	0.93
EL56RAW1481	1.8	0	2	3.4	1.8	3.8	4.5	2.5	34.9	72.6	8.5	52.4	1.33	7.6	0.92
EL57RAW1258		0	2			7.7	22.0								
EL58RAW1248	3.2	0	2	3.6	1.9			5.3	30.9	312.4	17.7	72.6	1.53	7.5	0.90
EL58RAW1443	2.5	1	2	5.5	3.1	8.5	35.8	6.4	64.6	740.0	27.2	122.9	1.45	10.7	0.91
EL59RAW1207	3	1	2	4.6	3.1	5.9	4.8	5.2	42.2	248.3	15.8	74.5	1.44	8.6	0.84
EL59RAW1502	1.6	0	3	4.3	1.8	8.8	6.5	4.3	39.0	173.7	13.2	66.3	1.54	8.6	0.90
EL59RAW1514	2.5	0	2	3.5	1.9	8.7	3.2	3.5	31.3	113.6	10.7	52.5	1.37	7.3	0.94
EL60RAW1239	2.4	1	2	5.5	3.0	7.6	13.4	4.1	33.8	161.7	12.7	64.3	1.59	8.1	0.88
EL60RAW1379	8	1	2	6.6	3.6	6.8	2.5	3.8	37.3	184.9	13.6	69.8	1.34	7.8	0.95
EL60RAW1462	3.5	1	3	6.7	3.2	8.4	80.3	2.9	31.3	92.1	9.6	51.9	1.33	7.2	0.94
EL61RAW1447	1.5	1	3	5.3	3.0	8.1	28.7	4.7	50.1	324.5	18.0	86.3	1.35	9.1	0.92

key	size cm	LN stat	grade	NPI	YBCGPI	sum Vv	VMI	SDNP	mean NA	VNA	SDNA	NA10	D1:D2	NDmax	shape
EL61RAW1453	1.8	1	2	4.4	3.0	6.5	2.6	3.7	70.6	305.4	17.5	103.0	1.31	10.7	0.93
EL61RAW1486	4	1	3	6.8	3.2	4.9	4.6	6.4	66.5	779.2	27.9	123.2	1.35	10.4	0.93
EL63RAW1293	4	0	3	4.8	2.0	4.0	1.4	7.3	46.2	781.8	28.0	112.9	1.44	8.8	0.92
EL64RAW1252	1.8	0	2	3.4	1.8	4.6	2.5	3.6	28.5	126.7	11.3	55.7	1.48	7.1	0.90
EL64RAW1548	1	0	1	2.2	1.2			3.0	30.6	78.6	8.9	50.4	1.57	7.7	0.85
EL65RAW1538	1.7	1	3		3.0	8.2	3.4	5.1	102.6	762.3	27.6	155.5	1.26	12.7	0.92
EL71RAW1243		0	2					3.1	25.2	70.2	8.4	44.6	1.40	6.6	0.93
EL73RAW1205	4.5	2	1			8.2	0.7	2.6	29.0	53.9	7.3	43.8	1.55	7.5	0.91
EL73RAW1616	2.5	0	3		1.9	8.2	13.8	3.4	36.0	118.3	10.9	57.0	1.52	8.2	0.87
EL75RAW1217			2			8.0	1.4	5.1	60.6	475.7	21.8	108.6	1.48	10.5	0.89

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade					
EL30RAW1634						2	3	1					
EL32RAW1567						3	1.5	0					
EL33RAW1376						2	1.8	0			Key		
EL33RAW1496						3	4	0					
EL33RAW1606						3	2.5	0				LOH	
EL35RAW1579						2	1.5	1					
EL36RAW1464						1	1.3	0				Retained	
EL37RAW1228						2	8	1					
EL37RAW1328						2	2.9	1				NI	
EL37RAW1532						3	2.1	1					
EL38RAW1324						3	2.5	1			?	Failed	
EL39RAW1457						3	2.2	0					
EL39RAW1500						2	2	1				Not tested	
EL39RAW1530						1	2.5	1					
EL40RAW1236						3	5.2	0					
EL40RAW1345		?				3		2					
EL40RAW1373						3	2.5	0					
EL40RAW1505						3	1.5	0					
EL41RAW1255						3	2.5	1					
EL42RAW1445						2	5	1					
EL43RAW1221						2	2.5	1					
EL43RAW1466						3	2	0					

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade					
EL43RAW1526						2	4	1					
EL43RAW1531						3	1.5	0					
EL44RAW1242						3	3	1					
EL44RAW1350						3	1.8	1					
EL45RAW1201						1		0					
EL45RAW1244						1	2.5	0					
EL45RAW1455						3	3	0					
EL45RAW1459						3	2.5	0					
EL45RAW1478						1	1.3	0					
EL45RAW1574						3	2	0					
EL46RAW1264						2	13.5	1					
EL46RAW1435						2	2	0					
EL46RAW1444						3	3	0					
EL47RAW1216						2		1					
EL47RAW1237						3	2	1					
EL47RAW1450						3	2.5	1					
EL48RAW1449						1	1.5	0					
EL49RAW1436						3		1					
EL49RAW1479						3	3	1					
EL50RAW1213						2	1.6	0					
EL51RAW1506						1	2	0					
EL52RAW1351						1	1.4	0					

256

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade					
EL52RAW1441						2	1.7	1					
EL52RAW1465						2	6	1					
EL52RAW1535						2	2	0					
EL53RAW1347						1	3	1					
EL53RAW1546						3	3	1					
EL54RAW1240						2	1.8	0					
EL54RAW1524						3	4	0					
EL55RAW1209						3		1					
EL55RAW1232						3	2.5	1					
EL55RAW1469						3	3	1					
EL55RAW1492						1	1.3	0					
EL55RAW1509						2	1.4	0					
EL56RAW1481						2	1.8	0					
EL57RAW1258						2		0					
EL58RAW1248						2	3.2	0					
EL58RAW1443						2	2.5	1					
EL59RAW1207						2	3	1					
EL59RAW1502						3	1.6	0					
EL59RAW1514						2	2.5	0					
EL60RAW1239						2	2.4	1					
EL60RAW1379						2	8	1					
EL60RAW1462		?				3	3.5	1					

key	ERTA	D6S193	D6S186	p53	D16S413	LN status	size cm	grade					
EL61RAW1447						3	1.5	1					
EL61RAW1453						2	1.8	1					
EL61RAW1486						3	4	1					
EL63RAW1293						3	4	0					
EL64RAW1252						2	1.8	0					
EL64RAW1548			?			1	1	0					
EL65RAW1538						3	1.7	1					
EL71RAW1243						2		0					
EL73RAW1205						1	4.5	2					
EL73RAW1616						3	2.5	0					
EL75RAW1217								2					

APPENDIX B

LIST OF COMMONLY USED SOLUTIONS

Solution	Components			
Digestion Buffer (microdissected sections)	Tris-MCl pH 7.6	1M	1μl	
	EDTA pH 8	0.5M	20μl	
	U.P. water		8.8μl	
	Proteinase K		20μl	
Digestion Buffer (whole sections)	KCl	1M	50000μl	
	Tris pH 8.3	1M	1000μl	
	MgCl₂	1M	250μl	
	Tween 20		450μl	
	NP40		450μl	
	U.P. water		92.9ml	
	Proteinase K		500μg/ml	
Eosin	1% aqueous water soluble eosin			
Ethidium Bromide	Ethidium Bromide		100 mg	
	U.P. water		10mls	
Loading Buffer	Xylene cyanol		1mg	
	Bromophenol Blue		1mg	
	TBE x 10		1ml	
	Glycerol		7ml	
	U.P. water		2 ml	

Solution	Components		
Mayers Haematoxylin	Haematoxylin		0.1%
	Aluminium Ammonium Sulphate dodecahydrate		5%
	Sodium Iodide		0.02%
	HCl		0.1%
	Chloral hydrate		5%
P.A.G.E gel (12%)	Acrylamide/bisacrylamide 40% solution (19:1 ratio)		15ml
	TBE x 1 buffer		5 ml
	U.P. water		30ml
	Ammonium persulphate 10%		400µl
	TEMED		25µl
Reaction Buffer x 10 (P.C.R.)	Tris-HCl pH 8.8	1M	2250µl
	(NH$_4$)$_2$SO$_4$	1M	550µl
	MgCl$_2$	1M	225µl
	dNTPs	100mM	100µl each
	B.S.A.	20mg/ml	275µl
	β mercaptoethanol	14.3M	23.4µl
	EDTA pH 8.0	10mM	2.2µl
	U.P. water	up to	5ml
T.B.E. x 10 buffer	Tris		108g
	Boric Acid		55g
	EDTA	0.5M	40ml

APPENDIX C

COMMONLY USED CHEMICALS

Chemical	Company	Catalogue Number
Acetic acid (glacial)	Nen Tech	2789
Acrylamide/bis acrylamide (40% solution) (19:1 ratio)	Sigma	A-2917
Albumin B.S.A.	Bochringer Mannheim	711454
Ammonium persuphate $(NH_4)_2S_2O_8$	Sigma	A-6761
Ammonium Sulphate $(NH_4)_2SO_4$	Sigma	A-4418
Aluminium Ammonium Sulphate dodecahydrate	Aldrich	20,256-8
Boric Acid	Sigma	B-6768
Bromophenol blue	Sigma	B-5525
Chloral hydrate		
Deoxynucleotide Triphosphate set (dNTPs)	Boehringer	711454
EDTA	BDH	10093SV
Eosin yellowish	BDH Gurr	34197
Ethanol	Aldrich	18,738-0
Ethidium Bromide	Sigma	E-2515
Glycerol	Sigma	G-5516
Haematoxylin	Sigma	H 3136
HCl	Aldrich	38,0 11-3
ISEPAL CA-630 (DP40)	Sigma	I-3021

Chemical	Company	Catalogue Number
Potassium Chloride	Sigma	P 9333
LE Agarose	FMC Seakem	50002
Magnesium Chloride 1M	Sigma	M-1028
β-Mercaptoethanol	Sigma	H 3148
Methanol	Aldrich	17,995-7
Nu Sieve Agarose	FMC	50084
Proteinase K	Boehringer	161519
Taq Polymerase	Promega	M 1665
TEMED	Sigma	T-7024
Tris Ultra pure	GibcoBRL	15504-020
Triton X-100	WinLab	292782
Tween-20	Sigma	P-1379
U.P. water	(distilled sterilized water)	
Xam neutral	BDM Gurr	36119
Xylene	Neu Tech	1307

REFERENCES

Aaltomaa S, Lipponen P, Eskelinen M, Kosma VM, Marin S, Alhava E, Syrjanen K.
Hormone receptor status and mitotic activity as risk factors for recurrence and death in female breast carcinoma.
Anticancer Res. 1991 Sep-Oct **11: 5** 1701-6.

Aaltomaa S, Lipponen P, Eskelinen M.
Demographic prognostic factors in breast cancer.
Acta Oncol. 1992a **31:6** 635-40.

Aaltomaa S, Lipponen P, Eskelinen M, Kosma VM, Marin S, Alhava E, Syrjanen K.
Mitotic indexes as prognostic predictors in female breast cancer.
J Cancer Res Clin Oncol. 1992b **118:1** 75-81.

Aaltomaa S, Lipponen P, Eskelinen M, Kosma VM, Marin S, Alhava E, Syrjanen K.
Prognostic factors after 5 years follow-up in female breast cancer.
Oncology. 1992c **49:2** 93-8

Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, De La Chapelle A
Clues to the pathogenesis of familial colorectal cancer.
Science 1993 May 7 **260:5109** 812-6

Abu Bakr S, Al-Suleiman SA
Attitude towards fertility control in the Qatif Region Eastern Province, Saudi Arabia
Saudi Med J 1990 **11:1** 64-66

Adami HO, Persson I, Ekbom A, Wolk A, Ponten J, Trichopoulos D.
The aetiology and pathogenesis of human breast cancer.
Mutat Res. 1995 Dec **333:1-2** 29-35

Adebamowo CA, Adekunle O.
Case-controlled study of the epidemiological risk factors for breast cancer in Nigeria.
Br J Surg. 1999 May **86:5** 665-8.

Adeyinka A, Pandis N, Bardi G, Bonaldi L, Mertens F, Mitelman F, Heim S.
A subgroup of breast carcinomas is cytogenetically characterized by trisomy 12.
Cancer Genet Cytogenet. 1997 Sep **97:2** 119-21

Adeyinka A, Mertens F, Idvall I, Bondeson L, Ingvar C, Mitelman F, Pandis N
Different patterns of chromosomal imbalances in metastasising and non-metastasising primary breast carcinomas.
Int J Cancer 1999 Aug 20 **84:4** 370-5

Adib SM, Mufarrij AA, Shamseddine AI, Kahwaji SG, Issa P, el-Saghir NS.
Cancer in Lebanon: an epidemiological review of the American University of Beirut
Medical Center Tumor Registry Report (1983-1994).

Adnane J, Gaudray P, Simon MP, Simony-Lafontaine J, Jeanteur P, Theillet C
Proto-oncogene amplification and human breast tumor phenotype.
Oncogene 1989 Nov **4:11** 1389-95

Aherne WA, Dunnill MS
Morphometry
Edward Arnold (Pub) 1982

Ajrim DS
Cancer at King Khalid University Hospital, Riyadh
Ann Saudi Med 1992 **12:** 76-82

Akhtar SS, Abu Bakr MA, Dawi SA, Huq IU.
Cancer in Libya--a retrospective study (1981-1985).
Afr J Med Med Sci. 1993 Mar **22:1** 17-24.

Albain KS, Allred DC, Clark GM.
Breast cancer outcome and predictors of outcome: are there age differentials?
J Natl Cancer Inst Monogr. 1994 **16** 35-42.

Al-Idrissi HY, Ibrahim EM, Kurashi NY, Sowayan SA
Breast cancer in a low-risk population. The influence of age and menstrual status on disease pattern and survival in Saudi Arabia.
Int J Cancer 1992 Aug 19 **52:1** 48-51

Allred DC, Clark GM, Molina R, Tandon AK, Schnitt SJ, Gilchrist KW, Osborne CK, Tormey DC, McGuire WL.
Overexpression of HER-2/neu and its relationship with other prognostic factors change during the progression of in situ to invasive breast cancer.
Hum Pathol. 1992 Sep **23:9** 974-9

Al-Rehaimi AA, Bjorntorp P.
Obesity and fat distribution in women from Saudi Arabia.
Int J Obes Relat Metab Disord. 1992 Dec **16:12** 1017-9

Al Saigh A, Allum MM, Khan KA, Al Hawsawi ZM
Pattern of caner in Madinah Al-Munawara region
Ann Saudi Med 1995 **15:4** 350-353

Al-Saleh MS
Analysis of clinical and histological features of breast cancer in Saudi Arabia
Saudi Med J 1994 **15:5** 385-388

Al-Shehri SN, Farag MK, Baldo MH, al-Mazrou YY, Aziz KM.
Overview on breastfeeding patterns in Saudi Arabia.
J Trop Pediatr. 1995 **41 Suppl 1** 38-44

Amari M, Suzuki A, Moriya T, Yoshinaga K, Amano G, Sasano H, Ohuchi N, Satomi S, Horii A
LOH analyses of premalignant and malignant lesions of human breast: frequent LOH in 8p, 16q, and 17q in atypical ductal hyperplasia.
Oncol Rep 1999 Nov-Dec **6:6** 1277-80

Ambros RA, Trost RC.
Cellularity in breast carcinoma.
Am J Clin Pathol. 1990 Jan **93:1** 98-100

Amer AH
Pattern of cancer in Saudi Arabia: A personal experience based on the management of 1000 patients.
KFSH Med Journal 1982 **2:** 203-215

American Joint Committee on Cancer
Manual for staging for breast carcinoma
1989 3rd Edition

Amir H, Kwesigabo G, Aziz MR, Kitinya JN.
Breast cancer and conservative surgery in sub Saharan Africa.
East Afr Med J. 1996 Feb **73:2** 83-7

Amr SS, Sa'di ARM, Ilahi F, Sheikh SS
The spectrum of breast diseases in Saudi Arab females: A 26 year pathological survey at Dhahran health center.
Ann Saudi Med 1995 **15:** 125-132

Anderson BO, Senie RT, Vetto JT, Wong GY, McCormick B, Borgen PI.
Improved survival in young women with breast cancer.
Ann Surg Oncol. 1995 Sep **2:5** 407-15

Armitage P, Doll R
Stochastic models for carcinogenesis.
In Neyman J (ed) 1961 "Proceedings of the Fourth Berkley Symposium on Mathematical Statistics and Probability." **Vol 4** 19-38

Armstrong B, Doll R.
Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices.
Int J Cancer. 1975 Apr 15 **15:4** 617-31

Artuso M, Esteve A, Brésil H, Vuillaume M, Hall J
The role of the Ataxia telangiectasia gene in the p53, WAF1/CIP1(p21)- and GADD45-mediated response to DNA damage produced by ionising radiation.
Oncogene 1995 Oct 19 **11:8** 1427-35

Attallah NL, Sharkawi NJ, Cambell JJ
Age at menarche of schoolgirls in the ASIR Region of Saudi Arabia, with a note on adult heights and weights.
Saudi Med J 1990 **11:1** 59-63

Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Hutzler P, Hofler H, Werner M
Intratumoral heterogeneity in breast carcinoma revealed by laser-microdissection and comparative genomic hybridization.
Cancer Genet Cytogenet 1999 Apr 15 **110:2** 94-102

Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Welzl G, Hofler H, Werner M.
Extensive ductal carcinoma In situ with small foci of invasive ductal carcinoma: evidence of genetic resemblance by CGH.
Int J Cancer. 2000 Jan 1 **85:1** 82-6

Aziz H, Hussain F, Sohn C, Mediavillo R, Saitta A, Hussain A, Brandys M, Homel P, Rotman M.
Early onset of breast carcinoma in African American women with poor prognostic factors.
Am J Clin Oncol. 1999 Oct **22:5** 436-40

Baak JP, Kurver PH, De Snoo-Niewlaat AJ, De Graef S, Makkink B, Boon ME
Prognostic indicators in breast cancer—morphometric methods.
Histopathology. 1982 May **6:3** 327-39.

Baak JPA, Oort J
A manual of morphometry in diagnostic pathology
Springer-Verlag (Pub) 1983

Baak JP, Van Dop H, Kurver PH, Hermans J
The value of morphometry to classic prognosticators in breast cancer.
Cancer. 1985 Jul 15 **56:2** 374-82

Baak JP, Wisse-Brekelmans EC, Kurver PH, van Gorp LH, Voorhorst FJ, Miettinen OS.
Regional differences in breast cancer survival are correlated with differences in
differentiation and rate of proliferation.
Hum Pathol. 1992 Sep **23:9** 989-92.

Bahl V.
Cancer and ethnic minorities--the Department of Health's perspective.
Br J Cancer Suppl. 1996 Sep **29** S2-10.

Barker RM, Baker MR.
Incidence of cancer in Bradford Asians.
J Epidemiol Community Health. 1990 Jun **44:2** 125-9

Barnes DE, Tomkinson AE, Lehmann AR, Webster AD, Lindahl T
Mutations in the DNA ligase I gene of an individual with immunodeficiencies and cellular
hypersensitivity to DNA-damaging agents.
Cell 1992a May 1 **69:3** 495-503

Barnes DM, Bartkova J, Camplejohn RS, Gullick WJ, Smith PJ, Millis RR
Overexpression of the c-erbB-2 oncoprotein: why does this occur more frequently in ductal
carcinoma in situ than in invasive mammary carcinoma and is this of prognostic
significance?
Eur J Cancer 1992b **28:2-3** 644-8

Beato M.
Gene regulation by steroid hormones.
Cell. 1989 Feb 10 **56:3** 335-44

Beckmann MW, Picard F, An HX, van Roeyen CR, Dominik SI, Mosny DS, Schnurch HG,
Bender HG, Niederacher D
Clinical impact of detection of loss of heterozygosity of BRCA1 and BRCA2 markers in
sporadic breast cancer.
Br J Cancer. 1996 May **73:10** 1220-6.

Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis
SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA
Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome.
Science. 1999 Dec 24 **286:5449** 2528-31

Ben-Ze'ev A
Cytoskeletal and adhesion proteins as tumor suppressors.
Curr Opin Cell Biol 1997 Feb **9:1** 99-108

Beral V, Hermon C, Kay C, Hannaford P, Darby S, Reeves G.
Mortality associated with oral contraceptive use: 25 year follow up of cohort of 46 000
women from Royal College of General Practitioners' oral contraception study.
BMJ. 1999 Jan 9 **318:7176** 96-100

Bergers E, Jannink I, van Diest PI, Cuesta MA, Meyer S, van Mourik JC, Baak JP.
The influence of fixation delay on mitotic activity and flow cytometric cell cycle variables.
Hum Pathol. 1997 Jan **28:1** 95-100

Bernardino J, Apiou F, Gerbault-Seureau M, Malfoy B, Dutrillaux B.
Characterization of recurrent homogeneously staining regions in 72 breast carcinomas.
Genes Chromosomes Cancer. 1998 Oct **23:2** 100-8.

Bernstein L, Ross RK, Lobo RA, Hanisch R, Krailo MD, Henderson BE
The effects of moderate physical activity on menstrual cycle patterns in adolescence: implications for breast cancer prevention.
Br J Cancer 1987 Jun **55:6** 681-5

Bertheau P, Steinberg SM, Cowan K, Merino MJ.
Breast cancer in young women: clinicopathologic correlation.
Semin Diagn Pathol. 1999 Aug;16 **3** 248-56.

Bethwaite PB, Koreth J, Herrington CS, McGee JO.
Loss of heterozygosity occurs at the D11S29 locus on chromosome 11q23 in invasive cervical carcinoma.
Br J Cancer. 1995 Apr **71:4** 814-8

Bhopal R.
Is research into ethnicity and health racist, unsound, or important science?
BMJ. 1997 Jun 14 **314:7096** 1751-6

Bieche I, Champeme MH, Matifas F, Hacene K, Callahan R, Lidereau R.
Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer.
Lancet. 1992 Jan 18 **339:8786** 139-43

Bieche I, Nogues C, Lidereau R.
Overexpression of BRCA2 gene in sporadic breast tumours.
Oncogene. 1999 Sep 16 **8:37** 5232-8.

Biesterfeld S, Noll I, Noll E, Wohltmann D, Bocking A.
Mitotic frequency as a prognostic factor in breast cancer.
Hum Pathol. 1995 Jan **26:1** 47-52.

Bilanges B, Varrault A, Basyuk E, Rodriguez C, Mazumdar A, Pantaloni C, Bockaert J, Theillet C, Spengler D, Journot L.
Loss of expression of the candidate tumor suppressor gene ZAC in breast cancer cell lines and primary tumors.
Oncogene. 1999 Jul 8 **18:27** 3979-88.

Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A.
Phyto-oestrogens: where are we now?
Br J Nutr. 1998 May **79:5** 393-406.

Birchmeier C, Birchmeier W
Molecular aspects of mesenchymal-epithelial interactions.
Annu Rev Cell Biol 1993: **9** 511-40

Bjerregaard B, Kung'u A.
Breast cancer in Kenya: a histopathologic and epidemiologic study.
East Afr Med J. 1992 Jan **69:1** 22-6.

Blamey RW, Bishop HM, Blake JR, Doyle PJ, Elston CW, Haybittle JL, Nicholson RI, Griffiths K
Relationship between primary breast tumor receptor status and patient survival.
Cancer. 1980 Dec 15 **46(12 Suppl)** 2765-9.

Bodmer W, Bishop T, Karran P
Genetic steps in colorectal cancer
Nat Genet 1994 Mar **6:3** 217-9

Bonneton C, Larue L, Thiery JP
Current data on the role of APC protein in the origin of colorectal cancer
Bull Cancer 1997 Nov **84:11** 1053-60

Bonsing BA, Devilee P, Cleton-Jansen AM, Kuipers-Dijkshoorn N, Fleuren GJ, Cornelisse CJ.
Evidence for limited molecular genetic heterogeneity as defined by allelotyping and clonal
analysis in nine metastatic breast carcinomas.
Cancer Res. 1993 Aug 15 **53:16** 3804-11

Borg A, Hedenfalk IA, Vallon-Christersson J, Johannsson O, Olsson H, Duggan DJ, Chen Y,
Bittner M, Kallioniemi O-P, Trent JM
Molecular characteristics of inherited breast tumors (abstract)
Breast-cancer-research 2000 **2 (Suppliment 1)** S6

Botha JL, Manku-Scott TK, Moledina F, Williams A.
Indirect discrimination and breast screening.
Ethn Dis. 1993 Spring **3:21** 89-95

Boveri T
Zur Frage der Entstehung maligner Tumoren
Gustav Fischer, Jena (pub) 1914

Boyer-Chammard A, Taylor TH, Anton-Culver H.
Survival differences in breast cancer among racial/ethnic groups: a population-based study.
Cancer Detect Prev. 1999 **23:6** 463-73.

Breast Cancer Linkage Consortium.
Pathology of familial breast cancer: differences between breast cancers in carriers of
BRCA1 or BRCA2 mutations and sporadic cases
Lancet. 1997 May 24 **349:9064** 1505-10

Breslow NE, Day NE
Statistical methods in cancer research. Vol 1- The analysis of case control studies
IARC Scientific Publications 1980 no.32

Brinton LA, Hoover R, Fraumeni JF Jr.
Menopausal oestrogens and breast cancer risk: an expanded case-control study.
Br J Cancer. 1986 Nov **54:5** 825-32

Brinton LA, Daling JR, Liff JM, Schoenberg JB, Malone KE, Stanford JL, Coates RJ, Gammon
MD, Hanson L, Hoover RN.
Oral contraceptives and breast cancer risk among younger women.
J Natl Cancer Inst. 1995a Jun 7 **87:11** 827-35

Brinton LA, Potischman NA, Swanson CA, Schoenberg JB, Coates RJ, Gammon MD, Malone
KE, Stanford JL, Daling JR.
Breastfeeding and breast cancer risk.
Cancer Causes Control. 1995b May **6:3** 199-208.

Brinton LA, Benichou J, Gammon MD, Brogan DR, Coates R, Schoenberg JB.
Ethnicity and variation in breast cancer incidence.
Int J Cancer. 1997 Nov 4 **73:3** 349-55

Brown JM, Benson EA, Jones M
Confirmation of a long-term prognostic index in breast cancer
The Breast 1993 **2:** 144-147

Buell P.
Changing incidence of breast cancer in Japanese-American women.
J Natl Cancer Inst. 1973 Nov **51:5** 1479-83

Buerger H, Otterbach F, Simon R, Schafer KL, Poremba C, Diallo R, Brinkschmidt C, Dockhorn-Dworniczak B, Boecker W
Different genetic pathways in the evolution of invasive breast cancer are associated with distinct morphological subtypes.
J Pathol. 1999a Dec **189:4** 521-6.

Buerger H, Otterbach F, Simon R, Poremba C, Diallo R, Decker T, Riethdorf L, Brinkschmidt C, Dockhorn-Dworniczak B, Boecker W.
Comparative genomic hybridization of ductal carcinoma in situ of the breast-evidence of multiple genetic pathways.
J Pathol. 1999b Mar **187:4** 396-402.

Bullough WS
Mitotic activity in the tissue of dead mice, and in tissues kept in physiological salt solution.
Exp Cell Res 1950 **1:** 410-420

Cade J, Thomas E, Vail A.
Case-control study of breast cancer in south east England: nutritional factors.
J Epidemiol Community Health. 1998 Feb **52:2** 105-10

Caligo MA, Polidoro L, Ghimenti C, Campani D, Cecchetti D, Bevilacqua G.
A region on the long arm of chromosome 16 is frequently deleted in metastatic node-negative breast cancer.
Int J Oncol. 1998 Jul **13:1** 177-82.

Callahan R, Cropp C, Merlo GR, Diella F, Venesio T, Lidereau R, Cappa AP, Lisicia DS.
Genetic and molecular heterogeneity of breast cancer cells.
Clin Chim Acta. 1993 Jul 30 **217:1** 63-73.

Campbell FC, Blamey RW, Elston CW, Nicholson RI, Griffiths K, Haybittle JL.
Oestrogen-receptor status and sites of metastasis in breast cancer.
Br J Cancer. 1981 Sep **44:3** 456-9

Cardoso MC, Leonhardt H, Nadal-Ginard B
Reversal of terminal differentiation and control of DNA replication: cyclin A and Cdk2 specifically localize at subnuclear sites of DNA replication.
Cell 1993 Sep 24 **74:6** 979-92

Carson DA, Lois A
Cancer progression and p53.
Lancet 1995 Oct 14 **346:8981** 1009-11

Carstens PH, Greenberg RA, Francis D, Lyon H
Tubular carcinoma of the breast. A long term follow-up.
Histopathology. 1985 Mar **9:3** 271-80

Carter CL, Allen C, Henson DE
Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases.
Cancer. 1989 Jan 1 **63:1** 181-7.

Castagnetta LA, Traina A, Liquori M, Marasà L, Amodio R, Di Falco M, Miele M, Rausa L, Carruba G
Quantitative image analysis of estrogen and progesterone receptors as a prognostic tool for selecting breast cancer patients for therapy.
Anal Quant Cytol Histol 1999 Feb **21:1** 59-62

Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE, Boyd J, Lubin MB, Deshano ML, Brody LC, et al
Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer.
Nat Genet. 1994 Dec **8:4** 387-91

Cavalli LR, Cavalieri LM, Ribeiro LA, Cavalli IJ, Silveira R, Rogatto SR.
Cytogenetic evaluation of 20 primary breast carcinomas.
Hereditas. 1997 **126:3** 261-8.

Caygill CP, Charlett A, Hill MJ.
Fat, fish, fish oil and cancer.
Br J Cancer. 1996 Jul **74:1** 159-64.

Central department of statistics
Population census1397 AH- 1977 AD, detailed data administrative area of Makkah
Ministry of Finance And National Economy, Kingdom of Saudi Arabia 1977

Central department of statistics
Statistical year book 1413 AH - 1993 AD
Ministry of Finance And National Economy, Kingdom of Saudi Arabia 1993 **29:**

Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B
14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage
Nature 1999 Oct 7 **401:6753** 616-20

Chappell SA, Walsh T, Walker RA, Shaw JA.
Loss of heterozygosity at chromosome 6q in preinvasive and early invasive breast carcinomas.
Br J Cancer. 1997 **75:9** 1324-9

Chaudary MA, Hayward JL, Bulbrook RD, Yoshida M, Miura S, Murai JT, Takatani O.
A comparison of epidemiological characteristics in breast cancer patients and normal women in Great Britain and Japan: results of a prospective study.
Breast Cancer Res Treat. 1991 May **18 Suppl 1** S19-22

Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore D 2d, Smith HS
Heterogeneity for allelic loss in human breast cancer.
J Natl Cancer Inst. 1992 Apr 1 **84:7** 506-10

Chuaqui RF, Zhuang Z, Emmert-Buck MR, Liotta LA, Merino MJ
Analysis of loss of heterozygosity on chromosome 11q13 in atypical ductal hyperplasia and in situ carcinoma of the breast.
Am J Pathol 1997 Jan **150:1** 297-303

Chung M, Chang HR, Bland KI, Wanebo HJ.
Younger women with breast carcinoma have a poorer prognosis than older women.
Cancer. 1996 Jan 1 **77:1** 97-103.

Cioce V, Castronovo V, Shmookler BM, Garbisa S, Grigioni WF, Liotta LA, Sobel ME.
Increased expression of the laminin receptor in human colon cancer.
J Natl Cancer Inst. 1991 Jan 2 **83:1** 29-36.

Ciolino HP, Daschner PJ, Wang TT, Yeh GC.
Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells.
Biochem Pharmacol. 1998 Jul 15 **56:2** 197-206.

Clark GM, McGuire WL
Progesterone receptors and human breast cancer.
Breast Cancer Res Treat. 1983 **3:2** 157-63

Clark GM, McGuire WL.
Steroid receptors and other prognostic factors in primary breast cancer.
Semin Oncol. 1988 Apr **15:2 Suppl 1** 20-5.

Clemmesen J
Carcinoma of the breast. Results from statistical research.
Br J Radiol 1948 **21:** 583-590

Cleton-Jansen AM, Moerland EW, Kuipers-Dijkshoorn NJ, Callen DF, Sutherland GR, Hansen B, Devilee P, Cornelisse CJ.
At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer.
Genes Chromosomes Cancer. 1994 Feb **9:2** 101-7.

Cleton-Jansen AM, Moerland HW, Callen DF, Doggett NA, Devilee P, Cornelisse CJ.
Mapping of the breast basic conserved gene (D16S444E) to human chromosome band 16q24.3.
Cytogenet Cell Genet. 1995 **68:1-2** 49-51

Cline MJ, Battifora H, Yokota J
Proto-oncogene abnormalities in human breast cancer: correlations with anatomic features and clinical course of disease.
J Clin Oncol 1987 Jul **5:7** 999-1006

Coates RJ, Uhler RJ, Hall HI, Potischman N, Brinton LA, Ballard-Barbash R, Gammon MD, Brogan DR, Daling JR, Malone KE, Schoenberg JB, Swanson CA.
Risk of breast cancer in young women in relation to body size and weight gain in adolescence and early adulthood.
Br J Cancer. 1999 Sep **81:1** 167-74.

Colditz GA, Frazier AL.
Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus.
Cancer Epidemiol Biomarkers Prev. 1995 Jul-Aug **4:5** 567-71.

Contesso G, Jotti GS, Bonadonna G.
Tumor grade as a prognostic factor in primary breast cancer.
Eur J Cancer Clin Oncol. 1989 Mar **25:3** 403-9

Croce CM, Nowell PC
Molecular basis of human B cell neoplasia.
Blood 1985 Jan **65:1** 1-7

Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH, Reid BJ
A p53-dependent mouse spindle checkpoint.
Science 1995 Mar 3 **267:5202** 1353-6

Daniel WW
Biostatistics: A foundation for analysis in the health sciences
Wiley series in probability and mathematical statistics-Applied 1991 **Fifth Edition.** *Wiley* (Pub)

Davis BW, Gelber RD, Goldhirsch A, Hartmann WH, Locher GW, Reed R, Golouh R, Save-Soderbergh J, Holloway L, Russell I, et al
Prognostic significance of tumor grade in clinical trials of adjuvant therapy for breast cancer with axillary lymph node metastasis.
Cancer 1986 Dec 15 **58:12** 2662-70

Davis FG, Persky VW, Ferre CD, Howe HL, Barrett RE, Haenszel WM.
Cancer incidence of Hispanics and non-Hispanic whites in Cook County, Illinois.
Cancer. 1995 Jun 15 **75:12** 2939-45

De Deckere EA
Possible beneficial effect of fish and fish n-3 polyunsaturated fatty acids in breast and colorectal cancer.
Eur J Cancer Prev. 1999 Jul **8:3** 213-21.

De Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR
A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia.
Nature 1982 Dec 23 **300:5894** 765-7

De la Rochefordiere A, Asselain B, Campana F, Scholl SM, Fenton J, Vilcoq JR, Durand JC, Pouillart P, Magdelenat H, Fourquet A.
Age as prognostic factor in premenopausal breast carcinoma.
Lancet. 1993 Apr 24 **341:8852** 1039-43.

Delesse A
Procede mechanique pour determines la composition des roches
CR Acadamy of Science (Paris) 1847 **25:** 544

De Lisi C
The age incidence of female breast cancer -simple models and the analysis of epidemiological patterns
Mathematical Biosci 1977 **37:** 245-266

Del Senno L, Aguiari GL, Piva R.
Dinucleotide repeat polymorphism in the human estrogen receptor (ESR) gene.
Hum Mol Genet. 1992 Aug;1 **5** 354.

Deng G, Chen LC, Schott DR, Thor A, Bhargava V, Ljung BM, Chew K, Smith HS.
Loss of heterozygosity and p53 gene mutations in breast cancer.
Cancer Res. 1994 Jan 15 **54:2** 499-505

De Potter CR, Beghin C, Makar AP, Vandekerckhove D, Roels HJ
The neu-oncogene protein as a predictive factor for haematogenous metastases in breast cancer patients.
Int J Cancer 1990 Jan 15 **45:1** 55-8

Devilee P, van Vliet M, van Sloun P, Kuipers Dijkshoorn N, Hermans J, Pearson PL, Cornelisse CJ
Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q.
Oncogene 1991 Sep **6:9** 1705-11

Devilee P, Cornelisse CJ
Somatic genetic changes in human breast cancer.
Biochim Biophys Acta 1994 Dec 30 **1198:2-3** 113-30

Devilee P, Schuuring E, van de Vijver MJ, Cornelisse CJ
Recent developments in the molecular genetic understanding of breast cancer.
Crit Rev Oncog 1994 **5:2-3** 247-70

De Waard F
The epidemiology of breast cancer: review and prospects.
Int J Cancer. 1969 Sep 15 **4:5** 577-86

De Waard F.
Premenopausal and postmenopausal breast cancer: one disease or two?
J Natl Cancer Inst. 1979 Sep **63:3** 549-52.

Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP.
Pten is essential for embryonic development and tumour suppression.
Nat Genet. 1998 Aug **19:4** 348-55.

Dixon JM, Anderson TJ, Page DL, Lee D, Duffy SW
Infiltrating lobular carcinoma of the breast.
Histopathology. 1982 Mar **6:2** 149-61

Djuric Z, Depper JB, Uhley V, Smith D, Lababidi S, Martino S, Heilbrun LK.
Oxidative DNA damage levels in blood from women at high risk for breast cancer are associated with dietary intakes of meats, vegetables, and fruits.
J Am Diet Assoc. 1998 May **98:5** 524-8

Doll R, Peto R.
The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today.
J Natl Cancer Inst. 1981 Jun **66:6** 1191-308.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS.
'Touchdown' PCR to circumvent spurious priming during gene amplification.
Nucleic Acids Res. 1991 Jul 25 **19:14** 4008

Donaldson LJ, Taylor JB.
Patterns of Asian and non-Asian morbidity in hospitals.
Br Med J (Clin Res Ed). 1983 Mar 19 **286:6369** 949-51

Donaldson LJ, Clayton DG.
Occurrence of cancer in Asians and non-Asians.
J Epidemiol Community Health. 1984 Sep **38:3** 203-7

Dorion-Bonnet F, Mautalen S, Hostein I, Longy M.
Allelic imbalance study of 16q in human primary breast carcinomas using microsatellite markers.
Genes Chromosomes Cancer. 1995 Nov **14:3** 171-81

Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, Weinberg RA
Physical interaction of the retinoblastoma protein with human D cyclins.
Cell 1993 May 7 **73:3** 499-511

Dufer J, Liautaud-Roger F, Barbarin D, Coninx P.
Nucleus image analysis as a possible prognostic tool in grading breast cancer.
Biomed Pharmacother. 1993 **47:4** 131-5.

Dulic V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper JW, Elledge SJ, Reed SI
p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest.
Cell 1994 Mar 25 **76:6** 1013-23

Dupont WD, Page DL
Risk factors for breast cancer in women with proliferative breast disease.
N Engl J Med 1985 Jan 17 **312:3** 146-51

Dutrillaux B, Gerbault-Seureau M, Zafrani B.
Characterization of chromosomal anomalies in human breast cancer. A comparison of 30 paradiploid cases with few chromosome changes.
Cancer Genet Cytogenet 1990 **49:2** 203-217

Dutrillaux B, Gerbault-Seureau M, Zafrani B.
Characterization of chromosomal anomalies in human breast cancer. A comparison of 30 paradiploid cases with few chromosome changes.
Cancer Genet Cytogenet. 1990 Oct 15 **49:2** 203-17.

Easton D, Ford D, Peto J
Inherited susceptibility to breast cancer.
Cancer Surv 1993 **18:** 95-113

Edwards MJ, Gamel JW, Vaughan WP, Wrightson WR
Infiltrating ductal carcinoma of the breast: the survival impact of race.
J Clin Oncol. 1998 Aug **16:8** 2693-9.

Eley JW, Hill HA, Chen VW, Austin DF, Wesley MN, Muss HB, Greenberg RS, Coates RJ, Correa P, Redmond CK, et al.
Racial differences in survival from breast cancer. Results of the National Cancer Institute Black/White Cancer Survival Study.
JAMA. 1994 Sep 28 **272:12** 947-54.

El-Hazmi MAF, Warsy AS
Prevalence of obesity in the Saudi population
Ann Saudi Med 1997 **17:** 302-306

Ellis PS, Whitehead R.
Mitosis counting--a need for reappraisal.
Hum Pathol. 1981 Jan **12:1** 3-4.

Ellis LM, Fidler IJ
Angiogenesis and breast cancer metastasis.
Lancet. 1995 Aug 12 **346:8972** 388-90

El Saghir NS, Adib S, Mufarrij A, Kahwaji S, Taher A, Issa P, Shamseddine AI.
Cancer in Lebanon: analysis of 10,220 cases from the American University of Beirut Medical Center.
J Med Liban. 1998 Jan-Feb **46:1** 4-11.

Elston CW, Ellis IO
Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up.
Histopathology. 1991 Nov **19:5** 403-10

El-Tamer MB, Homel P, Wait RB.
Is race a poor prognostic factor in breast cancer?
J Am Coll Surg. 1999 Jul **189:1** 41-5

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjold M, Gustafsson JA
Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern.
J Clin Endocrinol Metab. 1997 Dec **82:12** 4258-65

Erlich HA, Gelfand D, Sninsky JJ.
Recent advances in the polymerase chain reaction.
Science. 1991 Jun 21 **252:5013** 1643-51

Escot C, Theillet C, Lidereau R, Spyratos F, Champeme MH, Gest J, Callahan R
Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas.
Proc Natl Acad Sci U S A 1986 Jul **83:13** 4834-8

Eskelinen M, Lipponen P, Aaltomaa S, Kosma VM, Syrjanen K, Alhava E.
Breast cancer in young women: clinical, histological and morphometric prognostic factors.
Anticancer Res. 1992 Jul-Aug **12:4** 1237-42

Esteve J, Benhamou E, Raymond
Statistical methods in cancer research vol IV: Descriptive epidemiology
IARC Scientific Publications 1994 **no.128**

Eyfjord JE, Thorlacius S, Steinarsdottir M, Valgardsdottir R, Ogmundsdottir HM, Anamthawat-Jonsson K
p53 abnormalities and genomic instability in primary human breast carcinomas.
Cancer Res. 1995 Feb 1 **55:3** 646-51

Ezzat A, Raja M, Te O, Michels D, Bazarbashi S
Frequency and distribution of 22,836 adult cancer cases referred to King Faisal Specialist Hospital and Research Centre.
Ann Saudi Med 1966 **16:** 152-158

Ezzat A, Raja MA, Zwaan F, Brigden M, Rostom A, Bazarbashi S.
The lack of age as a significant prognostic factor in non-metastatic breast cancer.
Eur J Surg Oncol. 1998 Feb **24:1** 23-7.

Ezzat AA, Ibrahim EM, Raja MA, Al-Sobhi S, Rostom A, Stuart RK
Locally advanced breast cancer in Saudi Arabia: high frequency of stage III in a young population.
Med Oncol 1999 Jul **16:2** 95-103

Farewell VT
Statistical methods and mathematical models for research in breast disease
Commentaries on research in breast disease 1979 **1:** 193-232

Fearon ER, Vogelstein B
A genetic model for colorectal tumorigenesis.
Cell. 1990 Jun 1 **61:5** 759-67

Fearon ER
Genetic alterations underlying colorectal tumorigenesis.
Cancer Surv. 1992 **12:**119-36

Feigelson HS, Ross RK, Yu MC, Coetzee GA, Reichardt JK, Henderson BE.
Genetic susceptibility to cancer from exogenous and endogenous exposures.
J Cell Biochem Suppl. 1996 **25:** 15-22.

Ferguson DJ, Meier P, Karrison T, Dawson PJ, Straus FH, Lowenstein FE
Staging of breast cancer and survival rates. An assessment based on 50 years of experience with radical mastectomy.
JAMA. 1982 Sep 17 **248:11** 1337-41

Fialkow PJ
Clonal origin of human tumors.
Biochim Biophys Acta 1976 Oct 12 **458:3** 283-321

Field JK, Kiaris H, Howard P, Vaughan ED, Spandidos DA, Jones AS
Microsatellite instability in squamous cell carcinoma of the head and neck.
Br J Cancer 1995 May **71:5** 1065-9

Fisher ER, Palekar AS, Redmond C, Barton B, Fisher B
Pathologic findings from the National Surgical Adjuvant Breast Project (protocol no. 4). VI. Invasive papillary cancer.
Am J Clin Pathol. 1980 Mar **73:3** 313-22

Fisher CJ, Egan MK, Smith P, Wicks K, Millis RR, Fentiman IS.
Histopathology of breast cancer in relation to age.
Br J Cancer. 1997 **75:4** 593-6.

Forsyth IA
The mammary gland.
Baillieres Clin Endocrinol Metab. 1991 Dec **5:4** 809-32

Fowble BL, Schultz DJ, Overmoyer B, Solin LJ, Fox K, Jardines L, Orel S, Glick JH.
The influence of young age on outcome in early stage breast cancer.
Int J Radiat Oncol Biol Phys. 1994 Aug 30 **30:1** 23-33.

Fraumeni JF Jr, Mason TJ
Cancer mortality among Chinese Americans, 1950-69.
J Natl Cancer Inst. 1974 Mar **52:3** 659-65.

Frebourg T, Friend SH
Cancer risks from germline p53 mutations.
J Clin Invest 1992 Nov **90:5** 1637-41

Frierson HF Jr, Wolber RA, Berean KW, Franquemont DW, Gaffey MJ, Boyd JC, Wilbur DC.
Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson
histologic grading scheme for infiltrating ductal carcinoma.
Am J Clin Pathol. 1995 Feb **103:2** 195-8

Fujii H, Szumel R, Marsh C, Zhou W, Gabrielson E.
Genetic progression, histological grade, and allelic loss in ductal carcinoma in situ of the
breast.
Cancer Res. 1996 Nov 15 **56:22** 5260-5

Fujii S, Takeshima Y, Arihiro K, Kaneko M, Inai K
Microsatellite instability in breast cancers with special reference to patients' age and
bilaterality.
Hiroshima J Med Sci 1998 Sep **47:3** 89-97

Futreal PA, Soderkvist P, Marks JR, Iglehart JD, Cochran C, Barrett JC, Wiseman RW.
Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast
carcinoma using microsatellite length polymorphisms.
Cancer Res. 1992 May 1 **52:9** 2624-7.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM,
Haugen-Strano A, Swensen J, Miki Y,
BRCA1 mutations in primary breast and ovarian carcinomas.
Science. 1994 Oct 7 **266:5182** 120-2

Gadbois DM, Bradbury EM, Lehnert BE
Control of radiation-induced G1 arrest by cell-substratum interactions.
Cancer Res 1997 Mar 15 **57:6** 1151-6

Gail M, Rimer B.
Risk-based recommendations for mammographic screening for women in their forties.
J Clin Oncol. 1998 Sep **16:9** 3105-14.

Galea MH, Blamey RW, Elston CE, Ellis IO.
The Nottingham Prognostic Index in primary breast cancer.
Breast Cancer Res Treat. 1992 **22:3** 207-19.

Garcia I, Dietrich PY, Aapro M, Vauthier G, Vadas L, Engel E
Genetic alterations of c-myc, c-erbB-2, and c-Ha-ras protooncogenes and clinical
associations in human breast carcinomas.
Cancer Res 1989 Dec 1 **49:23** 6675-9

Gasparini G, Weidner N, Bevilacqua P, Maluta S, Dalla Palma P, Caffo O, Barbareschi M,
Boracchi P, Marubini E, Pozza F
Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel
invasion are relevant prognostic markers in node-negative breast carcinoma.
J Clin Oncol. 1994 Mar **12:3** 454-66

Gastrin G.
Programme to encourage self-examination for breast cancer.
Br Med J. 1980 Jul 19 **281:6234** 193.

Gayther SA, de Foy KA, Harrington P, Pharoah P, Dunsmuir WD, Edwards SM, Gillett C, Ardern-Jones A, Dearnaley DP, Easton DF, Ford D, Shearer RJ, Kirby RS, Dowe AL, Kelly J, Stratton MR, Ponder BA, Barnes D, Eeles RA
The frequency of germ-line mutations in the breast cancer predisposition genes BRCA1 and BRCA2 in familial prostate cancer.
Cancer Res 2000 **60:45** 4513-4518

Gebhart E, Bruderlein S, Augustus M, Siebert E, Feldner J, Schmidt W.
Cytogenetic studies on human breast carcinomas.
Breast Cancer Res Treat. 1986 **8:2** 125-38

German J
Genes which increase chromosomal instability in somatic cells and predispose to cancer.
Prog Med Genet 1972 **8** 61-101

Gibas Z, Prout GR Jr, Connolly JG, Pontes JE, Sandberg AA
Nonrandom chromosomal changes in transitional cell carcinoma of the bladder.
Cancer Res 1984 Mar **44:3** 1257-64

Gillett D, Kennedy C, Carmalt H.
Breast cancer in young women.
Aust N Z J Surg. 1997 Nov **67:11** 761-4

Gilliland FD.
Ethnic differences in cancer incidence: a marker for inherited susceptibility?
Environ Health Perspect. 1997 Jun **105 Suppl 4** 897-900.

Girard F, Strausfeld U, Fernandez A, Lamb NJ
Cyclin A is required for the onset of DNA replication in mammalian fibroblasts.
Cell 1991 Dec 20 **67:6** 1169-79

Goel AK, Seenu V, Shukla NK, Raina V.
Breast cancer presentation at a regional cancer centre.
Natl Med J India. 1995 Jan-Feb **8:1** 6-9.

Gonzalez R, Silva JM, Dominguez G, Garcia JM, Martinez G, Vargas J, Provencio M, Espana P, Bonilla F.
Detection of loss of heterozygosity at RAD51, RAD52, RAD54 and BRCA1 and BRCA2 loci in breast cancer: pathological correlations.
Br J Cancer. 1999 Oct **81:3** 503-9.

Gottlieb S.
HRT raises risk of rare, treatable breast cancer.
BMJ. 1999 Jun 19 **318:7199** 1645B.

Goulding H, Abdul Rashid NF, Robertson JF, Bell JA, Elston CW, Blamey RW, Ellis IO.
Assessment of angiogenesis in breast carcinoma: an important factor in prognosis?
Hum Pathol. 1995 Nov **26:11** 1196-200

Gretarsdottir S, Tryggvadottir L, Jonasson JG, Sigurdsson H, Olafsdottir K, Agnarsson BA, Ogmundsdottir H, Eyfjord JE.
TP53 mutation analyses on breast carcinomas: a study of paraffin-embedded archival material.
Br J Cancer. 1996 Aug; **74:4** 555-61

Grulich AE, McCredie M, Coates M.
Cancer incidence in Asian migrants to New South Wales, Australia.
Br J Cancer. 1995 Feb **71:2** 400-8

Guan LS, Rauchman M, Wang ZY
Induction of Rb-associated protein (RbAp46) by Wilms' tumor suppressor WT1 mediates growth inhibition.
J Biol Chem 1998 Oct 16 **273:42** 27047-50

Haapasalo H, Pesonen E, Collan Y.
Volume corrected mitotic index (M/V-INDEX). The standard of mitotic activity in neoplasms.
Pathol Res Pract. 1989 Nov **185:5** 551-4.

Haenszel W, Kurihara M.
Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States.
J Natl Cancer Inst. 1968 Jan **40:1** 43-68

Hainsworth PJ, Raphael KL, Stillwell RG, Bennett RC, Garson OM
Cytogenetic features of twenty-six primary breast cancers.
Cancer Genet Cytogenet 1991 Jun **53:2** 205-18

Hakama M.
The peculiar age specific incidence curve for cancer of the breast--Clemmesen's hook.
Acta Pathol Microbiol Scand. 1969 **75:3** 370-4.

Hakama M.
Age-adjustment of incidence rates in cancer epidemiology.
Acta Pathol Microbiol Scand Suppl. 1970 **213** 7-47

Hakama M, Riihimaki H.
End results of breast cancer patients in Finland 1953-1968.
Ann Clin Res. 1974 Apr **6:2** 115-21

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC
Linkage of early-onset familial breast cancer to chromosome 17q21.
Science. 1990 Dec 21 **250:4988** 1684-9

Hall NR, Fish DE, Hunt N, Goldin RD, Guillou PJ, Monson JR
Is the relationship between angiogenesis and metastasis in breast cancer real?
Surg Oncol. 1992 Jun **1:3** 223-9

Hankey BF, Miller B, Curtis R, Kosary C.
Trends in breast cancer in younger women in contrast to older women.
J Natl Cancer Inst Monogr. 1994 **16** 7-14

Hannon GJ, Beach D
p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest [see comments]
Nature 1994 Sep 15 **371:6494** 257-61

Hansen MF, Cavenee WK
Genetics of cancer predisposition.
Cancer Res 1987 Nov 1 **47:21** 5518-27

Hansen LL, Yilmaz M, Overgaard J, Andersen J, Kruse TA.
Allelic loss of 16q23.2-24.2 is an independent marker of good prognosis in primary breast cancer.
Cancer Res. 1998 May 15 **58:10** 2166-9.

Harland JO, White M, Bhopal RS, Raybould S, Unwin NC, Alberti KG.
Identifying Chinese populations in the UK for epidemiological research: experience of a name analysis of the FHSA register. Family Health Services Authority.
Public Health. 1997 Sep **111:5** 331-7.

Harris H, Miller OJ, Klein G, Worst P, Tachibana T
Suppression of malignancy by cell fusion.
Nature 1969 Jul 26 **223:204** 363-8

Harris M, Howell A, Chrissohou M, Swindell RI, Hudson M, Sellwood RA
A comparison of the metastatic pattern of infiltrating lobular carcinoma and infiltrating duct carcinoma of the breast.
Br J Cancer. 1984 Jul **50:1** 23-30

Harris CC, Hollstein M
Clinical implications of the p53 tumor-suppressor gene.
N Engl J Med. 1993 Oct 28 **329:18** 1318-27

Harris CC.
The 1995 Walter Hubert Lecture--molecular epidemiology of human cancer: insights from the mutational analysis of the p53 tumour-suppressor gene.
Br J Cancer. 1996 Feb **73:3** 261-9..

Hartmann A, Blaszyk H, Saitoh S, Tsushima K, Tamura Y, Cunningham JM, McGovern RM, Schroeder JJ, Sommer SS, Kovach JS.
High frequency of p53 gene mutations in primary breast cancers in Japanese women, a low-incidence population.
Br J Cancer. 1996 Apr **73:8** 896-901.

Hartwell LH, Weinert TA
Checkpoints: controls that ensure the order of cell cycle events.
Science 1989 Nov 3 **246:4930** 629-34

Hassan I, Onukak EE, Mabogunje OA.
Breast cancer in Zaria, Nigeria.
J R Coll Surg Edinb. 1992 Jun **37:3** 159-61.

Hastie ND
Wilms' tumour gene and function.
Curr Opin Genet Dev 1993 Jun **3:3** 408-13

Hedeen AN, White E, Taylor V.
Ethnicity and birthplace in relation to tumor size and stage in Asian American women with breast cancer.
Am J Public Health. 1999 Aug **89:8** 1248-52.

Heintz NH, Leslie KO, Rogers LA, Howard PL.
Amplification of the c-erb B-2 oncogene and prognosis of breast adenocarcinoma.
Arch Pathol Lab Med. 1990 Feb **114:2** 160-3.

Hemler ME, Elices MJ, Parker C, Takada Y
Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions.
Immunol Rev 1990 Apr **114:** 45-65

Henderson BE, Ross RK, Lobo RA, Pike MC, Mack TM
Re-evaluating the role of progestogen therapy after the menopause.
Fertil Steril. 1988 May **49:5** Suppl 2 9S-15S.

Henson DE, Ries L, Freedman LS, Carriaga M
Relationship among outcome, stage of disease, and histologic grade for 22,616 cases of breast cancer. The basis for a prognostic index.
Cancer. 1991 Nov 15 **68:10** 2142-9.

Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW, Vogelstein B
14-3-3 sigma is a p53-regulated inhibitor of G2/M progression.
Mol Cell 1997 Dec **1:1** 3-11

Hermsen MA, Baak JP, Meijer GA, Weiss JM, Walboomers JW, Snijders PJ, van Diest PJ.
Genetic analysis of 53 lymph node-negative breast carcinomas by CGH and relation to clinical, pathological, morphometric, and DNA cytometric prognostic factors.
J Pathol. 1998 Dec **186:4** 356-62

Hirose K, Tajima K, Hamajima N, Inoue M, Takezaki T, Kuroishi T, Yoshida M, Tokudome S.
A large-scale, hospital-based case-control study of risk factors of breast cancer according to menopausal status.
Jpn J Cancer Res. 1995 Feb **86:2** 146-54.

Hollstein M, Sidransky D, Vogelstein B, Harris CC
p53 mutations in human cancers.
Science 1991 Jul 5 **253:5015** 49-53

Holmes MD, Hunter DJ, Colditz GA, Stampfer MJ, Hankinson SE, Speizer FE, Rosner B, Willett WC.
Association of dietary intake of fat and fatty acids with risk of breast cancer.
JAMA. 1999 Mar 10 **281:10** 914-20

Honda H, Ohi Y, Umekita Y, Takasaki T, Kuriwaki K, Ohyabu I, Yoshioka T, Yoshida A, Taguchi S, Ninomiya K, Akiba S, Nomura S, Sagara Y, Yoshida H.
Obesity affects expression of progesterone receptors and node metastasis of mammary carcinomas in postmenopausal women without a family history.
Pathol Int. 1999 Mar **49:3** 198-202.

Hori A, Sasada R, Matsutani E, Naito K, Sakura Y, Fujita T, Kozai Y
Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against human basic fibroblast growth factor.
Cancer Res. 1991 Nov 15 **51:22** 6180-4

Houlston RS, McCarter E, Parbhoo S, Scurr JH, Slack J
Family history and risk of breast cancer.
J Med Genet. 1992 Mar **29:3** 154-7.

Huang CS, Chen YC, Wang M, Lin JK, Chang KJ.
HER-2/neu overexpression in Chinese breast cancers: correlation with other prognostic factors.
J Formos Med Assoc. 1995 Nov **94:11** 660-5.

Hunter CP, Redmond CK, Chen VW, Austin DF, Greenberg RS, Correa P, Muss HB, Forman MR, Wesley MN, Blacklow RS, et al.
Breast cancer: factors associated with stage at diagnosis in black and white women. Black/White Cancer Survival Study Group.
J Natl Cancer Inst. 1993 Jul 21 **85:14** 1129-37.

Hunter DJ, Willett WC.
Nutrition and breast cancer.
Cancer Causes Control. 1996 Jan **7:1** 56-68

Hussain MA, Ali S, Tyagi SP, Reza H
Incidence of cancer breast at Aligarh.
J Indian Med Assoc. 1994 Sep **92:9** 296-7

Hynes RO
Integrins: versatility, modulation, and signaling in cell adhesion.
Cell 1992 Apr 3 **69:1** 11-25

Ibrahim EM
Pattern and prognosis of breast cancer: Data from the Eastern Province of Saudi Arabia
Saudi Med J 1991 **12:3** 227-231

Ibrahim EM, al-Mulhim FA, al-Amri A, al-Muhanna FA, Ezzat AA, Stuart RK, Ajarim D.
Breast cancer in the eastern province of Saudi Arabia.
Med Oncol. 1998 Dec **15:4** 241-7

Iida A, Isobe R, Yoshimoto M, Kasumi F, Nakamura Y, Emi M
Localization of a breast cancer tumour-suppressor gene to a 3-cM interval within chromosomal region 16q22.
Br J Cancer 1997 **75:2** 264-7

Ijaduola TG, Smith EB.
Pattern of breast cancer among white-American, African-American, and nonimmigrant west-African women.
J Natl Med Assoc. 1998 Sep **90:9** 547-51.

Ingram D, Sanders K, Kolybaba M, Lopez D.
Case-control study of phyto-oestrogens and breast cancer.
Lancet. 1997 Oct 4 **350:9083** 990-4.

Innis MA, Myambo KB, Gelfand DH, Brow MA.
DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA.
Proc Natl Acad Sci U S A. 1988 Dec, **85:24** 9436-40.

Innocente SA, Abrahamson JL, Cogswell JP, Lee JM
p53 regulates a G2 checkpoint through cyclin B1.
Proc Natl Acad Sci U S A 1999 Mar 2 **96:5** 2147-52

Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M
Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis.
Nature 1993 Jun 10 **363:6429** 558-61

Iscovich JM, Parkin DM
The risk of cancer in three generations of young Israelis: A study of migrants and their descendants
IARC technical report 1998 **27**:

Iwase H, Greenman JM, Barnes DM, Bobrow L, Hodgson S, Mathew CG.
Loss of heterozygosity of the oestrogen receptor gene in breast cancer.
Br J Cancer. 1995 Mar **71:3** 448-50.

Iwase H, Iwata H, Toyama T, Hara Y, Omoto Y, Kobayashi S.
Prognostic significance of loss of heterozygosity in breast cancer
Gan To Kagaku Ryoho. 1998 Apr **25 Suppl 3** 426-30

Jain VK, Judde JG, Max EE, Magrath IT
Variable IgH chain enhancer activity in Burkitt's lymphomas suggests an additional, direct mechanism of c-myc deregulation.
J Immunol 1993 Jun 15 **150:12** 5418-28

Jannink I, van Diest PJ, Baak JP.
Comparison of the prognostic value of mitotic frequency and mitotic activity index in breast cancer
The Breast 1996 **5:** 31-36

Jeffreys AJ, Wilson V, Thein SL
Hypervariable 'minisatellite' regions in human DNA.
Nature 1985 Mar 7-13 **314:6006** 67-73

Jones DY, Schatzkin A, Green SB, Block G, Brinton LA, Ziegler RG, Hoover R, Taylor PR.
Dietary fat and breast cancer in the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study.
J Natl Cancer Inst. 1987 Sep **79:3** 465-71

Kahan E, Ibrahim AS, El Najjar K, Ron E, Al-Agha H, Polliack A, El-Bolkainy MN.
Cancer patterns in the Middle East--special report from the Middle East Cancer Society.
Acta Oncol. 1997 **36:6** 631-6

Kashiwaba M, Tamura G, Suzuki Y, Maesawa C, Ogasawara S, Sakata K, Satodate R.
Epithelial-cadherin gene is not mutated in ductal carcinomas of the breast.
Jpn J Cancer Res. 1995 Nov **86:11** 1054-9

Kawasaki ES
Sample preparation from blood, cells, and other fluids, PCR protocols: a guide to methods and applications
Innis Gelfand DH, Sninsky JJ, White TJ (ed). *Academic press* (pub) 1990 146-152

Kelsey JL, Horn-Ross PL.
Breast cancer: magnitude of the problem and descriptive epidemiology.
Epidemiol Rev. 1993 **15:1** 7-16

Kerangueven F, Noguchi T, Coulier F, Allione F, Wargniez V, Simony-Lafontaine J, Longy M, Jacquemier J, Sobol H, Eisinger F, Birnbaum D
Genome-wide search for loss of heterozygosity shows extensive genetic diversity of human breast carcinomas.
Cancer Res. 1997 Dec 15 **57:24** 5469-74

Kerr JF, Wyllie AH, Currie AR
Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.
Br J Cancer 1972 Aug **26:4** 239-57

Khan A, Hussain NK, Al-Saigh A, Malatani T Sheikha AA
Pattern of cancer at ASIR Central Hospital, Abha Saudi Arabia.
Ann Saudi Med 1991 **11:** 285-288

Khlat M.
Cancer in Mediterranean migrants--based on studies in France and Australia.
Cancer Causes Control. 1995 Nov **6:6** 525-31

Khlat M, Bouchardy C, Parkin DM.
Cancer mortality in immigrants from the Near East in Australia
Rev Epidemiol Sante Publique. 1993 **41:3** 208-17

Kingston HM
ABC of clinical genetics. Genetics of cancer.
BMJ 1989 Apr 22 **298:6680** 1086-9

Kinlen LJ.
Meat and fat consumption and cancer mortality: A study of strict religious orders in Britain.
Lancet. 1982 Apr 24 **1:8278** 946-9

Kirchweger R, Zeillinger R, Schneeberger C, Speiser P, Louason G, Theillet C
Patterns of allele losses suggest the existence of five distinct regions of LOH on chromosome 17 in breast cancer.
Int J Cancer. 1994 Jan 15 **56:2** 193-9

Kliewer EV, Smith KR
Breast cancer mortality among immigrants in Australia and Canada.
J Natl Cancer Inst. 1995 Aug 2 **87:15** 1154-61

Knudson AG Jr
Mutation and cancer: statistical study of retinoblastoma.
Proc Natl Acad Sci U S A 1971 Apr **68:4** 820-3

Knudson AG Jr
Hereditary cancer, oncogenes, and antioncogenes.
Cancer Res 1985 Apr **45:4** 1437-43

Kodama M, Kodama T, Kodama M
The genesis of breast cancer is a two-step phenomenon. I. Differential effects of aging on the cancer incidence in the United Kingdom and Japan.
Anticancer Res. 1992 Jan-Feb **12:1** 145-51

Koenders PG, Beex LV, Kienhuis CB, Kloppenborg PW, Benraad TJ
Epidermal growth factor receptor and prognosis in human breast cancer: a prospective study.
Breast Cancer Res Treat. 1993 **25:1** 21-7.

Koff A, Ohtsuki M, Polyak K, Roberts JM, Massagué J
Negative regulation of G1 in mammalian cells: inhibition of cyclin E-dependent kinase by TGF-beta.
Science 1993 Apr 23 **260:5107** 536-9

Kollias J, Elston CW, Ellis IO, Robertson JF, Blamey RW.
Early-onset breast cancer--histopathological and prognostic considerations.
Br J Cancer. 1997 **75:9** 1318-23.

Komarova EA, Diatchenko L, Rokhlin OW, Hill JE, Wang ZJ, Krivokrysenko VI,Feinstein E, Gudkov AV
Stress-induced secretion of growth inhibitors: a novel tumor suppressor function of p53.
Oncogene 1998 Sep 3 **17:9** 1089-96

Koreth J, Bethwaite PB, McGee JO.
Mutation at chromosome 11q23 in human non-familial breast cancer: a microdissection microsatellite analysis.
J Pathol. 1995 May **176:1** 11-8

Koriech OM, Al-Kuhaymi R
Profile of cancer in Riyadh Armed Forces Hospital
Ann Saudi Med 1994 **14:2** 187-194

Korsmeyer SJ
Bcl-2: an antidote to programmed cell death.
Cancer Surv 1992 **15:** 105-18

Koscielny S, Le MG, Tubiana M.
The natural history of human breast cancer. The relationship between involvement of axillary lymph nodes and the initiation of distant metastases.
Br J Cancer. 1989 May **59:5** 775-82.

Koscielny S, Tubiana M, Le MG, Valleron AJ, Mouriesse H, Contesso G, Sarrazin D
Breast cancer: relationship between the size of the primary tumour and the probability of metastatic dissemination.
Br J Cancer. 1984 Jun **49:6** 709-15

Koskinen PJ, Alitalo K
Role of myc amplification and overexpression in cell growth, differentiation and death.
Semin Cancer Biol 1993 Feb **4:1** 3-12

Kossoff MB.
Ultrasound of the breast.
World J Surg. 2000 Feb **24:2** 143-57

Kremmidiotis G, Baker E, Crawford J, Eyre HJ, Nahmias J, Callen DF.
Localization of human cadherin genes to chromosome regions exhibiting cancer-related loss of heterozygosity.
Genomics. 1998 May 1 **49:3** 467-71

Krieger N, van den Eeden SK, Zava D, Okamoto A.
Race/ethnicity, social class, and prevalence of breast cancer prognostic biomarkers: a study of white, black, and Asian women in the San Francisco bay area.
Ethn Dis. 1997 Spring-Summer **7:2** 137-49

Kronqvist P, Collan Y, Kuopio T, Kujari H.
Nuclear morphometry in breast cancer: the influence of sampling rules and freezing of samples.
Mod Pathol. 1995 Feb **8:2** 187-92.

Kronqvist P, Kuopio T, Collan Y.
Morphometric grading of invasive ductal breast cancer. I. Thresholds for nuclear grade.
Br J Cancer. 1998 Sep **78:6** 800-5..

Kumar NB, Hart WR
Metastases to the uterine corpus from extragenital cancers. A clinicopathologic study of 63 cases.
Cancer. 1982 Nov 15 **50:10** 2163-9.

Kumar V, Green S, Staub A, Chambon P
Localisation of the oestradiol-binding and putative DNA-binding domains of the human oestrogen receptor.
EMBO J. 1986 Sep **5:9** 2231-6

Kumar NB, Lyman GH, Allen K, Cox CE, Schapira DV.
Timing of weight gain and breast cancer risk.
Cancer. 1995 Jul 15 **76:2** 243-9.

Ladekarl M, Jensen V.
Quantitative histopathology in lymph node-negative breast cancer. Prognostic significance of mitotic counts.
Virchows Arch. 1995 **427:3** 265-70

Lagios MD, Margolin FR, Westdahl PR, Rose MR
Mammographically detected duct carcinoma in situ. Frequency of local recurrence following tylectomy and prognostic effect of nuclear grade on local recurrence.
Cancer. 1989 Feb 15 **63:4** 618-24

Lahiri DK, Zhang A, Nurnberger JI Jr.
High-resolution detection of PCR products from a microsatellite marker using a nonradioisotopic technique.
Biochem Mol Med. 1997 Feb **60:1** 70-5

Lakhani SR, Collins N, Stratton MR, Sloane JP
Atypical ductal hyperplasia of the breast: clonal proliferation with loss of heterozygosity on chromosomes 16q and 17p.
J Clin Pathol 1995 Jul **48:7** 611-5

Lakhani SR.
The transition from hyperplasia to invasive carcinoma of the breast.
J Pathol. 1999 Feb **187:3** 272-8.

Lakhani SR, Chaggar R, Davies S, Jones C, Collins N, Odel C, Stratton MR, O'Hare MJ
Genetic alterations in 'normal' luminal and myoepithelial cells of the breast.
J Pathol 1999 Dec **189:4** 496-503

Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, Seal S, Barfoot R, Collins N, Bignell G, Patel S, Hamoudi R, Larsson C, Wiseman RW, Berchuck A, Iglehart JD, Marks JR, Ashworth A, Stratton MR, Futreal PA.
BRCA2 mutations in primary breast and ovarian cancers.
Nat Genet. 1996 Jun **13:2** 238-40

Lander ES, Botstein D
Strategies for studying heterogeneous genetic traits in humans by using a linkage map of restriction fragment length polymorphisms.
Proc Natl Acad Sci U S A. 1986 Oct **83:19** 7353-7.

Landers JP, Spelsberg TC
New concepts in steroid hormone action: transcription factors, proto-oncogenes, and the cascade model for steroid regulation of gene expression.
Crit Rev Eukaryot Gene Expr. 1992 **2:1** 19-63.

Lane DP, Benchimol S.
p53: oncogene or anti-oncogene?
Genes Dev. 1990 Jan **4:1** 1-8.

Lane DP
Cancer. A death in the life of p53
Nature 1993 Apr 29 **362:6423** 786-7

Lane DP
p53 and human cancers.
Br Med Bull 1994 Jul **50:3** 582-99

Langston AA, Malone KE, Thompson JD, Daling JR, Ostrander EA.
BRCA1 mutations in a population-based sample of young women with breast cancer.
N Engl J Med. 1996 Jan 18 **334:3** 137-42

Larson RA, Williams SF, Le Beau MM, Bitter MA, Vardiman JW, Rowley JD
Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) or t(16;16) has a favorable prognosis.
Blood 1986 Dec **68:6** 1242-9

Lasko D, Cavenee W, Nordenskjold M
Loss of constitutional heterozygosity in human cancer.
Annu Rev Genet. 1991 **25:** 281-314.

Lasky LA
Selectins: interpreters of cell-specific carbohydrate information during inflammation.
Science. 1992 Nov 6 **258:5084** 964-9

La Vecchia C, Favero A, Franceschi S.
Monounsaturated and other types of fat, and the risk of breast cancer.
Eur J Cancer Prev. 1998 Dec **7:6** 461-4.

Lawson JS, Field AS, Champion S, Tran D, Ishikura H, Trichopoulos D.
Low oestrogen receptor alpha expression in normal breast tissue underlies low breast cancer incidence in Japan.
Lancet. 1999 Nov 20 **354:9192** 1787-8

Lee SW
H-cadherin, a novel cadherin with growth inhibitory functions and diminished expression in human breast cancer.
Nat Med 1996 Jul **2:7** 776-82

Lee SW, Reimer CL, Campbell DB, Cheresh P, Duda RB, Kocher O
H-cadherin expression inhibits in vitro invasiveness and tumor formation in vivo.
Carcinogenesis. 1998 Jun **19:6** 1157-9.

Lee CH, Smith RC, Levine JA, Troiano RN, Tocino I.
Clinical usefulness of MR imaging of the breast in the evaluation of the problematic mammogram.
AJR Am J Roentgenol. 1999 Nov **173:5** 1323-9

Lesser ML, Rosen PP, Kinne DW
Multicentricity and bilaterality in invasive breast carcinoma.
Surgery. 1982 Feb **91:2** 234-40

Ley K, Gaehtgens P, Fennie C, Singer MS, Lasky LA, Rosen SD
Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo.
Blood. 1991 Jun 15 **77:12** 2553-5

Li FP, Fraumeni JF Jr
Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?
Ann Intern Med. 1969 Oct **71:4** 747-52

Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R.
Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome.
Nat Genet. 1997 May **16:1** 64-7

Lindblom A, Rotstein S, Skoog L, Nordenskjold M, Larsson C
Deletions on chromosome 16 in primary familial breast carcinomas are associated with development of distant metastases.
Cancer Res. 1993 Aug 15 **53:16** 3707-11.

Liotta LA, Saidel MG, Kleinerman J
The significance of hematogenous tumor cell clumps in the metastatic process.
Cancer Res. 1976 Mar **36:3** 889-94.

Litt M, Luty JA.
A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene.
Am J Hum Genet. 1989 Mar **44:3** 397-401.

Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, Papadopoulos N, Fujiwara T, Jen J, Kinzler KW, Wyllie AH, Vogelstein B, Dunlop MG
Genetic instability occurs in the majority of young patients with colorectal cancer
Nat Med 1995 Apr **1:4** 348-52

Liu PK, Kraus E, Wu TA, Strong LC, Tainsky MA
Analysis of genomic instability in Li-Fraumeni fibroblasts with germline p53 mutations.
Oncogene. 1996 Jun 6 **12:11** 2267-78

Longacre TA, Bartow SA
A correlative morphologic study of human breast and endometrium in the menstrual cycle.
Am J Surg Pathol 1986 Jun **10:6** 382-93

Love JM, Knight AM, McAleer MA, Todd JA.
Towards construction of a high resolution map of the mouse genome using PCR-analysed microsatellites.
Nucleic Acids Res. 1990 Jul 25 **18:14** 4123-30.

Lynch HT, Schuelke GS, Kimberling WJ, Albano WA, Lynch JF, Biscone KA, Lipkin ML, Deschner EE, Mikol YB, Sandberg AA, Elston RC, Bailey-Wilson JE, Danes BS
Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). II. Biomarker studies.
Cancer. 1985 Aug 15 **56:4** 939-51

Lynch HT, Watson P, Lynch JF
Epidemiology and risk factors.
Clin Obstet Gynecol. 1989 Dec **32:4** 750-60.

MacMahon B, Cole P, Lin TM, Lowe CR, Mirra AP, Ravnihar B, Salber EJ, Valaoras VG, Yuasa S.
Age at first birth and breast cancer risk.
Bull World Health Organ. 1970 **43:2** 209-21.

Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH
Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms.
Science. 1990 Nov 30 **250:4985** 1233-8

Manton KG, Stallard E
A two-disease model of female breast cancer: mortality in 1969 among white females in the United States.
J Natl Cancer Inst. 1980 Jan **64:1** 9-16.

Manton KG, Stallard E.
Demographics (1950-1987) of breast cancer in birth cohorts of older women.
J Gerontol. 1992 Nov **47 Spec No** 32-42

Mariani-Costantini R, Escot C, Theillet C, Gentile A, Merlo G, Lidereau R, Callahan R
In situ c-myc expression and genomic status of the c-myc locus in infiltrating ductal carcinomas of the breast.
Cancer Res 1988 Jan 1 **48:1** 199-205

Marmorstein LY, Ouchi T, Aaronson SA.
The BRCA2 gene product functionally interacts with p53 and RAD51.
Proc Natl Acad Sci U S A. 1998 Nov 10 **95:23** 13869-74.

Mars WM, Saunders GF
Chromosomal abnormalities in human breast cancer.
Cancer Metastasis Rev. 1990 Jul **9:1** 35-43.

Marsh DJ, Zheng Z, Zedenius J, Kremer H, Padberg GW, Larsson C, Longy M, Eng C.
Differential loss of heterozygosity in the region of the Cowden locus within 10q22-23 in follicular thyroid adenomas and carcinomas.
Cancer Res. 1997 Feb 1 **57:3** 500-3.

Marshall C
How do small GTPase signal transduction pathways regulate cell cycle entry?
Curr Opin Cell Biol 1999 Dec **11:6** 732-6

Martinelli G, Trabetti E, Farabegoli P, Buzzi M, Zaccaria A, Testoni N, Amabile M, Casartelli A, de Vivo A, Pignatti PF, Tura S.
'Fingerprinting' of HLA-DQA by polymerase chain reaction and heteroduplex analysis.
Mol Cell Probes. 1996 Apr **10:2** 123-7

Martinez-Hernandez A, Amenta PS
The basement membrane in pathology.
Lab Invest 1983 Jun **48:6** 656-77

Maskarinec G, Meng L, Kolonel LN.
Alcohol intake, body weight, and mortality in a multiethnic prospective cohort.
Epidemiology. 1998 Nov **9:6** 654-61

Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S.
Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene.
EMBO J. 1984 Dec 20 **3:13** 3257-62

Matsumura Y, Tarin D
DNA fingerprinting survey of various human tumors and their metastases.
Cancer Res 1992 Apr 15 **52:8** 2174-9

Mayer ME
Hormone replacement and breast cancer: implications of the Iowa Women's Health Study.
Cleve Clin J Med. 1999 Nov-Dec **66:10** 608-10, 613.

Mayhew TM, Cruz-Orive LM
Caveat on the use of the Delesse principle of areal analysis for estimating component volume densities
Journal of Microscopy 1974 **102:** 195

Maynard PV, Davies CJ, Blamey RW, Elston CW, Johnson J, Griffiths K.
Relationship between oestrogen-receptor content and histological grade in human primary breast tumours.
Br J Cancer. 1978 Dec **38:6** 745-8.

Mazars R, Spinardi L, BenCheikh M, Simony-Lafontaine J, Jeanteur P, Theillet C.
p53 mutations occur in aggressive breast cancer.
Cancer Res. 1992 Jul 15 **52:14** 3918-23.

McCarthy EP, Burns RB, Coughlin SS, Freund KM, Rice J, Marwill SL, Ash A, Shwartz M, Moskowitz MA
Mammography use helps to explain differences in breast cancer stage at diagnosis between older black and white women.
Ann Intern Med. 1998 May 1 **128:9** 729-36

McCormick F
Signal transduction. How receptors turn Ras on
Nature 1993 May 6 **363:6424** 15-6

McGrath JP, Capon DJ, Goeddel DV, Levinson AD
Comparative biochemical properties of normal and activated human ras p21 protein.
Nature 1984 Aug 23-29 **310:5979** 644-9

McGuire WL, Naylor SL.
Loss of heterozygosity in breast cancer: cause or effect?
J Natl Cancer Inst. 1989 Dec 6 **81:23** 1764-5.

McPherson K, Vessey MP, Neil A, Doll R, Jones L, Roberts M
Early oral contraceptive use and breast cancer: results of another case-control study.
Br J Cancer. 1987 Nov **56:5** 653-60

McPherson K, Steel CM, Dixon JM
ABC of breast diseases. Breast cancer and epidemiology, risk factors and genetics.
BMJ 1994 Oct 15 **309:6960** 1003-6

Menon M, Teh CH, Chua CL.
Clinical and social problems in young women with breast carcinoma.
Aust N Z J Surg. 1992 May **62:5** 364-7.

Merchant WJ, Millis RR, Smith P, Chaudary MA, Barnes DM
Expression of c-erbB2 and p53 protein is similar in breast cancer from British and Japanese women.
Int J Cancer. 1999 Jun 21 **84:3** 278-83

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell W, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K
A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1.
Science. 1994 Oct 7 **266:5182** 66-71

Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y
Mutation analysis in the BRCA2 gene in primary breast cancers.
Nat Genet. 1996 Jun **13:2** 245-7

Miki Y, Katagiri T, Nakamura Y.
Infrequent mutation of the H-cadherin gene on chromosome 16q24 in human breast cancers.
Jpn J Cancer Res. 1997 Aug **88:8** 701-4

Mikuz G, Dietze O.
Nuclear morphometric and cytophotometric studies of breast cancer. Comparison of the cytophotometric study results with the histological grading
Wien Klin Wochenschr. 1982 Jul 9 **94:14** 368-70

Mittendorf R, Longnecker MP, Newcomb PA, Dietz AT, Greenberg ER, Bogdan GF, Clapp RW, Willett WC.
Strenuous physical activity in young adulthood and risk of breast cancer (United States).
Cancer Causes Control. 1995 Jul **6:4** 347-53.

Moerland E, Breuning MH, Cornelisse CJ, Cleton-Jansen AM.
Exclusion of BBC1 and CMAR as candidate breast tumour-suppressor genes.
Br J Cancer. 1997 **76:12** 1550-3

Mohla S, Sampson CC, Khan T, Enterline JP, Leffall L Jr, White JE.
Estrogen and progesterone receptors in breast cancer in Black Americans: Correlation of receptor data with tumor differentiation.
Cancer. 1982 Aug 1 **50:3** 552-9.

Mokhtar AM, Bedeiwy A.
Clinico-pathological study of carcinoma of breast at King Abdulaziz University Hospital
King Abdulaziz Med J 1982 **2:3** 25-34

Moolgavkar SH, Stevens RG, Lee JA.
Effect of age on incidence of breast cancer in females.
J Natl Cancer Inst. 1979 Mar **62:3** 493-501

Moolgavkar SH, Day NE, Stevens RG.
Two-stage model for carcinogenesis: Epidemiology of breast cancer in females.
J Natl Cancer Inst. 1980 Sep **65:3** 559-69.

Morabia A, Costanza MC
International variability in ages at menarche, first livebirth, and menopause. World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives.
Am J Epidemiol 1998 Dec 15 **148:12** 1195-205

Motawy MM, Qulali M, Al Jarallah M, Gouet RJ, Sledge GYT, Omar N, Al-Safdyo Z
Aggressive and early onset of advanced stage breast cancer in Kuwait
UICC conference 1994 presented paper

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H.
Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction.
Cold Spring Harb Symp Quant Biol. 1986 **51 Pt 1** 263-73.

Munn KE, Walker RA, Varley JM.
Frequent alterations of chromosome 1 in ductal carcinoma in situ of the breast.
Oncogene. 1995 Apr 20 **10:8** 1653-7

Munn KE, Walker RA, Menasce L, Varley JM.
Allelic imbalance in the region of the BRCA1 gene in ductal carcinoma in situ of the breast.
Br J Cancer. 1996 Mar **73:5** 636-9.

Muleris M, Almeida A, Gerbault-Seureau M, Malfoy B, Dutrillaux B
Identification of amplified DNA sequences in breast cancer and their organization within homogeneously staining regions.
Genes Chromosomes Cancer. 1995 Nov **14:3** 155-63.

Nabi IR, Watanabe H, Raz A
Autocrine motility factor and its receptor: role in cell locomotion and metastasis.
Cancer Metastasis Rev. 1992 Mar **11:1** 5-20

Nagai MA, Pacheco MM, Brentani MM, Marques LA, Brentani RR, Ponder BAJ, Mulligan LM
Allelic loss on distal chromosome 17p is associated with poor prognosis in a group of Brazilian breast cancer patients.
Br J Cancer 1994 **69:** 754-758

Nagayama K, Watatani M.
Analysis of genetic alterations related to the development and progression of breast carcinoma.
Jpn J Cancer Res. 1993 Nov **84:11** 1159-64.

Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E, et al
Variable number of tandem repeat (VNTR) markers for human gene mapping.
Science. 1987 Mar 27 **235:4796** 1616-22

Narod SA
Genetics of breast and ovarian cancer.
Br Med Bull. 1994 Jul **50:3** 656-76

National Cancer Registry (NCR) 1994 report
Cancer incidence in Saudi Arabia 1994
Ministry of Health Kingdom of Saudi Arabia 1996

Neuhausen SL.
Ethnic differences in cancer risk resulting from genetic variation.
Cancer. 1999 Dec 1 **86:11 Suppl** 2575-82

Newman LA, Alfonso AE.
Age-related differences in breast cancer stage at diagnosis between black and white patients in an urban community hospital.
Ann Surg Oncol. 1997 Dec **4:8** 655-62.

NHSBSP
Pathology reporting in breast cancer screening 2nd Edition
NHSBSP publication 1995 no.3

Nicholson RI, McClelland RA, Gee JM.
Steroid hormone receptors and their clinical significance in cancer.
J Clin Pathol. 1995 Oct **48:10** 890-5.

Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, et al
Mutations of two PMS homologues in hereditary nonpolyposis colon cancer.
Nature 1994 Sep 1 **371:6492** 75-80

Nicoll A, Bassett K, Ulijaszek SJ.
What's in a name? Accuracy of using surnames and forenames in ascribing Asian ethnic identity in English populations.
J Epidemiol Community Health. 1986 Dec **40:4** 364-8.

Niederacher D, Picard F, van Roeyen C, An HX, Bender HG, Beckmann MW
Patterns of allelic loss on chromosome 17 in sporadic breast carcinomas detected by fluorescent-labeled microsatellite analysis.
Genes Chromosomes Cancer. 1997 Mar **18:3** 181-92

Nielsen KV, Blichert-Toft M, Andersen J
Chromosome analysis of in situ breast cancer.
Acta Oncol 1989 **28:6** 919-22

Nigro JM, Aldape KD, Hess SM, Tlsty TD
Cellular adhesion regulates p53 protein levels in primary human keratinocytes.
Cancer Res 1997 Sep 1 **57:17** 3635-9

Nixon AJ, Neuberg D, Hayes DF, Gelman R, Connolly JL, Schnitt S, Abner A, Recht A, Vicini F, Harris JR.
Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer.
J Clin Oncol. 1994 May **12:5** 888-94

Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H
Clonal analysis of human breast cancer by means of the polymerase chain reaction
Cancer Res 1992 Dec 1 **52:23** 6594-7

Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H.
Clonal analysis of predominantly intraductal carcinoma and precancerous lesions of the breast by means of polymerase chain reaction.
Cancer Res. 1994 Apr 1 **54:7** 1849-53.

Nomura AM, Kolonel LN, Hirohata T, Lee J.
The association of replacement estrogens with breast cancer.
Int J Cancer. 1986 Jan 15 **37:1** 49-53.

Noviello C, Courjal F, Theillet C.
Loss of heterozygosity on the long arm of chromosome 6 in breast cancer: possibly four regions of deletion.
Clin Cancer Res. 1996 Sep **2:9** 1601-6.

Nowell PC
The clonal evolution of tumor cell populations.
Science 1976 Oct 1 **194:4260** 23-8

Nowell PC, Hungerford DA
A minute chromosome in human chronic granulocytic leukemia
Science 1960 **132:** 1497-1499

Ochi H, Takeuchi J, Holyoke D, Sandberg AA
Possible specific chromosome changes in large bowel cancer.
Cancer Genet Cytogenet 1983 Sep **10:1** 121-2

Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M, Mori T
Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis.
Cancer Res. 1993 Apr 1 **53:7** 1696-701

Okamoto H, Ogawara T, Arihara F, Kobayashi K, Inoue S, Nagahori K, Yamamoto M, Sekikawa T, Matsumoto Y.
Usefulness of ultrasonography combined with conventional physical examination in mass screening for breast cancer: a retrospective study of Yamanashi Health Care Center results from 1989 to 1994.
Jpn J Cancer Res. 1996 Mar **87:3** 317-23

Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B
Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53.
Nature 1993 Apr 29 **362:6423** 857-60

Orphanos V, Santibanez-Koref M, McGown G, Hey Y, Rackstraw C, Boyle JM.
Physical mapping of 43 STSs to human chromosome 6.
Genomics. 1994 Mar 15 **20:2** 301-4.

Orphanos V, McGown G, Hey Y, Boyle JM, Santibanez-Koref M.
Proximal 6q, a region showing allele loss in primary breast cancer.
Br J Cancer. 1995 Feb **71:2** 290-3

Orrego C
Organizing a laboratory for PCR work. PCR protocols: a guide to methods and applications
Innis Gelfand DH, Sninsky JJ, White TJ (ed). *Academic press (pub)* 1990 447-454

Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE.
Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells.
Cancer Res. 1994 May 15 **54:10** 2552-5.

Page DL, Dupont WD, Rogers LW, Jensen RA, Schuyler PA
Continued local recurrence of carcinoma 15-25 years after a diagnosis of low grade ductal carcinoma in situ of the breast treated only by biopsy.
Cancer 1995 Oct 1 **76:7** 1197-200

Paget S
The distribution of secondary growth in cancer of the breast.
Lancet 1889 1: 571

Pandis N, Heim S, Bardi G, Idvall I, Mandahl N, Mitelman F
Whole-arm t(1;16) and i(1q) as sole anomalies identify gain of 1q as a primary
chromosomal abnormality in breast cancer
Genes Chromosomes Cancer 1992 Oct 5:3 235-8

Pandis N, Jin Y, Limon J, Bardi G, Idvall I, Mandahl N, Mitelman F, Heim S
Interstitial deletion of the short arm of chromosome 3 as a primary chromosome
abnormality in carcinomas of the breast.
Genes Chromosomes Cancer 1993 Mar 6:3 151-5

Pandis N, Idvall I, Bardi G, Jin Y, Gorunova L, Mertens F, Olsson H, Ingvar C, Beroukas K,
Mitelman F, Heim S.
Correlation between karyotypic pattern and clincopathologic features in 125 breast cancer
cases.
Int J Cancer. 1996 Apr 10 66:2 191-6.

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA,
Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P,
Lynch HT, Peltomaki P, Meckilin JP, De La Chapelle A.
Mutation of a mutL homolog in hereditary colon cancer
Science 1994 Mar 18 263:5153 1625-9

Parkin DM, Muir CS, Whelan SL, Gao Y-T, Ferlay J, Powell J (Ed)
Cancer Incidence in Five Continents VI
IARC scientific publications Publ 1992 120:

Parkin DM , Whelan SL, Ferlay J, Raymond L, Young J (Ed)
Cancer Incidence in Five Continents VII
IARC scientific publications Publ 1997 143:

Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, Sanford KK
Deficient DNA repair capacity, a predisposing factor in breast cancer.
Br J Cancer 1996 Jul 74:1 1-5

Patavino VM, Epifani AC, Avena C, Minelli L.
Epidemiology of breast carcinoma.
Ann Ig. 1995 Jul-Aug 7:4 257-66.

Patchefsky AS, Schwartz GF, Finkelstein SD, Prestipino A, Sohn SE, Singer JS, Feig SA
Heterogeneity of intraductal carcinoma of the breast.
Cancer. 1989 Feb 15 63:4 731-41

Patel U, Grundfest-Broniatowski S, Gupta M, Banerjee S
Microsatellite instabilities at five chromosomes in primary breast tumors.
Oncogene 1994 Dec 9:12 3695-700

Patel KJ, Yu VP, Lee H, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS,
Ponder BA, Venkitaraman AR.
Involvement of Brca2 in DNA repair.
Mol Cell. 1998 Feb 1:3 347-57.

Peacock SL, White E, Daling JR, Voigt LF, Malone KE.
Relation between obesity and breast cancer in young women.
Am J Epidemiol. 1999 Feb 15 **149:4** 339-46.

Peer PG, Verbeek AL, Mravunac M, Hendriks JH, Holland R
Prognosis of younger and older patients with early breast cancer.
Br J Cancer. 1996 Feb **73:3** 382-5

Petrangeli E, Lubrano C, Ortolani F, Ravenna L, Vacca A, Sciacchitano S, Frati L, Gulino A.
Estrogen receptors: new perspectives in breast cancer management.
J Steroid Biochem Mol Biol. 1994 Jun **49:4-6** 327-31

Phillips RL, Garfinkel L, Kuzma JW, Beeson WL, Lotz T, Brin B.
Mortality among California Seventh-Day Adventists for selected cancer sites.
J Natl Cancer Inst. 1980 Nov **65:5** 1097-1107

Pienta KJ, Coffey DS.
Correlation of nuclear morphometry with progression of breast cancer.
Cancer. 1991 Nov 1 **68:9** 2012-6

Pinder SE, Ellis IO, Elston CW.
Prognostic factors in primary breast carcinoma.
J Clin Pathol. 1995 Nov **48:11** 981-3.

Pippard EC, Hall AJ, Barker DJ, Bridges BA
Cancer in homozygotes and heterozygotes of ataxia-telangiectasia and xeroderma
pigmentosum in Britain.
Cancer Res 1988 May 15 **48:10** 2929-32

Piwnica-Worms H
Cell cycle. Fools rush in
Nature 1999 Oct 7 **401:6753** 535, 537

Potter JF, Dawkins DM, Terry P, Beevers DG
Breast cancer in blacks, Asians and whites in Birmingham.
Postgrad Med J 1983 Oct **59:696** 661-3

Pullman WE, Bodmer WF.
Cloning and characterization of a gene that regulates cell adhesion.
Nature. 1992 Apr 9 **356:6369** 529-32.

Qattan N, Omu AE
The experrience of a menopause clinic in Kuwait: implications for primary health care
Ann Saudi Med 1996 **16:2** 135-138

Rabadi S
Cancer at Dhahran Health centre in Saudi Arabia
Saudi Med J 1987 **7:** 288-293

Rabbitts TH, Lefranc MP, Stinson MA, Sims JE, Schroder J, Steinmetz M, Spurr NL, Solomon
E, Goodfellow PN
The chromosomal location of T-cell receptor genes and a T cell rearranging gene: possible
correlation with specific translocations in human T cell leukaemia.
EMBO J 1985 Jun **4:6** 1461-5

Rajan PB, Scott DJ, Perry RH, Griffith CD
p53 protein expression in ductal carcinoma in situ (DCIS) of the breast.
Breast Cancer Res Treat. 1997 Feb **42:3** 283-90

Rao GN.
Influence of diet on tumors of hormonal tissues.
Prog Clin Biol Res. 1996 **394** 41-56.

Rasmussen BB
Human mucinous breast carcinomas and their lymph node metastases. A histological review of 247 cases.
Pathol Res Pract. 1985 Oct **180:4** 377-82

Rebbeck TR
Inherited genetic predisposition in breast cancer. A population-based perspective.
Cancer. 1999 Dec 1 **86:11 Suppl** 2493-501

Reddy DE, Sandhu AK, DeRiel JK, Athwal RS, Kaur GP.
Identification of a gene at 16q24.3 that restores cellular senescence in immortal mammary tumor cells.
Oncogene. 1999 Sep 9 **18:36** 5100-017.

Remvikos Y, Beuzeboc P, Zajdela A, Voillemot N, Magdelenat H, Pouillart P
Correlation of pretreatment proliferative activity of breast cancer with the response to cytotoxic chemotherapy.
J Natl Cancer Inst. 1989 Sep 20 **81:18** 1383-7.

Resnitzky D, Gossen M, Bujard H, Reed SI
Acceleration of the G1/S phase transition by expression of cyclins D1 and E with an inducible system.
Mol Cell Biol 1994 Mar **14:3** 1669-79

Richards MA, Coleman RE, Hamsa R, Khaled H, el Mawla MG, Kadry I, Hablas A, Ramadan M, Allen D, Wang DY, et al
Advanced breast cancer in Egyptian women: clinical features and response to endocrine therapy. The Anglo-Egyptian Health Agreement Collaborative Study.
Eur J Surg Oncol 1992 Jun **18:3** 219-23

Richter GO, Dockerty MB, Clagett OT
Diffuse infiltrating scirrhous carcinoma of the breast. Special consideration of the single-filing phenomenon.
Cancer. 1967 Mar **20:3** 363-70.

Rifkin DB, Gross JL, Moscatelli D, Gabrielides C
The involvement of proteases and protease inhibitors in neovascularization.
Acta Biol Med Ger. 1981 **40:10-11** 1259-63

Rimm DL, Sinard JH, Morrow JS
Reduced alpha-catenin and E-cadherin expression in breast cancer.
Lab Invest. 1995 May7 **2:5** 506-12

Rios MA, Macias A, Perez R, Lage A, Skoog L
Receptors for epidermal growth factor and estrogen as predictors of relapse in patients with mammary carcinoma.
Anticancer Res. 1988 Jan-Feb **8:1** 173-6

Riyadh dept. of economic studies and statistics
Saudi Arabia food balance sheets (1974-1984)
Ministry of Agriculture and Water, Saudi Arabia 73-78

Robbins P, Pinder S, de Klerk N, Dawkins H, Harvey J, Sterrett G, Ellis I, Elston C.
Histological grading of breast carcinomas: a study of interobserver agreement.
Hum Pathol. 1995 Aug **26:8** 873-9.

Rose DP.
Dietary fatty acids and cancer.
Am J Clin Nutr. 1997 Oct **66:4 Suppl** 998S-1003S.

Rosen PP, Lesser ML, Senie RT, Duthie K
Epidemiology of breast carcinoma IV: age and histologic tumor type.
J Surg Oncol. 1982 Jan **19:1** 44-51.

Rosenquist CJ, Lindfors KK
Screening mammography beginning at age 40 years: a reappraisal of cost-effectiveness.
Cancer. 1998 Jun 1 **82:11** 2235-40

Rosselli F, Ridet A, Soussi T, Duchaud E, Alapetite C, Moustacchi E
p53-dependent pathway of radio-induced apoptosis is altered in Fanconi anemia.
Oncogene 1995 Jan 5 **10:1** 9-17

Roylance R, Gorman P, Harris W, Liebmann R, Barnes D, Hanby A, Sheer D.
Comparative genomic hybridization of breast tumors stratified by histological grade reveals new insights into the biological progression of breast cancer.
Cancer Res. 1999 Apr 1 **59:7** 1433-6

Ruano G, Pagliaro EM, Schwartz TR, Lamy K, Messina D, Gaensslen RE, Lee HC.
Heat-soaked PCR: an efficient method for DNA amplification with applications to forensic analysis.
Biotechniques. 1992 Aug **13:2** 266-74.

Rubinfeld B, Souza B, Albert I, Müller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P
Association of the APC gene product with beta-catenin.
Science 1993 Dec 10 **262:5140** 1731-4

Ruoslahti E, Pierschbacher MD
New perspectives in cell adhesion: RGD and integrins.
Science 1987 Oct 23 **238:4826** 491-7

Rusciano D, Burger MM
Why do cancer cells metastasize into particular organs?
Bioessays. 1992 Mar **14:3** 185-94

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA.
Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase.
Science. 1988 Jan 29 **239:4839** 487-91

Sainsbury JR, Farndon JR, Needham GK, Malcolm AJ, Harris AL
Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer.
Lancet. 1987 Jun 20 **1:8547** 1398-402

Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K, Nakamura Y
Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers.
Cancer Res. 1992 Oct 15 **52:20** 5815-7

Sananes S, Clough KB, Zafrani B, Fourquet A, Durand JC.
Intraductal cancer of the breast in women under age 35 years
Bull Cancer. 1996 May **83:5** 395-9.

Sanchez Y, Elledge SJ
Stopped for repairs.
Bioessays 1995 Jun **17:6** 545-8

Santibanez-Koref MF, Birch JM, Hartley AL, Jones PH, Craft AW, Eden T, Crowther D, Kelsey AM, Harris M
p53 germline mutations in Li-Fraumeni syndrome.
Lancet. 1991 Dec 14 **338:8781** 1490-1

Sariego J, Zrada S, Byrd M, Matsumoto T.
Breast cancer in young patients.
Am J Surg. 1995 Sep **170:3** 243-5

Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y.
Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer.
Cancer Res. 1990 Nov 15 **50:22** 7184-9

Sato T, Akiyama F, Sakamoto G, Kasumi F, Nakamura Y.
Accumulation of genetic alterations and progression of primary breast cancer.
Cancer Res. 1991 Nov 1 **51:21** 5794-9.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor MR
A single ataxia telangiectasia gene with a product similar to PI-3 kinase
Science 1995 Jun 23 **268:5218** 1749-53

Sawyers CL, Denny CT, Witte ON
Leukemia and the disruption of normal hematopoiesis.
Cell 1991 Jan 25 **64:2** 337-50

Schmutzler RK, Bierhoff E, Werkhausen T, Fimmers R, Speiser P, Kubista E, Krebs D, Zeillinger R, Wiestler OD, Von Deimling A.
Genomic deletions in the BRCA1, BRCA2 and TP53 regions associate with low expression of the estrogen receptor in sporadic breast carcinoma.
Int J Cancer. 1997 Jun 20 **74:3** 322-5.

Schulman BA, Lindstrom DL, Harlow E
Substrate recruitment to cyclin-dependent kinase 2 by a multipurpose docking site on cyclin A.
Proc Natl Acad Sci U S A 1998 Sep 1 **95:18** 10453-8

Schwab M, Varmus HE, Bishop JM, Grzeschik KH, Naylor SL, Sakaguchi AY, Brodeur G, Trent J
Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc.
Nature 1984 Mar 15-21 **308:5956** 288-91

Schwendel A, Richard F, Langreck H, Kaufmann O, Lage H, Winzer KJ, Petersen I, Dietel M.
Chromosome alterations in breast carcinomas: frequent involvement of DNA losses including chromosomes 4q and 21q.
Br J Cancer. 1998 Sep **78:6** 806-11

Sears HF, Janus C, Levy W, Hopson R, Creech R, Grotzinger P
Breast cancer without axillary metastases. Are there high-risk biologic subpopulations?
Cancer 1982 Nov 1 **50:9** 1820-7

Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, Hammond D
Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas.
N Engl J Med 1985 Oct 31 **313:18** 1111-6

Seery LT, Knowlden JM, Gee JM, Robertson JF, Kenny FS, Ellis IO, Nicholson RI.
BRCA1 expression levels predict distant metastasis of sporadic breast cancers.
Int J Cancer. 1999 Jun 21 **84:3** 258-62.

Serrano M, Hannon GJ, Beach D
A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4
Nature 1993 Dec 16 **366:6456** 704-7

Shaw JA, Walsh T, Chappell SA, Carey N, Johnson K, Walker RA
Microsatellite instability in early sporadic breast cancer
Br J Cancer 1996 **73:** 1393-1397

Sheng ZM, Marchetti A, Buttitta F, Champeme MH, Campani D, Bistocchi M, Lidereau R, Callahan R
Multiple regions of chromosome 6q affected by loss of heterozygosity in primary human breast carcinomas.
Br J Cancer. 1996 Jan **73:2** 144-7

Sherr CJ
Mammalian G1 cyclins.
Cell 1993 Jun 18 **73:6** 1059-65

Shibata D.
Extraction of DNA from paraffin-embedded tissue for analysis by polymerase chain reaction: new tricks from an old friend.
Hum Pathol. 1994 Jun **25:6** 561-3.

Shoupe D
Hormone replacement therapy: reassessing the risks and benefits.
Hosp Pract 1999 Aug 15 **34:8** 97-103, 107-8, 113-4.

Shtivelman E, Lifshitz B, Gale RP, Canaani E
Fused transcript of abl and bcr genes in chronic myelogenous leukaemia.
Nature 1985 Jun 13-19 **315:6020** 550-4

Silverstein MJ, Rosser RJ, Gierson ED, Waisman JR, Gamagami P, Hoffman RS, Fingerhut AG, Lewinsky BS, Colburn W, Handel N
Axillary lymph node dissection for intraductal breast carcinoma--is it indicated?
Cancer. 1987 May 15 **59:10** 1819-24

Simard J, Tonin P, Durocher F, Morgan K, Rommens J, Gingras S, Samson C, Leblanc JF, Belanger C, Dion F et al
Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families.
Nat Genet. 1994 Dec **8:4** 392-8

Simon A, Allais DP, Duroux JL, Basly JP, Durand-Fontanier S, Delage C.
Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships.
Cancer Lett. 1998 Jul 3 **129:1** 111-6.

Simpson JF, Dutt PL, Page DL.
Expression of mitoses per thousand cells and cell density in breast carcinomas: a proposal.
Hum Pathol. 1992 Jun **23:6** 608-11.

Singh B, Ittmann MM, Krolewski JJ.
Sporadic breast cancers exhibit loss of heterozygosity on chromosome segment 10q23 close to the Cowden disease locus.
Genes Chromosomes Cancer. 1998 Feb **21:2** 166-71.

Sjalander A, Birgander R, Saha N, Beckman L, Beckman G.
p53 polymorphisms and haplotypes show distinct differences between major ethnic groups.
Hum Hered. 1996 Jan-Feb **46:1** 41-8.

Skirnisdottir S, Eiriksdottir G, Baldursson T, Barkardottir RB, Egilsson V, Ingvarrson S.
High frequency of allelic imbalance at chromosome region 16q22-23 in human breast cancer: correlation with high PgR and low S phase.
Int J Cancer. 1995 Apr 21 **64:2** 112-6.

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL
Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene.
Science 1987 Jan 9 **235:4785** 177-82

Smith DB, Howell A, Harris M, Bramwell VH, Sellwood RA
Carcinomatous meningitis associated with infiltrating lobular carcinoma of the breast.
Eur J Surg Oncol. 1985 Mar **11:1** 33-6

Soler AP, Knudsen KA, Salazar H, Han AC, Keshgegian AA
P-cadherin expression in breast carcinoma indicates poor survival.
Cancer 1999 Oct 1 **86:7** 1263-72

Soliman AS, Bondy ML, Raouf AA, Makram MA, Johnston DA, Levin B.
Cancer mortality in Menofeia, Egypt: comparison with US mortality rates.
Cancer Causes Control. 1999 Oct **10:5** 349-54

Sommers CL, Gelmann EP, Kemler R, Cowin P, Byers SW
Alterations in beta-catenin phosphorylation and plakoglobin expression in human breast cancer cells.
Cancer Res. 1994 Jul 1 **54:13** 3544-52

Southern EM
Detection of specific sequences among DNA fragments separated by gel electrophoresis.
J Mol Biol. 1975 Nov 5 **98:3** 503-17

Stadel BV, Rubin GL, Webster LA, Schlesselman JJ, Wingo PA.
Oral contraceptives and breast cancer in young women.
Lancet. 1985 Nov 2 **2:8462** 970-3

Stallard S, Litherland JC, Cordiner CM, Dobson HM, George WD, Mallon EA, Hole D
Effect of hormone replacement therapy on the pathological stage of breast cancer: population based, cross sectional study.
BMJ. 2000 Feb 5 **320:7231** 348-9

Stanbridge EJ, Wilkinson J
Analysis of malignancy in human cells: malignant and transformed phenotypes are under separate genetic control..
Proc Natl Acad Sci U S A 1978 Mar **75:3** 1466-9

Steeg PS
Invasion and metastasis.
Curr Opin Oncol. 1992 Feb **4:1** 134-41

Steinnitz R, Parkin DM, Young JL, Bieber CA, Katz L (Ed)
Cancer incidence in Jewish migrants to Israel 1961-1981
IARC scientific publications 1989 **98:**

Stephens FO.
Breast cancer: aetiological factors and associations (a possible protective role of phytoestrogens).
Aust N Z J Surg. 1997 Nov **67:11** 755-60.

Stevens RG, Moolgavkar SH, Lee JA
Temporal trends in breast cancer.
Am J Epidemiol. 1982 May **115:5** 759-77

Stirling JW, Chandler JA
The fine structure of the normal, resting terminal ductal-lobular unit of the female breast.
Virchows Arch A Pathol Anat Histol 1976 Dec 27 **372:3** 205-26

Strathdee CA, Duncan AM, Buchwald M
Evidence for at least four Fanconi anaemia genes including FACC on chromosome 9.
Nat Genet 1992 Jun **1:3** 196-8

Stratton MR, Ford D, Neuhasen S, Seal S, Wooster R, Friedman LS, King MC, Egilsson V, Devilee P, McManus R, et al.
Familial male breast cancer is not linked to the BRCA1 locus on chromosome 17q.
Nat Genet. 1994 May **7:1** 103-7.

Stratton MR, Collins N, Lakhani SR, Sloane JP.
Loss of heterozygosity in ductal carcinoma in situ of the breast.
J Pathol. 1995 Feb **175:2** 195-201.

Stratton MR
The pathology of inherited breast tumours (abstract)
Breast-cancer-research 2000 2 (suppliment 1) S6-S6

Swift M, Reitnauer PJ, Morrell D, Chase CL
Breast and other cancers in families with ataxia-telangiectasia.
N Engl J Med 1987 May 21 **316:21** 1289-94

Swift M, Morrell D, Massey RB, Chase CL
Incidence of cancer in 161 families affected by ataxia-telangiectasia
N Engl J Med 1991 Dec 26 **325:26** 1831-6

Tabar L, Vitak B, Chen HH, Prevost TC, Duffy SW
Update of the Swedish Two-County Trial of breast cancer screening: histologic grade-specific and age-specific results.
Swiss Surg. 1999 **5:5** 199-204

Takeichi M
Cadherins: a molecular family important in selective cell-cell adhesion.
Annu Rev Biochem 1990 **59:** 237-52

Takita K, Sato T, Miyagi M, Watatani M, Akiyama F, Sakamoto G, Kasumi F, Abe R, Nakamura Y.
Correlation of loss of alleles on the short arms of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes.
Cancer Res. 1992 Jul 15 **52:14** 3914-7.

Tamimi TM, Al-Bar A,Al-Suhaimi S, Ibrahim E, Ibrahim A, Wosornu L, Gabriel GS
Lung cancer in the Eastern Region of Saudi Arabia: A population based study.
Ann Saudi Med 1995 **16:** 3-10

Tamura G, Masuda T, Satodate R, Ishida M, Saitoh K.
Nuclear morphometry of cancer cells in stage III breast cancer with special reference to prognosis
Gan No Rinsho. 1988 Jul **34:8** 977-80

Tandon P, Pathak VP, Zaheer A, Chatterjee A, Walford N
Cancer in the Gizan province of Saudi Arabia: An eleven year study.
Ann Saudi Med 1995 **15:** 14-20

Tarone RE, Chu KC.
Implications of birth cohort patterns in interpreting trends in breast cancer rates.
J Natl Cancer Inst. 1992 Sep 16 **84:18** 1402-10

Taylor AM
Ataxia telangiectasia genes and predisposition to leukaemia, lymphoma and breast cancer
Br J Cancer 1992 Jul **66:1** 5-9

Theile M, Seitz S, Arnold W, Jandrig B, Frege R, Schlag PM, Haensch W, Guski H, Winzer KJ, Barrett JC, Scherneck S.
A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer.
Oncogene. 1996 Aug 15 **13:4** 677-85.

Thein SL, Jeffreys AJ, Gooi HC, Cotter F, Flint J, O'Connor NT, Weatherall DJ, Wainscoat JS
Detection of somatic changes in human cancer DNA by DNA fingerprint analysis.
Br J Cancer 1987 Apr **55:4** 353-6

Theissig F, Baak JP, Schuurmans L, Haroske G, Meyer W, Kunze KD.
'Blind' multicenter evaluation of the prognostic value of DNA image cytometric and morphometric features in invasive breast cancer.
Anal Cell Pathol. 1996 Mar **10:2** 85-99

Thibodeau SN, Bren G, Schaid D
Microsatellite instability in cancer of the proximal colon
Science 1993 May 7 **260:5109** 816-9

Thomas DB.
Epidemiologic studies of cancer in minority groups in the western United States.
Natl Cancer Inst Monogr. 1979 Nov **53** 103-13.

Thomas DB, Persing JP, Hutchinson WB
Exogenous estrogens and other risk factors for breast cancer in women with benign breast diseases.
J Natl Cancer Inst. 1982 Nov **69:5** 1017-25.

Thompson AM, Anderson TJ, Condie A, Prosser J, Chetty U, Carter DC, Evans HJ, Steel CM.
p53 allele losses, mutations and expression in breast cancer and their relationship to clinico-pathological parameters.
Int J Cancer. 1992 Feb 20 **50:4** 528-32.

Thompson WD
Genetic epidemiology of breast cancer.
Cancer. 1994 Jul 1 **74(1 Suppl):** 279-87

Timpl R, Dziadek M
Structure, development, and molecular pathology of basement membranes.
Int Rev Exp Pathol 1986 **29:** 1-112

Todd JA, McAleer MA, Hearne C, Aitman TJ, Cornall RJ, Love JM
Mouse microsatellites
McPherson MJ, Quirke P, Taylor GR (ed)
PCR A pratical approach
IRL Press (pub) 1991 101-105

Toi M, Kashitani J, Tominaga T
Tumor angiogenesis is an independent prognostic indicator in primary breast carcinoma.
Int J Cancer. 1993 Sep 30 **55:3** 371-4.

Trichopoulos D, MacMahon B, Cole P.
Menopause and breast cancer risk.
J Natl Cancer Inst. 1972 Mar **48:3** 605-13.

Trichopoulou A, Katsouyanni K, Stuver S, Tzala L, Gnardellis C, Rimm E, Trichopoulos D.
Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece.
J Natl Cancer Inst. 1995 Jan 18 **87:2** 110-6

Trock BJ.
Breast cancer in African American women: epidemiology and tumor biology.
Breast Cancer Res Treat. 1996 **40:1** 11-24.

Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Watanabe S, Terada M, Yamamoto H.
Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast carcinoma. A retrospective analysis of 176 cases.
Cancer. 1990 Apr 15 **65:8** 1794-800

Tsuda H, Callen DF, Fukutomi T, Nakamura Y, Hirohashi S.
Allele loss on chromosome 16q24.2-qter occurs frequently in breast cancers irrespectively of differences in phenotype and extent of spread.
Cancer Res. 1994 Jan 15 **54:2** 513-7

Tsuda H, Takarabe T, Hirohashi S.
Correlation of numerical and structural status of chromosome 16 with histological type and grade of non-invasive and invasive breast carcinomas.
Int J Cancer. 1999 Aug 20 **84:4** 381-7

Tung HT, Tsukuma H, Tanaka H, Kinoshita N, Koyama Y, Ajiki W, Oshima A, Koyama H.
Risk factors for breast cancer in Japan, with special attention to anthropometric measurements and reproductive history.
Jpn J Clin Oncol. 1999 Mar **29:3** 137-46.

Van de Vijver MJ, Nusse R
The molecular biology of breast cancer.
Biochim Biophys Acta 1991 Apr 16 **1072:1** 33-50

Van Diest PJ, Baak JP, Matze-Cok P, Bacus SS.
Prediction of response to adjuvant chemotherapy in premenopausal lymph node positive breast cancer patients with morphometry, DNA flow cytometry and HER-2/neu oncoprotein expression. Preliminary results.
Pathol Res Pract. 1992 Apr **188:3** 344-9.

Van Laar T, Steegenga WT, Jochemsen AG, Terleth C, van der Eb AJ
Bloom's syndrome cells GM1492 lack detectable p53 protein but exhibit normal G1 cell-cycle arrest after UV irradiation.
Oncogene 1994 Mar **9:3** 981-3

Varley JM, Swallow JE, Brammar WJ, Whittaker JL, Walker RA
Alterations to either c-erbB-2(neu) or c-myc proto-oncogenes in breast carcinomas correlate with poor short-term prognosis.
Oncogene 1987 **1:4** 423-30

Varley JM, Brammar WJ, Lane DP, Swallow JE, Dolan C, Walker RA
Loss of chromosome 17p13 sequences and mutation of p53 in human breast carcinomas.
Oncogene 1991 Mar **6:3** 413-21

Velentgas P, Daling JR.
Risk factors for breast cancer in younger women.
J Natl Cancer Inst Monogr. 1994 **16** 15-24

Venkitaraman AR.
The breast cancer susceptibility gene, BRCA2: at the crossroads between DNA replication and recombination?
Philos Trans R Soc Lond B Biol Sci. 2000 Feb 29 **355:1394** 191-8.

Verma RS
Oncogenetics. A new emerging field of cancer.
Mol Gen Genet 1986 Dec **205:3** 385-9

Verma SP, Salamone E, Goldin B.
Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides.
Biochem Biophys Res Commun. 1997 Apr 28 **233:3** 692-6.

Vernon SW, Tilley BC, Neale AV, Steinfeldt L.
Ethnicity, survival, and delay in seeking treatment for symptoms of breast cancer.
Cancer. 1985 Apr 1 **55:7** 1563-71

Viel JF, Perarnau JM, Challier B, Faivre-Nappez I.
Alcoholic calories, red wine consumption and breast cancer among premenopausal women.
Eur J Epidemiol. 1997 Sep **13:6** 639-43

Viens P, Jacquemier J, Bardou VJ, Bertucci F, Penault-Llorca F, Puig B, Gravis G, Oziel-Taieb S, Resbeut M, Houvenaeghel G, Camerlo J, Birbaum D, Hassoun J, Maraninchi D
Association of angiogenesis and poor prognosis in node-positive patients receiving anthracycline-based adjuvant chemotherapy.
Breast Cancer Res Treat. 1999 Apr **54:3** 205-12

Vihko R, Apter D
Endocrine characteristics of adolescent menstrual cycles: impact of early menarche.
J Steroid Biochem 1984 Jan **20:1** 231-6

Vogel PM, Georgiade NG, Fetter BF, Vogel FS, McCarty KS Jr
The correlation of histologic changes in the human breast with the menstrual cycle.
Am J Pathol 1981 Jul **104:1** 23-34

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL.
Genetic alterations during colorectal-tumor development.
N Engl J Med. 1988 Sep 1 **319:9** 525-32.

Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R.
Allelotype of colorectal carcinomas.
Science. 1989 Apr 14 **244:4901** 207-11

Voogd AC, Crommelin MA, Repelaer van Driel OJ, Tutein Nolthenius-Puylaert MC, Vreugdenhil G, Coebergh JW
The importance of steroid receptors for the prognosis and hormone treatment of breast cancer patients: a retrospective study in Southeastern Netherlands
Ned Tijdschr Geneeskd 1998 Aug 1 **142:31** 1772-8

Walker RA, Gullick WJ, Varley JM
An evaluation of immunoreactivity for c-erbB-2 protein as a marker of poor short-term prognosis in breast cancer.
Br J Cancer 1989 Sep **60:3** 426-9

Walker RA, Dearing SJ, Lane DP, Varley JM.
Expression of p53 protein in infiltrating and in-situ breast carcinomas.
J Pathol. 1991 Nov **165:3** 203-11

Walker RA
Chp16 The breast
General and systemic pathology
Underwood JCE (Ed)
Churchill Livingstone (pub) 1992 457-484

Walker RA, Varley JM.
The molecular pathology of human breast cancer.
Cancer Surv. 1993 **16:** 31-57.

Walker AR, Walker BF, Stelma S.
Is breast cancer avoidable? Could dietary changes help?
Int J Food Sci Nutr. 1995 Nov **46:4** 373-81.

Walker RA, Lees E, Webb MB, Dearing SJ.
Breast carcinomas occurring in young women (< 35 years) are different.
Br J Cancer. 1996 Dec **74:11** 1796-800.

Walker RA, Jones JL, Chappell S, Walsh T, Shaw JA
Molecular pathology of breast cancer and its application to clinical management.
Cancer Metastasis Rev. 1997 Jun **16:1-2** 5-27.

Wang H, Thoresen SO, Tretli S
Breast cancer in Norway 1970-1993: a population-based study on incidence, mortality and survival.
Br J Cancer 1998 May **77:9** 1519-24

Wargotz ES, Silverberg SG
Medullary carcinoma of the breast: a clinicopathologic study with appraisal of current diagnostic criteria.
Hum Pathol. 1988 Nov **19:11** 1340-6.

Watanabe M, Imai H, Shiraishi T, Shimazaki J, Kotake T, Yatani R
Microsatellite instability in human prostate cancer.
Br J Cancer 1995 Sep **72:3** 562-4

Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin CH, Huang JS, Deuel TF
Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus.
Nature 1983 Jul 7-13 **304:5921** 35-9

Waterhouse J, Muir C, Correa P, Powell J (Ed)
Cancer incidence in five continents III
IARC scientific publications **15:** 1976

Waterhouse J, Muir C,Shanmugaratnam K, Powell J
Cancer incidence in five continents IV
IARC scientific publications **42:** 1982

Weber JL, May PE.
Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction.
Am J Hum Genet. 1989 Mar **44:3** 388-96.

Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G.
Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma.
J Natl Cancer Inst. 1992 Dec 16 **84:24** 1875-87

Weinberg RA
Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis.
Cancer Res 1989 Jul 15 **49:14** 3713-21

Weiss SE, Tartter PI, Ahmed S, Brower ST, Brusco C, Bossolt K, Amberson JB, Bratton J.
Ethnic differences in risk and prognostic factors for breast cancer.
Cancer. 1995 Jul 15 **76:2** 268-74.

Weiss HA, Brinton LA, Brogan D, Coates RJ, Gammon MD, Malone KE, Schoenberg JB, Swanson CA.
Epidemiology of in situ and invasive breast cancer in women aged under 45.
Br J Cancer. 1996 May **73:10** 1298-305

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M.
A second-generation linkage map of the human genome.
Nature. 1992 Oct 29 **359:6398** 794-801.

Welcsh PL, Owens KN and King M-C
Insights into the functions of BRCA1 and BRCA2
Trends in Genetics, 2000 **16:2** 69-74

Weston A, Pan CF, Ksieski HB, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, Brower ST, Senie RT, Wolff MS.
p53 haplotype determination in breast cancer.
Cancer Epidemiol Biomarkers Prev. 1997 Feb **6:2** 105-12.

Willen R, Pettersson BA
Pattern of malignant tumours in King Fahad Hospital, Al Baha, Saudi Arabia.
Saudi Med J 1989 **10:** 498-502

Wilson CA, Ramos L, Villasenor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen JJ, Scully R, Livingston D, Zuch RH, Kanter MH, Cohen S, Calzone FJ, Slamon DJ.
Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas.
Nat Genet. 1999 Feb **21:2** 236-40

Winstanley J, Cooke T, George WD, Murray G, Holt S, Croton R, Griffiths K, Nicholson R.
The long term prognostic significance of oestrogen receptor analysis in early carcinoma of the breast.
Br J Cancer. 1991 Jul **64:1** 99-101

Winter H, Cheng KK, Cummins C, Maric R, Silcocks P, Varghese C
Cancer incidence in the south Asian population of England (1990-92).
J Cancer Br 1999 Feb **79:3-4** 645-54

Wojcik BE, Spinks MK, Optenberg SA.
Breast carcinoma survival analysis for African American and white women in an equal-access health care system.
Cancer. 1998 Apr 1 **82:7** 1310-8.

Woodman R.
Controversy rekindled over pill and risk of breast cancer.
BMJ. 1999 Apr 24 **318:7191** 1095.

Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, et al.
Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13.
Science. 1994 Sep 30 **265:5181** 2088-90.

Wright DK, Manos MM
Sample preparation from parraffin-embedded tissue
PCR protocols: a guide to methods and applications
Innis Gelfand DH, Sninsky JJ, White TJ (ed)
Academic press (pub) 1990 153-158

Wu AH, Ziegler RG, Pike MC, Nomura AMY, West DW, Kolonel LN, Horn-Ross PL, Rosenthal JF, Hoover RN.
Menstrual and reproductive factors and risk of breast cancer in Asian-Americans.
Br J Cancer. 1996a Mar **73:5** 680-6.

Wu AH, Ziegler RG, Horn-Ross PL, Nomura AM, West DW, Kolonel LN, Rosenthal JF, Hoover RN, Pike MC.
Tofu and risk of breast cancer in Asian-Americans.
Cancer Epidemiol Biomarkers Prev. 1996b Nov **5:11** 901-6

Wynder EL, Rose DP, Cohen LA.
Diet and breast cancer in causation and therapy.
Cancer. 1986 Oct 15 **58:8 Suppl** 1804-13.

Yang X, Lippman ME
BRCA1 and BRCA2 in breast cancer.
Breast Cancer Res Treat. 1999 Mar **54:1** 1-10.

Yaremko ML, Kutza C, Lyzak J, Mick R, Recant WM, Westbrook CA.
Loss of heterozygosity from the short arm of chromosome 8 is associated with invasive behavior in breast cancer.
Genes Chromosomes Cancer. 1996 Jul **16:3** 189-95.

Yee CJ, Roodi N, Verrier CS, Parl FF
Microsatellite instability and loss of heterozygosity in breast cancer.
Cancer Res 1994 Apr 1 **54:7** 1641-4

Yokota T, Yoshimoto M, Akiyama F, Sakamoto G, Kasumi F, Nakamura Y, Emi M.
Localization of a tumor suppressor gene associated with the progression of human breast carcinoma within a 1-cM interval of 8p22-p23.1.
Cancer. 1999 Jan 15 **85:2** 447-52

Yood MU, Johnson CC, Blount A, Abrams J, Wolman E, McCarthy BD, Raju U, Nathanson DS, Worsham M, Wolman SR.
Race and differences in breast cancer survival in a managed care population.
J Natl Cancer Inst. 1999 Sep 1 **91:17** 1487-91

Yosida TH
Karyotype evolution and tumor development.
Cancer Genet Cytogenet. 1983 Feb **8:2** 153-79.

Yuan JM, Yu MC, Ross RK, Gao YT, Henderson BE.
Risk factors for breast cancer in Chinese women in Shanghai.
Cancer Res. 1988 Apr 1 **48:7** 1949-53.

Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong CP, Anderson KE, Kushi LH, Sellers TA, Folsom AR.
Well-done meat intake and the risk of breast cancer.
J Natl Cancer Inst. 1998 Nov 18 **90:22** 1724-9.

Ziegler RG, Hoover RN, Nomura AM, West DW, Wu AH, Pike MC, Lake AJ, Horn-Ross PL, Kolonel LN, Siiteri PK, Fraumeni JF Jr.
Relative weight, weight change, height, and breast cancer risk in Asian-American women.
J Natl Cancer Inst. 1996 May 15 **88:10** 650-60