BREAST CANCER EPIDEMIOLOGY: MAMMOGRAPHIC SCREENING AND MOLECULAR SUBTYPES

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Monserrat Puig i Vives / DOCTORAL THESIS

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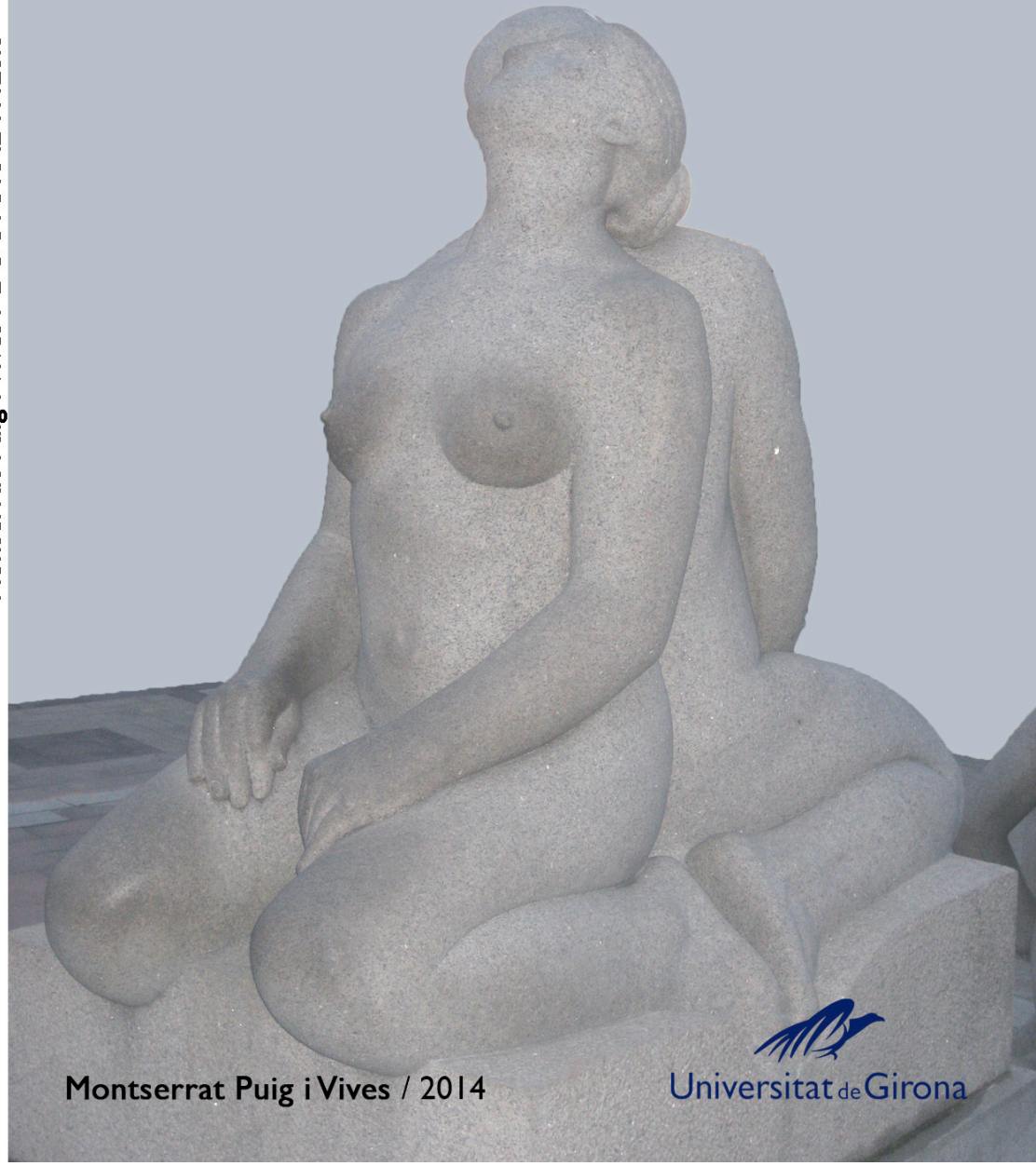

Montserrat Puig i Vives / 2014

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Doctoral Thesis

Breast cancer epidemiology: Mammograhpic screening and molecular subtypes

Montserrat Puig i Vives

2014

PhD Programme in Experimental Sciences and Sustainability

Directed by:

Dr. Marc Saez Zafra and Dr. Rafael Marcos Gragera

Thesis delivered to obtain the doctoral degree by the Universitat de Girona

El Dr. Marc Saez Zafra, de la Universitat de Girona i membre del Grup de Recerca en Estadística, Econometria i Salut (GRECS) i el Dr. Rafael Marcos Gragera, de la Universitat de Girona i epidemiòleg de la Unitat d'Epidemiologia i Registre de Càncer de Girona (UERCG) de l'Institut Català d'Oncologia (ICO),

CERTIFICA:

Que aquest treball, titulat "Breast cancer epidemiology: Mammographic screening and molecular subtypes", presentat per Montserrat Puig i Vives per optar al títol de Doctora per la Universitat de Girona, ha estat realitzat sota la seva direcció.

I, perquè així consti i tingui els efectes oportuns, signem aquest document.

Dr. Marc Saez Zafra

Dr. Rafael Marcos Gragera

Al pare, de qui tant he après i a qui dedico aquesta tesi

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List of publications

This thesis is presented as a compendium of articles.

ARTICLE 1

Title: Rapid increase in incidence of breast ductal carcinoma *in situ* in Girona, Spain 1983-2007

Authors: Puig-Vives M, Pollan M, Rue M, Osca-Gelis G, Saez M, Izquierdo A, Marcos-Gragera R.

Journal: The Breast. 2012 Oct;21(5):646-51

Impact factor (2011)**:** 2.491 (Q1 Obstetrics & Gynecology, position 16 of 79)

DOI: 10.1016/j.breast.2012.01.014

ARTICLE 2

Title: Proportion of breast cancer in women aged 50 to 69 years from Girona according to detection method

Authors: Puig-Vives M, Osca-Gelis G, Camprubí-Font C, Vilardell ML, Izquierdo A, Marcos-Gragera R

Journal: Med Clin (Barc). 2014 Oct 7;143(7):300-2. Epub 2013 Dec 28

Impact factor (2012)**:** 1.399 (Q2 Medicine, General & Internal, position 65 of 155)

DOI: 10.1016/j.medcli.2013.09.042

ARTICLE 3

Title: Distribution and prognosis of molecular breast cancer subtypes defined by immunohistochemical biomarkers in a Spanish population-based study

Authors: Puig-Vives M, Sánchez MJ, Sánchez-Cantalejo J, Torrella-Ramos A, Martos C, Ardanaz E, Chirlaque MD, Perucha J, Díaz JM, Mateos A, Machón M, Marcos-Gragera R

Journal: Gynecol Oncol. 2013 Sep; 130(3):609-14.

Impact factor (2012): 3.929 (Q1 Obstetrics & Gynecology, position 5 of 78)

DOI: 10.1016/j.ygyno.2013.05.039

ARTICLE 4

Title: Molecular subtypes and survival of breast cancer diagnosed within and outside a national mammographic screening program

Authors: M Puig-Vives, LA Akslen, Å Holen, L Solhaug, R Marcos-Gragera, G Ursin, S Hofvind

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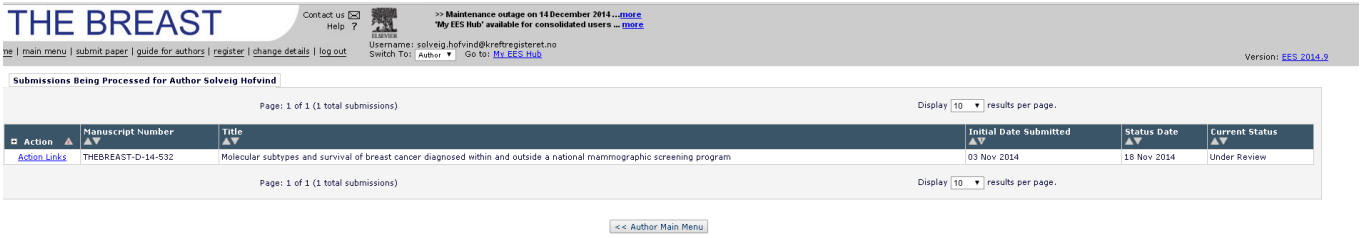

Abbreviations

Abbreviation	**Meaning**
ANOVA	One-way analysis of variance
ASR_W	Age-standardized to the world standard population
ASR_E	Age-standardized to the European standard population
BRCA1/2	Breast cancer gene 1 and 2
CI	Confidence interval
CK	Cytokeratin
COX-2	Cyclooxygenase-2
CR	Crude rate
DCIS	Ductal carcinoma *in situ* of the breast
DCO	Death certificate only
EAPC	Estimated annual percentage change
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
ENCR	European Network of Cancer Registries
ER	Estrogen receptor
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridization
HER1	Human epidermal growth factor receptor 1
HER2	Human epidermal growth factor receptor 2
HER3	Human epidermal growth factor receptor 3
HER4	Human epidermal growth factor receptor 4
HR	Hazard ratio
HRT	Hormone replacement therapy
IACR	International Association of Cancer Registries
IARC	International Agency for Research on Cancer
ICD-O-2	International Classification of Diseases for Oncology, second edition
ICD-O-3	International Classification of Diseases for Oncology, third edition
ICD-10	International Statistical Classification of Diseases, tenth edition
IDESCAT	*Institut d'Estadística de Catalunya*

IHC	Immunohistochemistry
LCIS	Lobulillar *in situ* carcinoma
M/I	Ratio of mortality and incidence
mTOR	Mammalian target of rapamycin
MV	Microscopic verification
NBCSP	Norwegian Breast Cancer Screening Programme
NPI	Nottingham Prognostic Index
PARP	Poly (adenosine disphosphate-ribose) polymerase
PDPCM	*Programa de Detecció Precoç del Càncer de Mama*
PR	Progesterone receptor
RER	Relative excess risk of death
SBR	Scarff, Bloom and Richardson
SD	Standard deviation
SERM	Selective estrogen receptor modulator
TNBC	Triple-negative breast cancers
TP53	Tumour protein p53
US	United States
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization's

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Summary

Summary

Breast cancer is the leading cancer site and the most common cause of death among women worldwide. Over recent decades, breast cancer incidence and survival rates have changed considerably in many countries due mainly to new prevention strategies, novel treatment approaches and changes in lifestyle. The aim of this thesis is to carry out an in-depth study of various aspects of breast cancer epidemiology via the analysis of different population-based datasets. It focuses on the following: incidence trends of breast ductal carcinoma *in situ* (DCIS) in Girona province, paying particular attention to recent changes in mammography use; identifying interval cancers, screen-detected cancers and non-screen-detected cancers in Girona in women aged 50-69; and evaluating the prognostic value of breast cancer molecular subtypes defined by immunohistochemistry (IHC) biomarkers and method of detection, through the analysis of population-based datasets including patients diagnosed in Spain and Norway. Firstly, we have confirmed that DCIS incidence in women resident in Girona province has increased over recent decades (1983–2007) in parallel with an increase in women undergoing periodical mammography. Proportions of screen-detected cancers, interval cancers and non-screen-detected cancers during the start-up phase of the mammographic screening programme (2002–2006) were found to be 42.2%, 5.8% and 52.2%, respectively. Secondly, we have found that luminal A-like was the most frequent subtype associated with the most favourable histopathological characteristics and the best survival rate, while triple-negative breast cancer was related to the most aggressive behaviour and had the lowest survival rate. These studies included women diagnosed with breast cancer in 2004-2005 in Spain and in 2005-2011 in Norway. Importantly, we have concluded that breast cancer molecular subtype defined by IHC biomarkers provides prognostic value, regardless of age, tumour size, histological grade, lymph node involvement and method of detection. And thirdly, we have demonstrated that screen-detected cancers have more favourable histopathological characteristics than non-screen-detected cancers. It is interesting to note that method of detection also provides prognostic value regardless of age, tumour size, histological grade, lymph node involvement and breast cancer molecular subtype defined by IHC biomarkers.

Resum

A nivell mundial, el càncer de mama és el càncer més freqüent i la principal causa de mortalitat per càncer entre les dones. Durant les últimes dècades, les taxes d'incidència i de supervivència del càncer de mama han canviat considerablement en molts països, degut principalment a noves estratègies de prevenció, nous enfocaments terapèutics i canvis en l'estil de vida. L'objectiu d'aquesta tesi és realitzar un estudi per aprofundir en diversos aspectes de l'epidemiologia del càncer de mama, a través de l'anàlisi de diferents bases de dades de cobertura poblacional. Es centra en els següents temes: la tendència de la incidència del carcinoma ductal *in situ* de mama (DCIS) a la província de Girona, prestant especial atenció als canvis recents en l'ús de la mamografia; la identificació dels càncers d'interval, els càncers detectats mitjançant el programa de cribratge i de la resta de càncers diagnosticats a Girona en dones de 50 a 69 anys; i l'avaluació del valor pronòstic tant dels subtipus moleculars de càncer de mama definits per biomarcadors determinats amb tècniques d'immunohistoquímica (IHC), com del mètode de detecció del càncer, utilitzant bases de dades poblacionals que inclouen pacients diagnosticades a Espanya i Noruega. En primer lloc, hem confirmat que la incidència del DCIS de les dones residents a la província de Girona ha incrementat en les últimes dècades (1983-2007), en paral·lel a l'augment de dones que es fan mamografies periòdicament. Les proporcions dels càncers detectats mitjançant el programa de cribatge, fora d'aquest i els càncers d'interval diagnosticats durant els primers anys després de l'inici del programa de cribatge (2002-2006) van ser del 42,2%, 52,2% i 5,8%, respectivament. En segon lloc, hem trobat que el subtipus més freqüent, associat a unes característiques histopatològiques més favorables i a una supervivència més elevada va ser el subtipus *luminal A-like*, i que el càncer de mama triple negatiu es va relacionar amb un comportament més agressiu i va tenir la supervivència més baixa. A aquests estudis s'hi van incloure dones diagnosticades amb càncer de mama el anys 2004 i 2005 a Espanya i del 2005 al 2011 a Noruega. És important destacar que el subtipus molecular de càncer de mama definit per biomarcadors determinats amb tècniques d'IHC proporciona valor pronòstic, independentment de l'edat, la mida del tumor, el grau histològic, l'afectació dels ganglis limfàtics i el mètode de detecció. I en tercer lloc, hem demostrat que els càncers detectats mitjançant el cribratge tenen unes característiques histopatològiques més favorables que els càncers detectats fora del programa. És interessant observar que el mètode de detecció del càncer també proporciona valor pronòstic independentment de l'edat, la mida del tumor, el grau histològic, l'afectació dels ganglis limfàtics i el subtipus molecular definit per biomarcadors determinats amb tècniques d'IHC.

Resumen

A nivel mundial, el cáncer de mama es el cáncer más frecuente y la principal causa de mortalidad por cáncer entre las mujeres. En las últimas décadas, las tasas de incidencia y de supervivencia del cáncer de mama han cambiado considerablemente en muchos países, debido principalmente a las nuevas estrategias de prevención, nuevos enfoques del tratamiento y cambios de estilo de vida. El objetivo de esta tesis es realizar un estudio para profundizar en diversos aspectos de la epidemiología del cáncer de mama, a través del análisis de diferentes bases de datos de cobertura poblacional. Se centra en los siguientes temas: la tendencia de la incidencia del carcinoma ductal *in situ* de mama (DCIS) en la provincia de Girona, prestando especial atención a los cambios recientes en el uso de la mamografía; la identificación de los cánceres de intervalo, los cánceres detectados mediante el programa de cribado y el resto de cánceres diagnosticados en Girona en mujeres de 50 a 69 años; y la evaluación del valor pronóstico de los subtipos moleculares del cáncer de mama definidos por biomarcadores determinados con técnicas de inmunohistoquímica (IHC), así como el método de detección del cáncer, utilizando bases de datos poblacionales que incluyen pacientes diagnosticadas en España y Noruega. En primer lugar, hemos confirmado que la incidencia del DCIS en las mujeres residentes en la provincia de Girona ha incrementado en las últimas décadas, en paralelo con el aumento de mujeres que se han realizado una mamografía periódicamente. Las proporciones de los cánceres detectados mediante el programa de cribado, fuera de este y los cánceres de intervalo diagnosticados durante los primeros años después del inicio del programa de cribado (2002-2006) fueron del 42,2%, 52,2% y 5,8%, respectivamente. En segundo lugar, en los dos estudios de base poblacional español y noruego, encontramos que el subtipo más frecuente, asociado a unas características histopatológicas más favorables y a una supervivencia más elevada fue el subtipo *luminal A-like*, y que el cáncer de mama triple negativo se relacionó con un comportamiento más agresivo y tuvo una supervivencia más baja. Estos estudios incluyen mujeres diagnosticadas con cáncer de mama los años 2004 y 2005 en España y del 2005 al 2011 en Noruega. Es importante destacar que el subtipo molecular de cáncer de mama definido por biomarcadores determinados con técnicas de IHC proporciona valor pronóstico, independientemente de la edad, el tamaño del tumor, el grado histológico, la afectación de los ganglios linfáticos y el método de detección. Y en tercer lugar, hemos demostrado que los cánceres detectados mediante el cribado tienen unas características histopatológicas más favorables que los cánceres detectados fuera del programa. Es interesante observar que el método de detección del cáncer también proporciona valor pronóstico independientemente de la edad, el tamaño del tumor, el grado histológico, la afectación ganglionar y el subtipo molecular de cáncer de mama definido por biomarcadores determinados con técnicas de IHC.

Contents

“Live as if you were to die tomorrow. Learn as if you were to live forever.”

Mahatma Gandhi

"Prevention is so much better than healing because it saves the labour of being sick."

Thomas Adams, 17th century British physician

Introduction

1. Breast cancer: natural history and histological classification

1.1. Anatomy of the breast

The breast is composed of adipose tissue and glandular tissue with a dense fibrous stroma (Figure 1) [1-3]. The glandular tissue consists of lobules that group together into 15-20 grape-like cluster lobes. These are connected with small ducts converging into larger collecting ducts that drain into the nipple. These ducts are formed by two cell layers (epithelial and myoepithelial) surrounded by fibroblast. The layer of myoepithelial cells is in contact with the basement membrane. Epithelial cells are responsible for milk synthesis and release into the lumen. Milk flows from the lobules through the ducts to the nipple. The breast also contains blood and lymphatic vessels. Most breast lymphatic drainage takes place through the axillary lymph nodes [4].

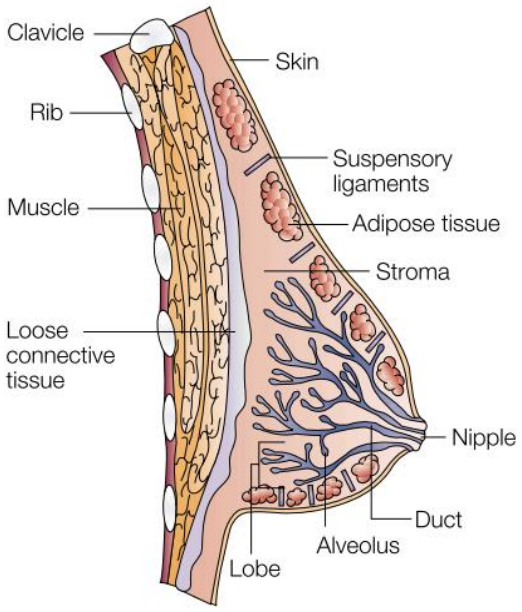

Figure 1. Anatomy of the human mammary gland. Taken from Ali *et al.*, 2002 [1].

1.2. The natural history of breast cancer

The natural history of breast cancer is not completely well-known. Different hypotheses have been suggested regarding breast carcinogenesis, the linear model traditionally being the most accepted. This model postulates that epithelial cells progressively evolve through the following non-obligatory phases: normal healthy breast tissue, hyperplasia, atypical hyperplasia, carcinoma *in situ* and invasive carcinoma (Figure 2) [3, 5].

This progression can take years or decades and requires the accumulation of genetic alterations. There is growing evidence that the carcinoma *in situ* is the direct precursor to most invasive breast cancers, and many of these cancers are indeed accompanied by an *in situ* component. Besides this, the two diseases show concordance in risk factors and genetic alterations,

suggesting that they are involved in the same disease process [3, 5-7]. Invasive tumour cells can penetrate through the basement membrane into stroma. Here, they have the potential to invade the vasculature and thereby reach regional lymph nodes or other sites, causing distant metastasis (see Section 5.3 of the Introduction).

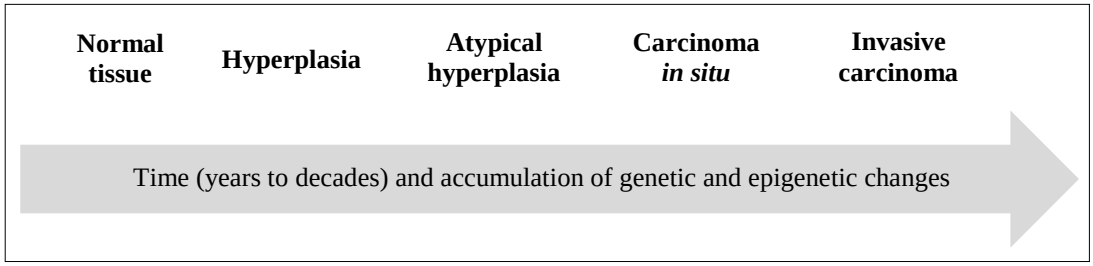

Figure 2. Linear model of breast carcinogenesis. Adapted from Allred, 2010 and Burstein *et al*., 2004 [3, 5].

1.3. The WHO histological classification of breast tumours

From a pathological point of view breast tumours are highly heterogeneous. The World Health Organization's (WHO) Classification of Breast Tumours divides this disease into the groups outlined below, each with different histological characteristics, prognoses and clinical manifestations [8]. This is the most recent breast cancer classification published by the WHO, from 2012.

– **Epithelial tumours**. Most breast tumours fall into this group, which is divided into:

  Invasive breast carcinoma: Invasive carcinoma of no special type, invasive lobular carcinoma, tubular carcinoma, cribriform carcinoma, mucinous carcinoma, carcinoma with medullary features, carcinoma with apocrine differentiation, carcinoma with signet-ring-cell differentiation, invasive micropapillary carcinoma, metaplastic carcinoma of no special type and rare types.

  Epithelial-myoepithelial tumours: Pleomorphic adenoma, adenomyoepithelioma and adenoid cystic carcinoma.

  Precursor lesions: Ductal carcinoma *in situ* (DCIS) and lobular neoplasia.

  Intraductal proliferative lesions: Usual ductal hyperplasia, columnar cell lesions including flat epithelial atypia and atypical ductal hyperplasia.

  Papillary lesions: Intraductal papilloma, intraductal papillary carcinoma, encapsulated papillary carcinoma and solid papillary carcinoma.

  Benign epithelial proliferations: Sclerosing adenosis, apocrine adenosis, microglandular adenosis, radial scar/complex sclerosing lesion and adenomas.

- **Mesenchymal tumours**: Nodular fasciitis, myofibroblastoma, desmoid-type fibromatosis, inflammatory myofibroblastic tumour, benign vascular lesions, pseudoangiomatous stromal hyperplasia, granular cell tumour, benign peripheral nerve-sheath tumours, lipoma, liposarcoma, angiosarcoma, rhabdomyosarcoma, osteosarcoma, leiomyoma and leiomyosarcoma.
- **Fibroepithelial tumours**: Fibroadenoma, phyllodes tumour and hamartoma.
- **Tumours of the nipple**: Nipple adenoma, syringomatous tumour and Paget disease of the nipple.
- **Malignant lymphoma**: Diffuse large B-cell lymphoma, Burkitt lymphoma, T-cell lymphoma, extranodal marginal-zone B-cell lymphoma of MALT type and follicular lymphoma.
- **Metastatic tumours**
- **Tumours of the male breast**: Gynaecomastia and carcinoma invasive and *in situ*.
- **Clinical patterns**: Inflammatory carcinoma and bilateral breast carcinoma.

According to the fourth edition of the WHO Classification of Tumours of the Breast, the term "infiltrating ductal carcinoma" should be replaced by "invasive carcinoma of no special type" [8]. The WHO suggests that there is no evidence that these tumours are derived exclusively from mammary ductal epithelium in distinction from lobular carcinomas. However, since "ductal" is still widely used, "invasive ductal carcinoma" is also accepted by the WHO as alternative terminology, and hence its use in the present thesis.

1.4. Invasive carcinoma of the breast

Invasive carcinoma of the breast is defined as a malignant tumour that has the ability to penetrate the basement membrane, invade adjacent tissues and regional nodes and even metastasize to distant sites. Invasive ductal carcinoma comprises the largest group of all invasive breast cancers [8].

The most common symptom of this disease is breast lumps, which can be associated with pain. Other possible signs are nipple abnormalities, such as discharge, retraction, distortion or eczema, but these are uncommon symptoms. Prior to the widespread use of mammography, most malignant carcinomas were diagnosed clinically. Nowadays, the proportion of asymptomatic cancers detected has risen considerably.

Invasive carcinomas of the breast are associated with different clinical behaviour and prognosis according to histopathological characteristics such as stage and age at diagnosis, histological grade, histology, hormonal receptors status and cell proliferation rate. Risk factors associated

with invasive breast cancer development, prevention and therapeutic approach to breast cancer will all be explained in the Introduction section.

1.5. Ductal carcinoma in situ of the breast (DCIS)

Carcinoma *in situ* refers to breast epithelial cells that have abnormal increased growth and accumulate within the ducts and lobules without evidence of invasion beyond the basement membrane. DCIS, also known as intraductal cancer, is the most common (80%-90%) type of *in situ* carcinoma of the breast [3]. It can be presented as a palpable breast mass or thickening or nipple discharge or after the diagnosis of Paget's disease of the nipple, but is generally not associated with clinical manifestations. Calcifications represent the most common mammographic presentation of DCIS. In fact, following the widespread of mammographic screening nearly 90% of DCIS are diagnosed while they are clinically occult [5].

The biology of DCIS is heterogeneous and poorly understood. Several histopathological classifications have been proposed to distinguish between different types of DCIS [3, 5, 8]. These classifications are based on nuclear morphology, architectural pattern of tumour growth (solid, papillary, micropapillary, or cribriform), and presence/absence of comedonecrosis (comedo, non-comedo). The first classification system is the most widely used, yielding three categories of low, intermediate and high nuclear grades. Low-grade DCIS is related to a low risk of recurrence and proliferation rate. Contrarily, high-grade DCIS is associated with aggressive tumour behaviour, high proliferation rate and well-differentiated tumours. A large proportion of DCIS displays complex combinations of nuclear grades and/or growth patterns.

Several biological and genomic characteristics distinguish DCIS from both normal breast tissue and benign proliferative breast lesions. These characteristics are often factors related to cell growth and differentiation, cytoskeletal function, intracellular transport of membranes and the surrounding microenvironment [5]. Contrarily, progression from DCIS to invasive breast cancer is not well-characterized. Cell behaviour, molecular pathways and gene expression profiles of DCIS and invasive breast cancer are similar [3]. In fact, intrinsic subtypes previously identified in invasive breast cancer can be also recognized in DCIS, although their prognosis value remains unclear [9, 10]. Biological differences responsible for invasion must exist, but there is a lack of effective means to distinguish which DCIS would develop into invasive breast cancer and how long they would remain latent. Thus, a high proportion of women diagnosed with DCIS receive some form of surgical treatment. Mastectomy, excision followed by radiotherapy and excision alone have all been proposed as appropriate treatment approaches for DCIS [4]. Also, some patients undergo contralateral prophylactic-mastectomy. Tamoxifen is often recommended as an effective therapy in women with *in situ* tumour expressing estrogen receptor (ER) and trastuzumab has been studied to treat DCIS that overexpress human epidermal growth factor receptor 2 (HER2) [6, 11]. The absence of a tool to distinguish between progressive and non-progressive DCIS may lead to overtreatment. There is a need to determine

whether these women definitively need to undergo surgery or whether all they really need is repeat mammography and to be treated as individuals with an elevated risk of the disease [12]. In small studies, ER and HER2 positivity have been inconsistently pointed to as markers for a decreased and increased risk of recurrence, respectively [6, 11]. Furthermore, high expression levels of p16, cyclooxygenase-2 (COX-2) and Ki-67 has been linked with a risk of subsequent invasive cancer [13]. In addition, it has to be considered that as well as progressing to invasive breast cancer, DCIS diagnosis is a marker for an increased chance of developing invasive breast cancer elsewhere in the ipsi- or contralateral breast. It has been estimated that between 14% and 50% of DCIS would evolve into invasive breast cancer if left untreated, whereas less than 10% of patients diagnosed with DCIS would subsequently develop invasive breast cancer elsewhere if treated by excision alone [6, 12]. A deeper understanding of the molecular mechanisms of invasion could lead to the development of new personalized therapeutic approaches to treat DCIS.

2. Epidemiology of breast cancer

Breast cancer is the leading cancer site and the most frequent cause of cancer death among women worldwide with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers) (Figure 3) [14]. In terms of mortality, it is estimated that around 522,000 women died from breast cancer in 2012. Female breast cancer incidence and mortality rates vary among countries (Figure 4). Estimated age-standardized incidence and mortality rates for breast cancer in Spain are similar to those in Portugal or Slovenia. In Catalonia, approximately 4841 women will be diagnosed with breast cancer and around 932 patients will die from this cancer in 2020 [15]. Breast cancer incidence rates have been slightly higher in Girona province than in the rest of Spain, with a rate ratio of 1.1 (95% CI: 1.1 to 1.2) in 2000-2004 (per 100,000 European standard population) [16].

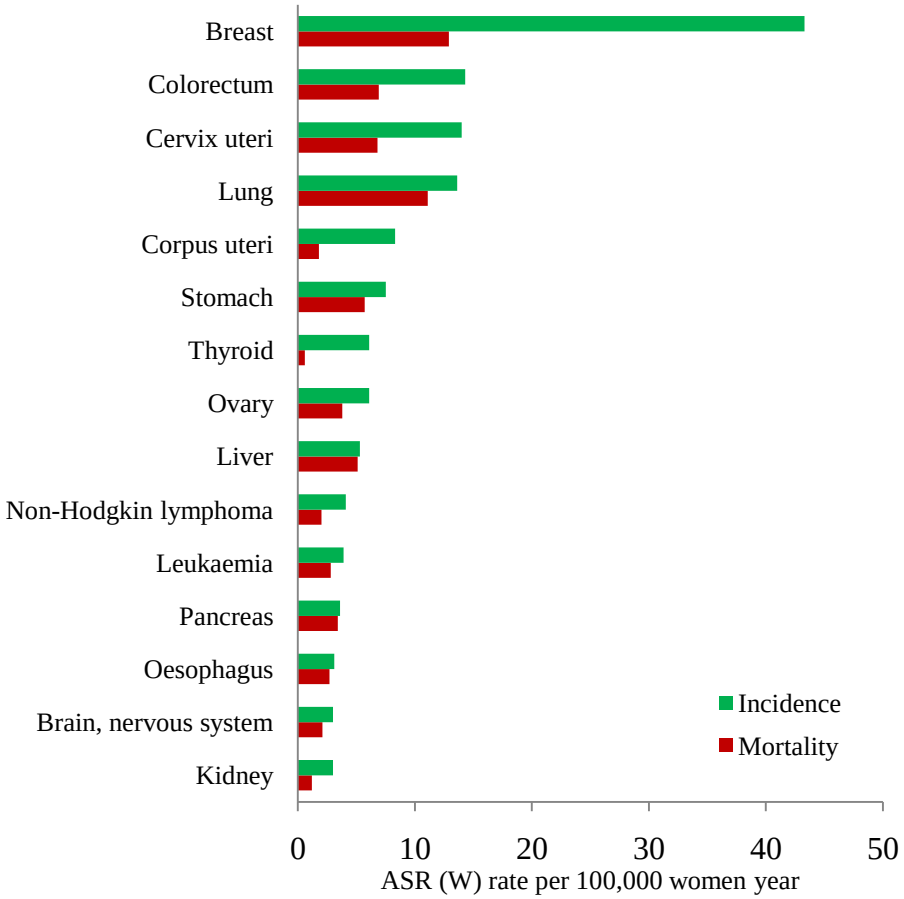

Figure 3. Estimated age-standardized incidence and mortality rates of cancer in women worldwide.
ASR_W: Age-standardized to the world standard population rate. Adapted from Globocan 2012 [14].

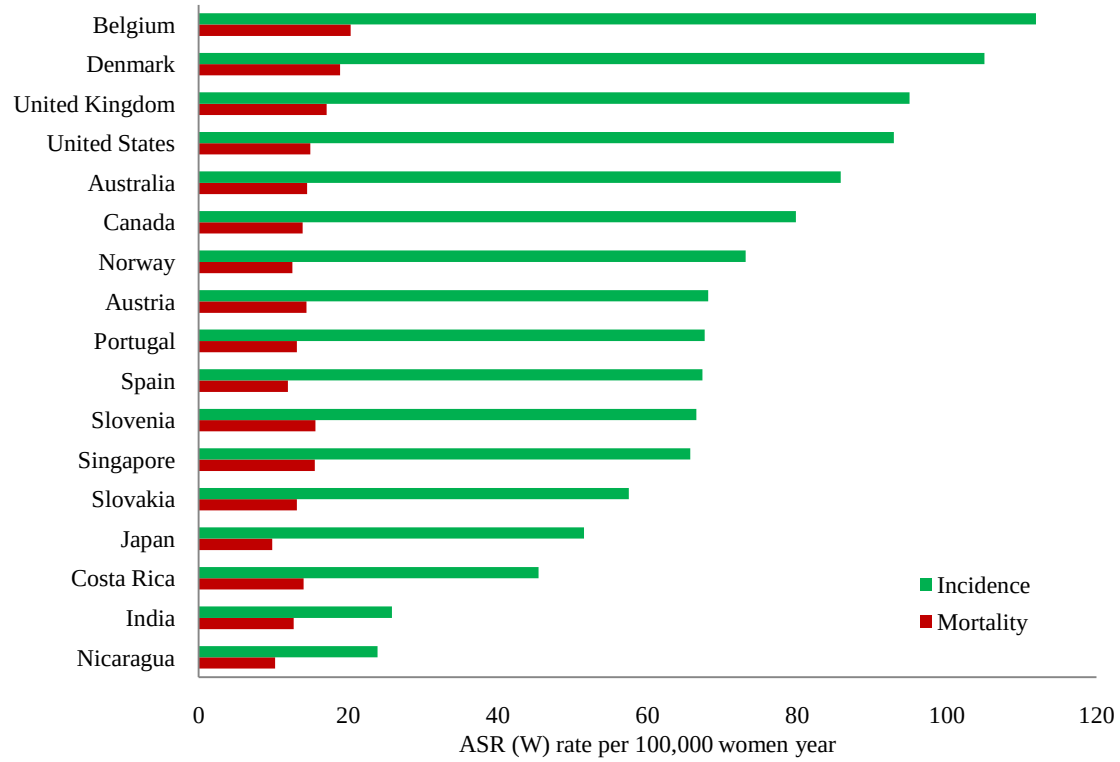

Figure 4. **Estimated age-standardized incidence and mortality rates for female breast cancer in different countries**. ASR_W: Age-standardized to the world standard population rate. Adapted from Globocan 2012 [14].

2.1. Invasive carcinoma of the breast

The invasive breast cancer incidence rate increased throughout the 1980s and '90s, before falling and then levelling off at the beginning of the 21st century, a trend described in many developed countries such as Australia [17], France [18], Norway [19], Spain [20] and the United States (US) [21, 22]. This pattern was mostly restricted to postmenopausal women and to tumours expressing ER. In Catalonia, breast cancer incidence rose by 2.2% (95% CI: 1.8% to 2.6%) from 1980 to 1999 [23]. This increase was more marked in women over 40, whereas incidence rates for young women remained stable. Following this period, a significant decrease of 1.5% was detected in overall breast cancer incidence (2000-2007) [24]. Similar incidence trends were observed in Spain, with the peculiarity that incidence in young women was still rising in 2004 [16]. Multiple factors might affect changes in breast cancer incidence. In many European countries and the US increasing incidence has been attributed to a rise in the use of menopausal hormone replacement therapy (HRT) among postmenopausal women in the '90s, and the decline to a drop in HRT prescription [17, 19, 21, 22]. However, in Spain the proportion of women using HRT has always been very low [25]. Prevalence of HRT use among women aged >40 years rose slightly from 0.7% in 1989 to 3.4% in 1999. According to the 2006 Spanish National Health Survey, the percentage of women using HRT was 5.3% in women aged 45-64 and 0.5% for women over 65 [26]. In the 2011-2012 Spanish survey, these rates decrease to 1.6% and 0.2%, respectively [27]. Consequently, it has been suggested that changes in HRT

prescription may not be the only key factor in explaining recent trends in breast cancer incidence [16].

The implementation of mammographic screening programmes has also been posited as influencing incidence trends [22, 28, 29]. The adoption of mammographic population-based screening programmes brings the date of diagnosis forward, resulting in a transitory increment in incidence rates. Once the programme is fully established, incidence rates usually decrease before stabilizing due to the pool of prevalent undiagnosed cases being notably reduced. This change in incidence trends produced by the introduction of a screening programme is known as **screening saturation**.

Despite the arguments presented above, the impact of HRT use and screening saturation remains disputed. Changes in lifestyle and reproductive factors may have also contributed to changes in incidence trends. These include physical inactivity, obesity in postmenopausal women, alcohol consumption, delayed childbearing, decline in fertility, early menarche and late menopause (see Section 3 of the Introduction).

Regarding survival, women diagnosed with breast cancer usually present high outcomes rates. A recently published article concluded that 5-year net survival was 81% in Europe and 84% in the US [30]. However, survival differs greatly according to many prognostic factors, such as stage at diagnosis time, for example. Survival rate for women diagnosed with distant metastasis is much lower than for patients with metastatic lymph nodes or localized disease [31]. This and other prognostic factors will be explained in Section 5 of the Introduction.

A steady downturn in breast cancer mortality has been described in many European countries and the US over the last 20-40 years [31, 32]. In Spain and Catalonia, a statistically significant rise in mortality trends was detected during the 1970s, ‘80s and the beginning of ‘90s, followed by an important decline [20, 24, 33, 34]. This fall has mainly been associated with increased access to more effective treatments and early detection.

2.2. Ductal carcinoma in situ of the breast (DCIS)

Women diagnosed with DCIS have about a 4-fold increased risk of developing an invasive breast cancer compared with women in the general population [35]. Given that incidence rates of DCIS are currently increasing in many developed countries, these tumours are clinically challenging and considered to be of growing importance to public health.

In some countries, DCIS incidence has been seen to stabilize after a sharp increase over recent decades, with incidence trends differing by histological type and age at diagnosis [36, 37]. In the US, an increase in the incidence of non-comedo DCIS, which are not associated with subsequent breast malignancy, has been reported in recent decades, whereas rates of comedo DCIS, which are associated with subsequent invasive breast cancer, decreased or held constant throughout the 1980s and ‘90s [37]. Furthermore, larger or restricted upward trends have been

observed in target age groups for mammographic screening [36, 38, 39]. In Norway, rates of DCIS in women aged 50-69 years, the target population for mammographic screening, steadily increased in the years prior to the start-up phase of the programme; they then peaked during its implementation, dropped, and then rose again.

Since the majority of DCIS lesions do not present breast lumps but are often visible on mammography, the increase in detected cases has mainly been attributed to the widespread adoption of mammographic screening over the past decade. Whereas DCIS now accounts for around 7.4%-21% of all newly diagnosed cases of breast cancer, prior to screening DCIS diagnosis was rather rare, representing less than 5% of all breast malignancies [31, 36, 38, 40]. Nevertheless, organized screening may not completely explain the upward trend and other factors may play an important role. The number of women attending opportunistic screening, improved detection methods, improved training and skills of the radiologist and changes in risk factors have to be considered when analysing DCIS incidence [38, 41].

It has been suggested that the marked upward trend in DCIS incidence may contribute, by earlier stage detection, to declining incidence of invasive breast cancer and consequently to reduced breast cancer mortality from this disease [38, 42]. However, there are no consistent data confirming that mammography detection of DCIS directly prevents breast cancer death [43]. In fact, breast cancer mortality after 10 years of DCIS diagnosis is around 1-2%, regardless of whether mastectomy or breast-conserving surgery is applied, thus revealing that DCIS is not a life-threatening disease *per se* [40].

As commented previously, risk factors for DCIS and invasive breast cancer are similar, suggesting that etiologic pathways may be shared between the two diseases [3, 5-7, 43]. Family history of breast cancer, nulliparity or delayed childbearing, late age at menopause, long-term use of postmenopausal HRT, obesity in postmenopausal women and high mammography breast density all increase the risk of both DCIS and invasive breast cancer. Contrarily, early menarche, high alcohol consumption and oral contraceptive use are not consistently linked with an increased risk of DCIS development but are associated with invasive breast cancer development.

3. Breast cancer risk factors

The aetiology of breast cancer is multifactorial, involving hormonal and reproductive factors, dietary and lifestyle factors, and others, as described in Table 1 [4, 8, 44, 45]. It is widely accepted on the basis of epidemiological studies that endogenous and exogenous estrogen play a key role in the development of breast tumours and the risk of breast cancer development is higher with increasing estrogen levels. Breast cancer incidence is low among young women, increases sharply during the premenopausal period and then peaks at 50-69 years old before dropping again, when synthesis of estrogen ceases. This incidence trend over the course of the lifetime differs from the majority of cancers, which usually show a greater risk of tumour development with age, and suggests the involvement of reproductive hormones in breast cancer aetiology [14].

Many hormonal and reproductive factors have been considered to be risk factors for breast cancer development in women. Earlier age at menarche and later age at menopause have consistently been associated with increased risk of breast cancer by raising lifetime exposure to endogenous estrogen. Furthermore, nulliparous women over around 45 years of age are at greater risk of the development of breast cancer compared to parous women. Oral contraceptives and HRT use are both associated with breast cancer development by increasing estrogen levels. The decline in breast cancer incidence in some developed countries at the beginning of this century has mainly been attributed to a decrease in HRT use (see Section 2.1 of the Introduction) [17, 19, 21, 22, 45]. Contrarily, it has been confirmed that young age at first full-term pregnancy, high parity and lactation, preferably more than 2 years, all have a protective effect [4, 8].

Hormonal and reproductive factors	Dietary and lifestyle factors	Other factors
↑ Early age at menarche	↑ High consumption of fat	↑ Family history of breast cancer
↑ Late age at menopause	↑ High consumption of alcohol	History of:
↑ Late age at first full-term birth	↑ Obesity (postmenopausal women)	↑ Atypical hyperplasia
↑ Nulliparity	↓ Physical activity	↑ DCIS
↑ Oral contraceptives	↓ High intake of vegetables	↑ LCIS
↑ HRT		↑ Benign breast disease
↓ High parity		↑ Type 2 diabetes
↓ Lactation		↑ Hyperinsulinemia
↓ Young age at first full-term birth		↑ Ionizing radiation

Table 1. **Risk and protective factors for breast cancer.** DCIS: Ductal carcinoma *in situ*; HRT: Hormone replacement therapy; LCIS: Lobulillar carcinoma *in situ*. Red arrow: Risk factors for breast cancer; green arrow: Protective factors for breast cancer. Adapted from DeVita *et al*., 2008, Lakhani *et al.*, 2012, Hamajima *et al.*, 2002 and Boyle *et al.*, 2003 [4, 8, 44, 45].

In terms of dietary factors, high intakes of vegetables are probably associated with a moderate protective effect for breast cancer. Epidemiologic studies have also confirmed the protective effect of physical activity with regard to breast cancer development. The influence of physical activity on breast cancer is of high interest for postmenopausal women, as in these women obesity is strongly associated with an elevated risk of breast cancer [46]. Following the menopause, adipose tissue is the major source of estrogen and obese postmenopausal women therefore have higher levels of endogenous estrogen and consequently a higher risk of breast cancer development. In addition, a high consumption of fat and alcohol are associated with an increased risk of breast cancer [4, 45]. The relationship between smoking and breast cancer has been found to be confounded by alcohol [44].

Genetic factors have to be taken into account when identifying women at high risk of breast cancer development. Risk varies with relationship to affected family members and number of affected and unaffected relatives [47]. Mutations in *BRCA1* (breast cancer gene 1) and/or *BRCA2* (breast cancer gene 2) are responsible for the majority of these cancers. De Sanjosé *et al.* described that these mutations explained about 10% of breast cancers diagnosed in Catalan women under 40 years of age [48].

Additional factors have been considered to increase the risk of breast cancer: women with a history of atypical hyperplasia, DCIS, lobulillar *in situ* carcinoma (LCIS) or other benign breast diseases, women with a history of ionizing radiation, women with high mammographic density and women previously diagnosed with type 2 diabetes or hyperinsulinemia [4, 45].

Risk and protective factors that determine the development of breast cancer differ according to molecular subtype [49-53]. This suggests that molecular subtype classification has to be taken into account in order to understand breast cancer aetiology. Population-based studies have found that reproductive factors such as early age at menarche, nulliparity and increasing age at first full-term birth are more strongly associated with positive than negative hormonal receptor tumours. An increase in the risk of basal-like breast cancer is described when increasing parity. Moreover, *BRCA1* mutation carriers and premenopausal African American women show a high prevalence of basal-like breast cancer [50, 51, 54, 55].

4. Prevention and screening for breast cancer

Primary breast cancer prevention consists in avoiding or reducing exposure to risk factors mentioned in the previous section or by increasing resistance to them, thus obtaining a decrease in breast cancer incidence. The objective is to avoid the disease. Secondary prevention is the early detection and treatment of the disease, meaning it must be applied during the non-detectable phase (Figure 5). Screening is the major component of secondary prevention because it can detect disease at an early stage and so increase the probability that a cancer may be cured. Finally, tertiary prevention refers to curing cancers that have developed and preventing cancer death. It is applied during the symptomatic phase (Figure 5) through treatment and rehabilitation programmes [45, 56]. This section focuses on secondary prevention.

At the end of the last century, many developed countries implemented an organized breast cancer population-based mammographic screening programme, including France [18], the Netherlands [36], Norway [57], Spain [58] and the United Kingdom [59]. Mammographic screening has been confirmed so far as the most effective method for breast screening [60]. The objective of screening for breast cancer is to reduce morbidity and mortality from the disease by detecting cancer at an early stage without adversely affecting healthy participants [61]. Screening is therefore based on the existence of an adequate treatment, which is more effective if begun earlier during the progression of the disease [59, 62].

Opportunistic breast screening coexists alongside organized population-based mammographic screening in many countries. Opportunistic screening is defined as screening that takes place outside an organized or population-based screening [61]. This type of screening may be recommended during check-ups by doctors at primary health care centres or in other health care settings. The prevalence of women attending opportunistic and organized screening varies substantially across countries and health systems.

Since mammographic screening programmes were first established, there has been debate regarding their potential benefits and adverse effects. The anticipated major benefit is a reduction in breast cancer mortality and the most commonly discussed adverse effect is overdiagnosis. Both of these are discussed in the following paragraphs.

4.1. Benefits of mammographic screening programmes

First of all, implementing a mammographic screening programme reduces inequality of access to the preventive test among the target population. Access to mammography becomes homogeneous for the whole population, regardless of income or educational level [63].

It is also widely accepted that breast cancer mortality has decreased since the introduction of mammographic screening programmes. However, it is not yet fully clear whether the drop in mortality can be attributed to screening, the improved treatment available in recent years or the

interaction of both factors. Naturally, this uncertainty is no reason to interrupt mammographic screening.

The effect of mammographic screening on breast cancer mortality differs between studies, although all of them observe an important reduction. In general, these studies suggest a relative risk reduction around 20% with mammography at 11 years of follow-up [60]. In particular, a meta-analysis of 11 randomized trials with 13 years of follow-up also estimated a 20% (95% CI: 11% to 27%) reduction in breast cancer mortality among women invited for screening [59]. A review of 20 European incidence-based mortality studies found a reduction of 26% (95% CI: 13% to 36%) after 6-11 years of follow-up among women invited to mammographic screening [64]. Additionally, a recent review of observational studies reported that the reduction in breast cancer mortality is even higher for women who are invited (25-31%) and actually screened (38-48%) [65]. Finally, in Norway the reduction in the rate of breast cancer death in recent years is described as being 10 points higher in screen-detected (28%) than in non-screen-detected women (18%) [66]. The difference in estimates of absolute risk reduction reported is one of the greatest sources of controversy regarding the value of mammographic screening. However, there is general agreement regarding the evidence that screening does have a beneficial effect on breast cancer mortality.

4.2. Biases related to mammographic screening programmes

Screen-detected cancers are breast cancers identified using the screening test, with or without further assessment in a member of the target population who was invited for and attended mammographic screening. These cancers have a more favourable prognosis than symptomatic cancers, even in long-term survival analyses [61, 67-71]. Generally, screen-detected cancers have a higher proportion of negative lymph nodes and small-sized, well-differentiated and hormonal receptor positive tumours. Whether the cancer is detected at screening or by symptoms is considered an independent prognostic factor beyond the stage shift [72]. However, some biases have to be considered when comparing mortality from breast cancer among screen-detected and non-screen-detected cancers.

Before a tumour can be detected through clinical signs and symptoms, it remains asymptomatic for an indeterminate period of time known as **sojourn time** or **detectable preclinical phase** and distinctive for each particular tumour (Figure 5). Sojourn time starts when the tumour is detectable by mammography. If women participate in a mammographic screening programme, the tumour will be detected before symptoms appear. The period between when a cancer is found by screening and when it would appear through clinical signs and symptoms is known as **lead time** (Figure 5) [62, 73, 74].

Overall survival is measured from date of diagnosis to date of death. In the example in Figure 5, the patient would survive 10 years if she did not participate in a screening programme, but 15

years if she did. This simply reflects earlier diagnosis; the natural history of the disease and time of death are unchanged. We cannot therefore consider it real improved overall survival.

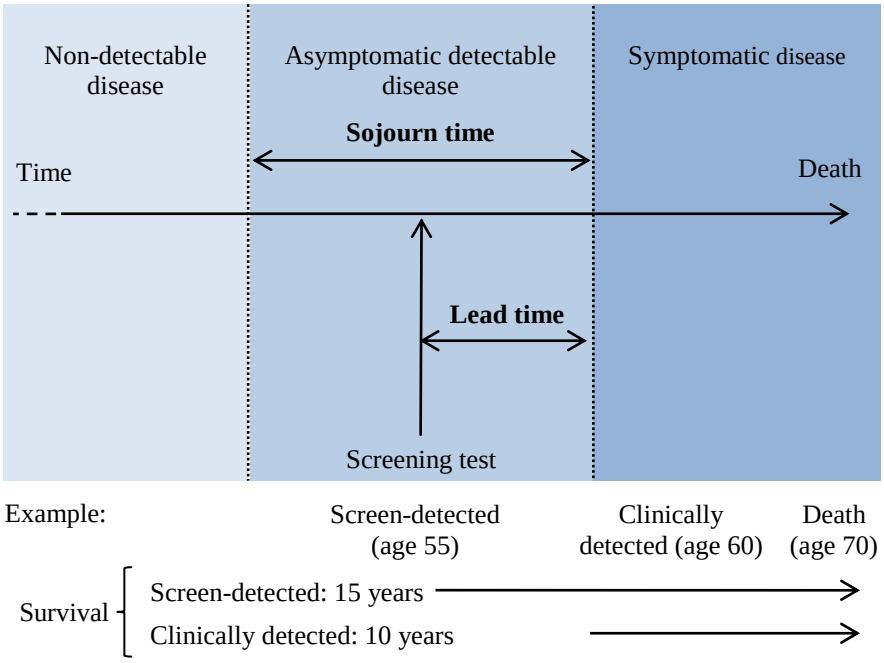

Figure 5. Overview of disease progression with the intervention of an early-detection screening test. Adapted from IARC (International Agency of Research on Cancer) handbooks of cancer prevention, 2002 [62].

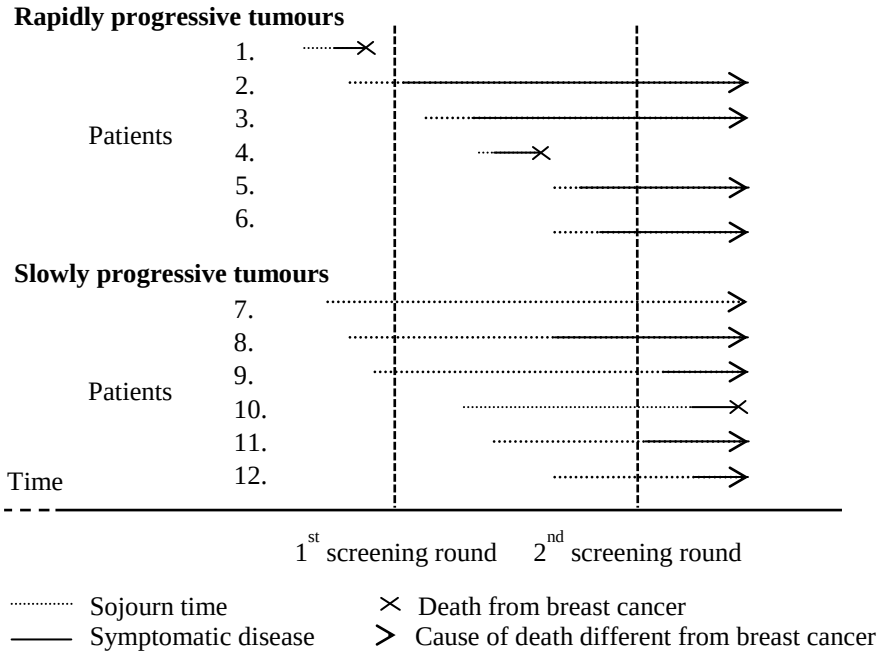

Figure 6. **Overview of rapidly and slowly progressive tumours in relation to breast cancer screening with 12 patient examples**. Adapted from Cox *et al.*, 2013 [74].

However, this scenario is not always exactly like the example because treatment approach and prognosis are different in early and advanced-detected breast cancers. As mentioned above, screening is based on the existence of an adequate treatment being more effective when applied in early-staged rather than advanced-stage diseases.

Furthermore, overestimation of survival among screen-detected women is influenced by the high proportion of slowly progressing tumours detected by screening. The probability of cancer detection is directly proportional to the length of sojourn time: the longer the sojourn time, the greater the chance of detecting the lesion (Figure 6). The length of sojourn time depends on the cancer progression rate. Patients with slowly progressive cancers have a longer sojourn time and are more likely to be diagnosed by screening than women with rapidly growing cancers. In addition, these cancers are usually less aggressive, with a low histological grade, and they are often associated with good prognosis. Contrarily, women with rapidly progressive tumours are more likely than average to die of their disease and less likely to have it detected by screening. Thus, screen-detected cancers are represented by a higher proportion of non-aggressive and slowly growing tumours than non-screen-detected breast cancer. This bias is known as **length bias** [62, 73, 74].

Finally, when examining the benefits of screening, aside from early diagnosis and the duration of the tumour's progression, patient characteristics and the health system must also be considered. Comorbidity, ethnicity and culture can influence participation in mammographic screening. Therefore, participants in a mammographic screening programme may have a different baseline risk for developing breast cancer and mortality to non-participants; this is known as **selection bias** [74, 75].

4.3. Adverse effects of mammographic screening programmes

Although many women will benefit from mammographic screening programmes, others will be affected by the inevitable adverse effects of it. The objective is therefore to minimize these. The most important are explained in this section: overdiagnosis, interval cancers, false-negative and false-positive cancers.

Some screen-detected cancers would not have been diagnosed in the absence of mammographic screening. These cancers are referred to as **overdiagnosis**. A cancer is overdiagnosed if it would never progress further or would evolve slowly enough that the patient would die from other causes than breast cancer. The tumour in both cases would not become clinically apparent during the patient's lifetime, thus it would not be life-threatening [62, 76]. If patient number 7 in Figure 6 attended screening and were diagnosed with breast cancer, it would be overdiagnosed. As depicted in Figure 7, cancer growth rate varies greatly between tumours [77]. Some screen-detected cancers might progress so slowly that they would never have been clinically apparent. Detection of these cancers turns women into patients, which means that they receive unnecessary treatment and their quality of life might deteriorate. However, clinicians are unable

to distinguish between overdiagnosed and non-overdiagnosed patients and treat all cases, leading to overtreatment. In Figure 7, tumour D represents a rapidly growing tumour, leading to distant metastasis and death in a short period of time. This case would not have benefitted from screening. Contrarily, tumour A exemplifies a tumour growing very slowly, remaining microscopic, undetectable and without morbidity during a woman's lifetime. This type of tumour tends to be DCIS or, if invasive, histological grade 1 or 2 rather than grade 3. If this woman were to attend mammographic screening, it would be a case of overdiagnosis. The women with tumours B and C would benefit from screening by bringing forward diagnosis to a time when they would still be curable.

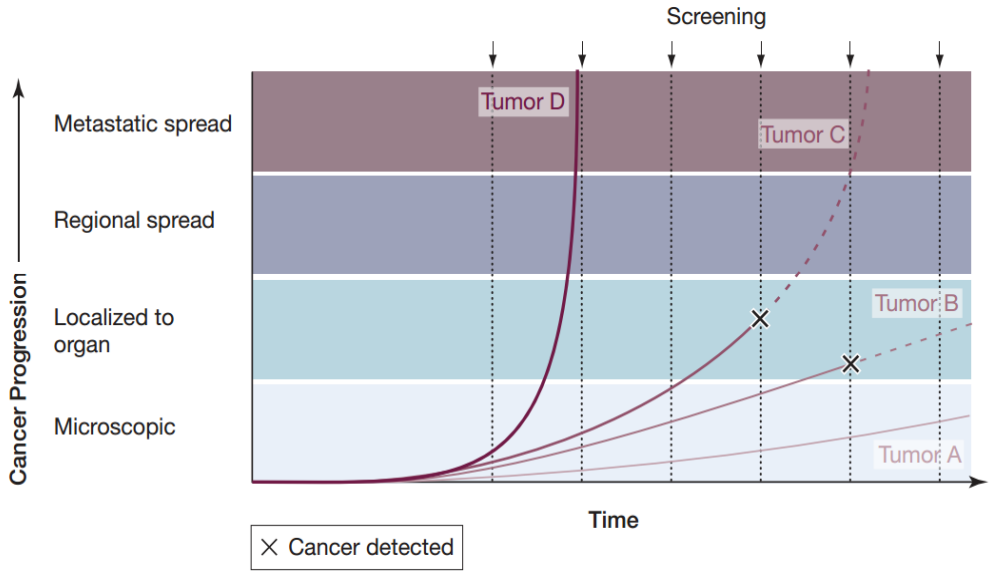

Figure 7. Varying screen detection capability in relation to tumour growth rate. Taken from Esserman *et al.*, 2009 [77].

Information regarding frequency of overdiagnosis is necessary to quantify the adverse effect of screening. However, estimating the rate of overdiagnosis is very complex and advanced statistical analyses are required with long follow-up times. This results in a wide variety of studies demonstrating different rates of overdiagnosis, ranging from about 0-54% [59, 60, 76, 78-81]. Although there is no consensus regarding this percentage, there is a general agreement that target women for screening need to be informed about its adverse effects as well as its benefits.

False-positive results are also an important concern in mammographic screening. A result is considered to be false-positive if breast cancer is not further diagnosed after recall for additional evaluation. Screening effectiveness evaluation should include assessing the frequency of false-positives. The main negative effects of false-positive results are lower attendance, anxiety and associated posterior excision biopsies [82].

Finally, another key component of quality control for screening programmes is **interval cancer** rate. As defined in European Guidelines for Quality Assurance in Breast Cancer Screening and Diagnosis, an interval cancer is a breast cancer arising after a negative screening episode (which may include assessment) and before the next scheduled screen round or within 24 months for women who have reached the upper age limit [61]. To identify interval cancers, a link is required between women participating in a screening programme and population-based cancer registries. In general, interval cancers are rapidly growing and aggressive tumours associated with a short sojourn time. In Figure 6, patient 4 represents an example of interval cancer. Compared with screen-detected cancers, interval cancers are related to poorer prognosis [83, 84]. European guidelines recommend interval cancers be classified into the following: true interval, occult, minimal signs, false-negative, and unclassifiable tumours [61]. For true interval cancers, the screening mammogram is normal and there is no reason for further assessment. The sojourn time for these cancers is under two years (screening period intervals), and they are therefore inevitable in mammographic screening. Contrarily, in the **false-negative** group (undetected cancers) an abnormality is clearly visible in the screening mammogram and additional assessment should be tested. Delays in diagnosing false-negative cancers may be due to reading or technical errors. Frequency of false-negatives should be estimated in a screening programme so as to minimize the rate and improve screening effectiveness. Distribution of histopathological characteristics, such as molecular subtypes, is represented differently within the interval cancer categories described above. Notably, the triple-negative phenotype is concentrated among true interval cancers and tumours with minimal signs [84, 85].

Although a debate still exists balancing the benefits and adverse effects of mammographic screening, many studies agree that the benefits outweigh the adverse effects, which is why it is recommended in many developed countries.

4.4. Mammography use and implementation of mammographic screening programmes

In Catalonia, mammography use started during the 1980s and spread throughout the ‘90s. In 1980, only 10 mammography devices were available, while in 2000 there were 134 [23]. The use of mammography as a preventive treatment for breast cancer in Catalonia has therefore increased in the last decades. The proportion of women over 20 undergoing mammography periodically in Catalonia was lower in 1994 (24.5%) than in 2002 (40.4%), 2006 (43.1%) and 2012 (49.1%) according to Catalan Health Surveys [86-88]. Mammography use has increased dramatically among women aged 50-69, from 26.9% in 1994 to 94.1% in 2012 [86, 89]. Prior to implementation of the organized population-based mammographic screening programme (*Programa de Detecció Precoç del Càncer de Mama*, PDPCM) in Catalonia, mammography use was higher among women aged 40-49 than those aged 50-69. Nowadays, the target population of the PDPCM (women aged 50-69) shows the largest proportion of women undergoing mammography periodically, as recommended by European guidelines. The highest

increase in participation was among those with a lower educational level, indicating that the introduction of an organized screening programme is associated with reduced inequality of access to an effective test [63].

It was in 1990 that the first organized population-based mammographic screening programme was implemented in Spain, namely in Navarra [58]. All Autonomous Regions followed suit and the entire Spanish target population has been covered since 2009. In Catalonia, the PDPCM began in the mid-1990s and has covered the entire target population since 2002 [63]. The National Health Service recommends a biennial mammography for all women aged 50-69. In Girona province, the PDPCM began in 1999 and was fully implemented in 2002.

Mammographic screening programmes are organized in rounds. During one round, the entire target population is invited to participate in the programme. The participation rate is then used to evaluate the overall programme. European guidelines define >70% as an acceptable level of participation and >75% as the desirable level [62]. In the first round in Girona province, the participation rate was 67%, in the second 70% and from the third to the fifth it remained at 68%, meaning participation has remained just below the acceptable level.

In some countries such as Norway mammographic screening programmes cover the entire country. The Norwegian Breast Cancer Screening Programme (NBCSP) started as a pilot project in one county in 1995, expanded gradually and became nationwide in 2005. The programme, which is administered by the Norwegian Cancer Registry, annually invites about 580,000 women aged 50-69 to biennial mammographic screening [57]. The compliance rate was 84% between 1996 and 2009 [75].

5. Prognostic factors of breast cancer

Although the majority of women diagnosed with breast cancer have good prognosis, some have poor prognosis and others have survival similar to the general population. Prognostic factors are used to determine which of these groups a patient might belong to. Prognostic markers are defined as tumour characteristics established at time of diagnosis that determine the natural evolution of the disease in the absence of treatment and are associated with outcome [4]. In breast cancer, the most common and well-documented prognostic factors are lymph node involvement, ER and progesterone receptor (PR) status, HER2 amplification, and tumour size, stage, histological grade, histology and proliferation rate. Some of the prognostic factors are also considered as predictive factors, suggesting the likely benefit of specific therapy; for example, ER positivity is associated with good prognosis and predicts the response to hormonal therapies.

5.1. Lymph node involvement

Nowadays, absence or presence of axillary lymph node metastasis is the most important prognostic factor for breast cancer and remains one of the most powerful markers for predicting relapse [4, 8]. The number of metastatic lymph nodes and levels of involvement are strongly associated with clinical outcome [90]. The technique used to identify the status of the sentinel lymph node was first implemented at the beginning of the 21st century and has become commonly used in clinical practice. The sentinel lymph node is the first lymph node to receive lymphatic drainage from a primary tumour. If it does not contain metastatic cells, it is highly probable that the other nodes will not contain tumour cells either and lymph node dissection is therefore usually not recommended [91].

5.2. Tumour size

After extent of axillary lymph node involvement, tumour size is the most important factor in predicting breast cancer outcome. Mostly, the larger the tumour is, the higher the rate of recurrence, distant metastasis and death from breast cancer. As described below, tumour size is classified according to the extension of the primary tumour (Table 2). Micrometastasis is defined as the extension of cancer cells beyond the basement membrane into the adjacent tissues, with a maximum dimension of 0.1 cm [91]. When more than one focus exists, the largest one is considered. In this scenario, we call the tumour multifocal or multicentric depending on the area affected. A tumour is multifocal when only one breast quadrant is involved and multicentric when two or more quadrants are involved.

5.3. TNM Classification System

The TNM breast cancer classification system divides the disease into groups, or so-called stages. This system was first described in the 1950s and last updated in 2009 in the 7th edition [91]. It is mainly used as a tool by clinicians to plan treatment and obtain prognosis information. This classification system is based on the size of the primary tumour (T), the presence or absence and extent of regional lymph nodes metastasis (N), and the presence or absence of distant metastasis (M). Clinical classification (pre-treatment) is termed cTNM and is essential for therapy selection. Postsurgical histopathological classification is known as pTNM and is used to guide adjuvant therapy and estimate prognosis. The prefix y is used in those cases where classification takes place during or after neoadjuvant treatment (ycTNM or ypTNM). The three components of the TNM classification are further divided, as shown in Table 2. Combining the categories of T, N and M, five stages are recognized. Stage 0 corresponds to *in situ* carcinomas and Paget disease, stage I (IA, IB) is associated with localized tumours, stages II (IIA, IIB) and III (IIIA, IIIB, IIIC) with regional metastasis and/or large tumours, and stage IV with tumours with distant metastasis. Survival rates are higher for women diagnosed with a localized breast cancer than for those where the disease has extended beyond the breast [31, 92].

pTNM	Subclassification	Description
T	X	Primary tumour cannot be assessed
	0	Occult carcinoma (no evidence of primary tumour)
	is	Carcinoma *in situ* or Paget disease
	1 (1mi, 1a, 1b, 1c)	≤2 cm, including micrometastasis
	2	>2cm to 5 cm
	3	>5 cm
	4 (4a, 4b, 4c, 4d)	Chest wall/skin ulceration, skin nodules, inflammatory
N	X	Regional lymph nodes cannot be assessed
	0	No regional lymph node metastasis
	1 (1mi, 1a, 1b, 1c)	Micrometastasis or metastasis in 1 – 3 nodes
	2 (2a, 2b)	Metastasis in 4 – 9 nodes
	3 (3a, 3b, 3c)	Metastasis in ≥10 nodes
M	0	No distant metastasis
	1	Distant metastasis

Table 2. Overview of the 7th TNM breast cancer classification system (pathological classification, pTNM). Adapted from Sobin L *et al*., [91].

5.4. Histological grade

Histological grade is based on the tumour's degree of differentiation and is a well-established prognostic factor in breast cancer [8, 93]. An important advantage of histological grade is that it can be determined by trained pathologists using a simple and low-cost method. The most widely-used system of grading is the Scarff, Bloom and Richardson (SBR) classification, modified by Elston and Ellis and based on the following three morphological characteristics [94]. First, the degree of tubule or gland formation; second, the nuclear pleomorphism, related to the shape of the cell and nuclei; and third, the mitotic index, which determines the tumour's rate of proliferation. Each of these characteristics gives a score from 1 to 3, leading to a histological grade categorized as grade 1 (well-differentiated), 2 (moderately-differentiated) or 3 (poorly-differentiated). Well-differentiated tumours tend to show very good outcomes, whereas poorly-differentiated tumours are usually associated with a high risk of recurrence and death [8, 93].

5.5. Histology

As explained in Section 1.3 of the Introduction, the WHO divides breast cancers according to their histological characteristics, updating the classification periodically [8]. Most tumours fall into the group of invasive breast ductal or lobular carcinomas; those remaining are classified into several groups associated with different prognoses. For example, prior to the use of neoadjuvant therapy, inflammatory tumours had a survival rate around 25%. Nowadays, this has increased to 50%, but is still very low compared with ductal carcinoma or lobular carcinoma. Contrarily, pure tubular carcinoma has an excellent long-term prognosis, usually reaching the same level of survival as women from the general population.

5.6. Hormonal receptors

George Thomas Beatson was first to recognize and demonstrate the relationship between estrogen and breast cancer [95]. In his article published in The Lancet in 1896, he showed that bilateral oophorectomy in premenopausal women can result in disease regression. Since then, thousands of studies have explained the function of hormonal receptors and their role in breast cancer.

The ER is a member of the nuclear receptor superfamily which is activated by the hormone 17β-estradiol. This binding results in a conformational change that enables binding to DNA and the formation of co-activator and/or co-repressor multiprotein complexes. These complexes facilitate the gene transcription that stimulates cell growth, proliferation and survival [96]. Two main forms of ER exist, ERα and ERβ, which are encoded by separate genes located on different chromosomes. Each receptor has distinct tissue expression patterns, post-traslational modifications, and cellular localization in normal and disease states. However, both are normally present in the mammary gland [97].

PR also plays an important role in breast cancer development. It is a member of the nuclear receptor superfamily, which is activated by progesterone. This binding is followed by conformational changes of the receptor, recruitment of co-activators and co-repressors and binding to DNA, leading to the upregulation of target gene transcription [98].

Currently, ER and PR are recognized as prognostic factors and the most important predictive factors for endocrine treatment (see Section 7 of the Introduction) [4, 8]. Patients whose tumours express ER and/or PR are associated with a better prognosis than women with a complete absence of ER and/or PR expression. Only hormone receptor positive patients benefit significantly from endocrine therapy.

Currently, assays for ER and PR are performed using immunohistochemistry (IHC) techniques. The cut-off for ER and PR positivity may vary between laboratories. For many years ≥10% was commonly used as the cut-off point, but it has recently been recommended that ER and PR assays be considered positive if there are at least 1% positive tumour nuclei in the sample [99].

5.7. Human epidermal growth factor receptor 2 (HER2)

HER2 (also known as ErbB-2, ERBB2, HER2/neu) is located on chromosome 17 and is a member of the family of tyrosine kinase receptors, which comprises four members: HER1 (also known as EGFR, epidermal growth factor receptor), HER2, HER3 and HER4 [100]. All members have an extracellular ligand-binding region, a transmembrane segment and an intracellular kinase domain. HER receptors ligands are members of the EGF (Epidermal growth factor) family. HER2 does not bind to a specific ligand, but rather tends to form homodimers or heterodimers with other HER receptors (Figure 8). Dimerization induces activation of the intrinsic kinase domain, resulting in phosphorylation on specific tyrosine residues, which leads to activation of intracellular signalling pathways involved in enhanced cell growth, survival and cell differentiation. Overexpression of HER2 in tumours (not only in breast cancer) leads to constitutive activation of HER2 [100, 101].

HER2 gene amplification or overexpression is the major predictive factor for the efficacy of trastuzumab (see Section 7 of the Introduction) [4, 8, 102]. Additionally, HER2 overexpression acts as an independent prognostic factor associated with poor clinical outcome. Along with ER and PR, HER2 overexpression is routinely assessed in clinical practice. A guideline for defining HER2 overexpression was developed some years ago. IHC for HER2 is considered negative when the score is 0 or 1+, equivocal when 2+ and positive when 3+. Fluorescence *in situ* hybridization (FISH) for HER2 is performed to verify HER2 status in cases with a result of IHC 2+ [103].

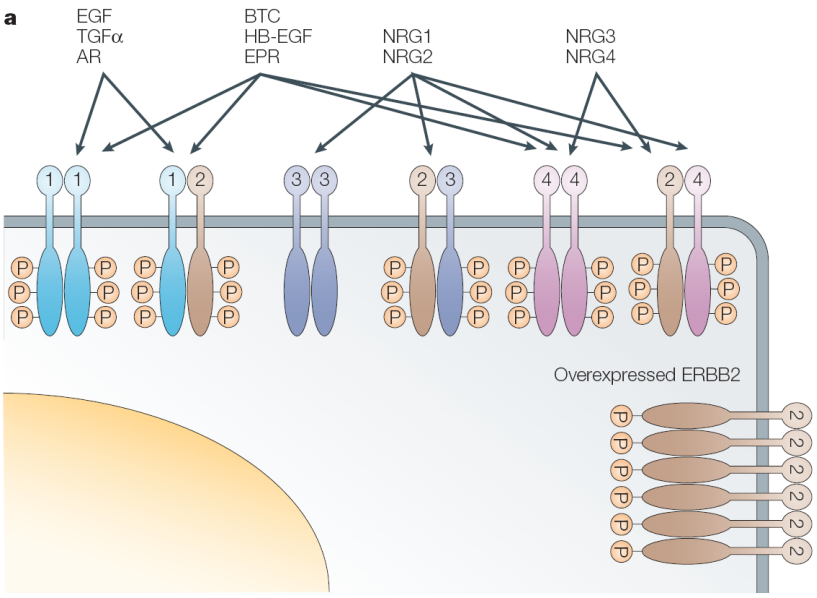

Figure 8. HER receptors 1, 2, 3 and 4, their ligands and the formation of homodimers and heterodimers. HER: Human epidermal growth factor receptor. Taken from Hynes *et al.*, 2005 [100].

5.8. Proliferation rate

Several biological markers have been evaluated for cell proliferation in breast cancer. Currently, one of the most widely used is IHC assessment of the Ki-67 antigen. The expression of Ki-67 varies in intensity throughout the cell cycle, reaching a peak during mitosis, which makes Ki-67 a very good marker for proliferation [104]. It is both a predictive and prognostic marker for breast cancer. A high Ki-67 score is associated with a higher chance of response to chemotherapy and also poor prognosis. As well as ER and PR, cut-off for Ki-67 is usually different among laboratories. In the case of Ki-67, <14% and <20% have been both proposed as a cut-off, showing the need to establish a standardized value [105, 106].

6. Breast cancer molecular subtypes

Numerous advances in diagnosis and therapeutic approaches in breast cancer have occurred over recent decades. However, it is estimated that around 522,000 women died from this disease worldwide in 2012 [14]. This reflects the lack of a complete understanding of breast cancer with respect to prevention strategies, treatment, disease progression, molecular pathways and genetic alterations. Breast cancer is a heterogeneous disease with regard to its clinical and biological behaviour. This complexity is partly reflected by the prognostic factors explained in the previous section. These parameters are currently used by clinicians to predict prognosis and decide treatment strategies, but they do not provide a complete understanding of the biology of the disease [101]. Breast cancer molecular subtypes may help to explain this heterogeneity.

6.1. Breast cancer intrinsic subtypes

Hierarchical clustering analyses have emerged during the last decade to suggest that part of the phenotypic diversity of breast tumours may be accompanied by a corresponding diversity in gene expression patterns. These analyses enable the molecular taxonomy of breast cancer to be improved. In 2000, Perou *et al*. identified a set of 496 genes, known as "intrinsic genes", that presented little variance in expression within repeated samples but high variance across different tumours, thus defining "intrinsic subtypes" [107]. This classification reflects genetic and biological alterations as well as cell biology behaviour, describing the intrinsic properties of the tumour. They also present relevant differences in incidence, survival and response to treatment, being considered both a predictive and prognostic factor [52, 55, 101, 108-110]. In fact, one might even consider them independent diseases. Intrinsic subtypes therefore represent an important tool for clinicians to complement and expand the information provided by classic histopathological factors in tailoring treatment and predicting prognosis.

As mentioned above, intrinsic subtypes are defined according to gene expression patterns. Table 3 summarizes the level of expression (high, moderate, low or absent) of luminal, HER2, basal and proliferation gene clusters in intrinsic subtypes [52, 107, 108, 110]. A major distinction is detected between tumours showing moderate to high and low to absent expression of luminal epithelial specific genes. At least two subtypes have been identified in the group of tumours expressing luminal specific genes: Luminal A and luminal B. On the other hand, tumours characterized by low to absent gene expression of hormonal receptors were split into two main subtypes: HER2-enriched and basal-like. In addition, a normal breast gene expression pattern has also been detected, typified by elevated expression of basal epithelial genes and many genes typically expressed in adipose tissue, as well as low expression of luminal epithelial genes.

Breast cancer intrinsic subtypes	Gene cluster expression			
	Luminal	**HER2**	**Basal**	**Proliferation**
Luminal A	High	Low-absent	Low-absent	Low
Luminal B	Moderate	High-low	Low-absent	High
HER2-enriched	Low-absent	High*	Low-absent	High
Basal-like	Low-absent	Low-absent	High	High

Table 3. Intrinsic subtypes classification of breast cancer. HER2: Human epidermal growth factor receptor 2. *Not all HER2-enriched tumours show HER2 amplification**.** Adapted from Perou *et al.*, 2011, Perou *et al.*, 2000, Sorlie *et al.*, 2001 and Sorlie *et al.*, 2003 [52, 107, 108, 110].

Tumours categorized as luminal give rise to the majority of breast tumours, the luminal A subtype being the most prevalent. These tumours present high expression of cytokeratins (CK) 8/18 and the cluster of transcription factors that include ER and PR, genes typically expressed by breast luminal cells [52, 101, 107-110]. The luminal A subtype shows a low to absent expression of the HER2 cluster genes. Contrarily, the luminal B subtype exhibits a greater proportion of HER2 positive tumours. One of the most marked differences between luminal subtypes is proliferation rate. Whereas luminal A tumours generally present low expression of proliferation associated genes, proliferation rate in luminal B is elevated [105]. Regarding treatment, luminal tumours are associated with endocrine sensitivity [101].

The HER2-enriched subtype is typified by a low expression of luminal and basal gene clusters and elevated expression of HER2 and proliferation gene clusters [52, 55, 101, 107, 108, 110]. Nevertheless, not all tumours that fall into this subtype are HER2 amplified and/or overexpressed [55].

Like the HER2-enriched subtype, the basal-like subtype is less frequent than luminal tumours. It exhibits high expression of basal and proliferation genes, and low to absent expression of luminal and HER2 gene clusters [107]. The basal cluster is composed of genes typically expressed by breast basal (and/or myoepithelial) epithelial cells, such as CK 5/6, HER1 or vimentin.

It is well-known that specific genetic alterations lead to the development of certain subtypes of breast cancer [110]. For example, many basal-like tumours show mutations in *BRCA1* and/or *TP53* (tumour protein p53) (about 80%), which are two important tumour suppressors [52, 55, 108]. In fact, the majority of *BRCA1* mutation carriers, if they develop breast cancer, often develop a basal-like tumour. Also, the HER2-enriched subtype shows a high proportion of cancers with *TP53* mutated, but *BRCA1* mutation carriers do not generally develop a breast cancer positive for HER2. Contrarily, only about 13% of luminal A tumours have a *TP53* mutation.

In terms of histopathological characteristics, it has consistently been confirmed that luminal A is associated with non-aggressive tumours and non-luminal cancers are related to aggressive tumours. The proportion of high-grade tumours is usually higher for basal-like and HER2-enriched than luminal cancers. As for tumour size, luminal A tends to have the smallest tumours of all intrinsic subtypes [52, 101].

Differences in patient outcome have also been described between intrinsic subtypes. Luminal A tumours show the highest survival rate among all subtypes measured as overall, breast-specific or relapse-free survival [101, 108-110]. In general terms, luminal B is associated with intermediate prognosis and hormonal receptor negative with the lowest. Outcomes for women diagnosed with an HER2-enriched cancer have improved greatly in recent decades due to the use of anti-HER2-target therapies. Prior to the use of this treatment, in combination with or sequentially after chemotherapy, the survival of patients with HER-enriched was similar to or lower than that of women diagnosed with basal-like breast cancer. Currently, basal-like tumours show the lowest survival and identifying effective targets for this subtype remains a challenge in breast cancer treatment [101, 109].

The complexity of gene expression patterns in breast tumours reveals how far we are from a comprehensive understanding of breast tumour heterogeneity. Progress in genomic studies may lead to definition of more homogeneous subtypes. In this context, the claudin-low subtype has recently been characterized by a low expression of genes involved in tight junctions (Claudin) and cell-cell adhesion, negativity for hormonal receptors expression and absence of HER2 overexpression [52, 55, 101, 111]. Claudin-low tumours share some biological and genomic characteristics with basal-like tumours. Further genetic and molecular studies are needed in order to better characterize this emerging subtype.

6.2. Breast cancer molecular subtypes defined by IHC biomarkers

One of the main drawbacks of intrinsic subtype profiling is that microarray-based tests are expensive and not accessible for the vast majority of patients. This limits the use of gene expression patterns as a routine diagnostic tool in the public health setting. Since intrinsic subtypes were first defined, many efforts have been made to obtain accurate IHC surrogate biomarkers for subtyping breast cancers [101, 106, 112, 113]. From here on, the term molecular subtypes refers to subtypes defined by IHC biomarkers, rather than intrinsic subtypes.

Initially, molecular subtypes were defined using a panel of three biomarkers: ER, PR and HER2. Luminal A is defined by ER and/or PR positive and HER2 negative, luminal B by ER and/or PR positive and HER2 positive, HER2 overexpressed as a lack of ER and PR expression but amplified HER2, and triple-negative breast cancers (TNBC) as an absence of ER and PR positivity and lack of HER2 overexpression. It was considered that TNBC would be equivalent to the basal-like subtype. Nevertheless, many studies have described the biological heterogeneity within triple-negative phenotypes [55, 114]. Although the vast majority of TNBC

fall into the basal-like phenotype, other intrinsic subtypes (HER2-enriched and Claudin-low subtypes) are also present. Prat *et al*. found that ER and HER2 status do not entirely recapitulate intrinsic subtypes [101] (Figure 9). In their data set, 83% of tumours classified as basal-like were ER-/HER2-, whereas 17% showed positivity for ER and/or HER2. In addition, 34% of the HER2-enriched cancers were HER-. Distinguishing between luminal B and luminal A represented a problem because both subtypes showed an elevated proportion of ER+/HER2- tumours. The St Gallen International Expert Consensus has worked during recent years to better describe a classification of breast cancer molecular subtypes and associated treatment. In 2009, for the first time it recommended the routine use of Ki-67 expression to guide treatment [115]. It then presented a surrogate definition of intrinsic subtypes of breast cancer in 2011, subsequently updated in 2013 [106, 113]. The most recent breast cancer molecular subtype classification is summarized in Table 4. To avoid confusion between intrinsic subtypes and those identified by IHC, the following terminology has been suggested for molecular subtypes: "luminal A-like", "luminal B-like HER2-", "luminal B-like HER2+", "HER2 positive" and "triple-negative breast cancer (TNBC)" [106]. Compared to luminal B-like, the luminal A-like subtype was restricted to tumours showing positivity for PR and low expression of Ki-67. Furthermore, the luminal B-like subtype was separated into two subgroups according to HER2 overexpression (luminal B-like HER2- and luminal B-like HER2+). HER2 positive and TNBC were both defined by an absence of ER and PR expression and HER2 was only overexpressed in tumours classified as HER2 positive.

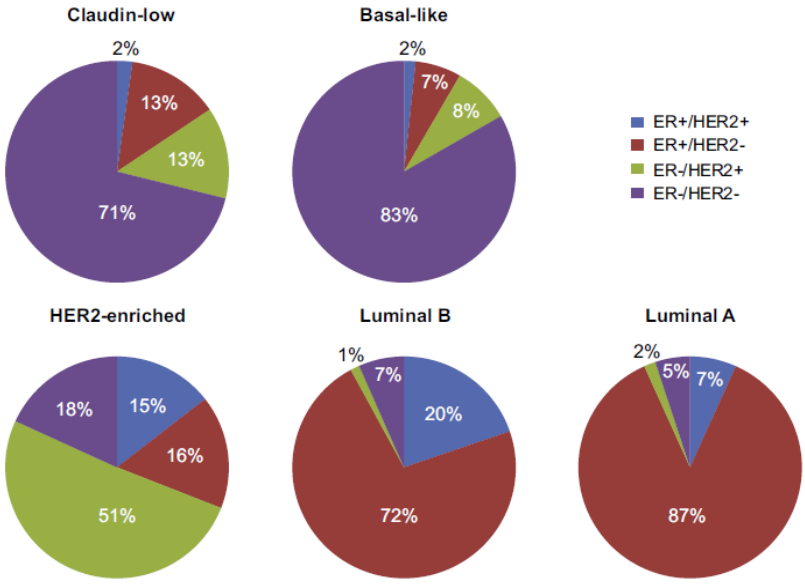

Figure 9. Distribution of ER+/HER2+, ER+/HER2-, ER-/HER2+ and ER-/HER2- clinical groups within each intrinsic subtype of breast cancer. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2. Taken from Prat *et al*., 2011 [101].

Molecular subtypes	Definition of molecular subtypes
Luminal A-like	ER and PR positive HER2 negative Ki-67 low
Luminal B-like (HER2 -)	ER positive HER2 negative And at least one of: Ki-67 high PR negative or low
Luminal B-like (HER2 +)	ER positive HER2 overexpressed or amplified Any Ki-67 Any PR
HER2 positive	HER2 overexpressed or amplified ER and PR absent
Triple-negative breast cancer (TNBC)	ER and PR absent HER2 negative

Table 4. Definition of breast cancer molecular subtypes suggested in the last St Gallen recommendation, 2013. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; PR: Progesterone receptor. Adapted from Goldhirsch *et al*., 2013 [106].

Many population-based studies have been published to show differences in incidence, risk factors, survival, therapeutic treatment responsiveness and prevalence between molecular subtypes [49-51, 54, 101, 105, 116-120]. The classification of molecular subtypes used in these studies was not exactly the same, mainly due to the lack of an international standard definition. In addition, the percentages of unclassified breast tumours and biomarkers used also differ among studies. Some use ER, PR and HER2, and others add CK5/6 and/or HER1 to the IHC panel for defining molecular subtypes (see Section 2 of the Discussion). Despite methodological differences, all studies agree that luminal A-like is the most frequent subtype, representing about 50-70% of the total number of breast cancers [54, 116-119]. Generally, the second most common subtype is luminal B-like, followed closely by TNBC and finally the HER2 positive subtype. However, the prevalence of breast cancer subtypes seems to vary among different races or ethnicities. For example, prevalence of the TNBC subtype was significantly higher in African American and black women, particularly in premenopausal women, than in non-African American and white patients [54, 120]. Also, the HER2 positive subtype is more frequent among Asian and Pacific Islander women than either European or African American populations [119, 120].

Differences are found when histopathological characteristics such as age at diagnosis, menopausal status, axillary lymph node involvement, tumour size and histological grade are compared according to molecular subtypes [54, 116-119]. Considering menopausal status, postmenopausal patients are more frequently diagnosed with HER2 positive and luminal tumours and premenopausal women with TNBC. In fact, women diagnosed with luminal A-like subtype are generally slightly older than women diagnosed with other subtypes, whereas an association has consistently been described between young age at diagnosis and TNBC [114]. Luminal A-like tumours tend to be smaller, with less lymph node involvement and lower histological grade and stage than the other molecular subtypes. Contrarily, HER2 positive, luminal B-like and triple-negative tumours are associated with an advanced stage (II, III or IV), lymph node involvement, large tumour size and high histological grade tumours. The elevated proportion of high histological grade tumours found in TNBC and HER2 positive subtypes is in concordance with the high expression of the proliferation gene cluster in microarray analyses detected in these subtypes [54]. The presence of *TP53* and/or *BRCA1* mutations also differs according to molecular subtypes. TNBC and HER2 positive subtypes contain a higher proportion of *TP53* mutated tumours than hormonal positive receptors [54]. Importantly, around 80% to 90% of *BRCA1*-associated tumours are classified as TNBC [114].

Use of adjuvant treatment also varies across breast cancer molecular subtypes. Hormonal positive receptors are predictive for response to endocrine therapy whereas positive HER2 are sensitive to target therapy with the specific monoclonal antibody trastuzumab (see Section 7 of the Introduction).

In terms of prognosis, results are also similar to those reported in earlier studies using gene expression profiling, reflecting the prognostic value of molecular subtypes. Overall and disease-specific survival is lower among TNBC and HER2 overexpressed subtypes than in hormonal receptor positive tumours [54, 116-119]. Luminal A-like is always associated with the best prognosis within all the molecular subtypes. Prognosis of HER2 positive subtype has notably improved since the introduction of trastuzumab, which was approved by the US Food and Drug Administration (FDA) for adjuvant treatment in 2005 [116, 121]. Currently, TNBC is associated with poor clinical outcomes, reflecting the high proliferative capacity and lack of specific target therapy for this subtype.

7. Therapeutic approaches to breast cancer

Prognostic and predictive factors including stage at diagnosis and status of hormonal receptors and HER2, are important in determining optimal treatment for breast cancer. Additionally, other factors such as age, family history of breast cancer, general condition of the patient, tumour focality, histology and tumour proliferation rate must also be considered. Recent advances in molecular biology relating to breast cancer have provided the basis for new targeted drug development that improves current therapeutic strategies. Some of the most recent drugs approved by the FDA and European Medicines Agency (EMA) target specific signalling pathways for breast cancer. Therapeutic approaches to breast cancer are briefly discussed in the following paragraphs.

Depending on when the treatment is administered it can be classified as either adjuvant (post-surgery) or neoadjuvant (pre-surgery). Palliative treatment is administered to improve the quality of life of patients who have a life-threatening disease, usually stage IV [122]. Patients most likely to benefit from neoadjuvant therapy are those with large tumours or with inflammatory tumours. The goal of adjuvant systemic therapy is to prevent the recurrence of breast cancer by eradicating micrometastatic deposits of tumour that are present at diagnosis. Chemotherapy, endocrine and biological therapies are usually administered as adjuvant treatment. Chemotherapy can also be administered as neoadjuvant therapy, as can endocrine and biological therapies on rare occasions.

Surgery is the most common treatment used in breast cancer and often the first to be applied if no distant metastases are detected [4]. During the second half of the last century, surgical treatment evolved notably towards less invasive surgery procedures, with first breast-conserving surgery and then local radiotherapy replacing radical mastectomy for some patients diagnosed with early-staged breast cancer. With regard to localized tumours, the risk of breast cancer recurrence is similar between mastectomy and conservative surgery followed by radiotherapy [123]. Similarly, sentinel lymph node dissection avoids many axillary lymph node dissections, highly benefitting women with no metastatic node involvement.

Many patients who undergo conservative surgery are also treated with **radiotherapy**, although women undergoing radical surgery or no surgery can also receive it. Radiotherapy consists in the use of controlled doses of high-energy radiation to damage the DNA of cancerous cells, thus leading to apoptosis [122].

Chemotherapy is the most common systemic treatment for cancer and involves the use of cytotoxic drugs to stop the proliferation and growth of cancerous cells. As with radiotherapy, chemotherapy has to be carefully planned because both treatments can also harm healthy cells, thus causing important side effects. Many chemotherapeutic agents have been developed and are administered in different combinations.

Hormonal receptors play important roles in the molecular pathways that lead to the survival and proliferation of breast tumours. **Endocrine therapy** targets these pathways in order to control cell cycle and tumour growth. This therapy is only effective if the tumour expresses ER and/or PR. Although endocrine treatment can be administered as neoadjuvant therapy, it is usually dosed after surgery. There are two main types of endocrine therapies: aromatase inhibitors and tamoxifen, which is a SERM (selective estrogen receptor modulator). Aromatase is an enzyme required to synthesize estrogen. By inhibiting aromatase the amount of estrogen in the body decreases, thus reducing tumour size. Tamoxifen was initially approved by the FDA in 1977 for treatment of metastatic breast cancer and later for prevention of breast cancer development in women at high risk [124-126]. It has been shown that this drug can notably reduce the risk of ER-positive breast cancer development [127]. Tamoxifen blocks the binding of estrogen to its receptor in cancerous cells, also leading to a reduction of tumour growth. Generally, tamoxifen is administered over 5 years as an effective prevention of breast cancer recurrence and death.

Finally, **biological therapy** is the most recently developed treatment strategy, subsequent to new knowledge regarding signalling transduction pathways in breast cancer. This therapy acts together with the immune system to reduce the size of the tumour. Importantly, side effects are minimized through this therapy due to it being a targeted approach [122]. Some of the most important specific-targeted drugs for treating breast cancer are monoclonal antibodies or tyrosine kinase inhibitors. Trastuzumab is a monoclonal antibody targeting the extracellular domain of the HER2 protein (Figure 10). Although some HER2 overexpressing tumours present resistance, the binding of trastuzumab to HER2 blocks HER2 dimerization and with it the downstream signalling pathways that lead to cell growth, survival and cell differentiation [100, 101]. Trastuzumab was approved by the FDA in 1998 as a first-line treatment in combination with paclitaxel for HER2 positive metastatic breast cancer. Many studies have reported that trastuzumab has contributed to reduced rates of breast cancer mortality and recurrence [101, 116, 121].

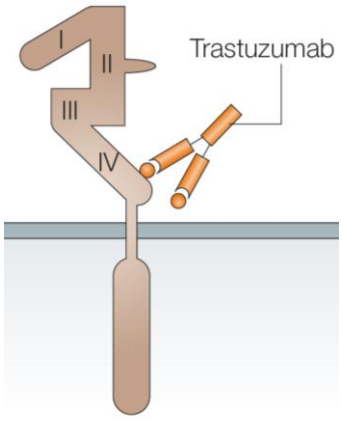

Figure 10. Trastuzumab binds domain IV of HER2. HER2: Human epidermal growth factor receptor 2. Taken from Hynes *et al.*, 2005 [100].

The main goal of approaches to breast cancer treatment is to identify effective targets to tailor treatment in breast cancer patients. Classifying patients according to molecular subtypes guides the selection of systemic adjuvant therapies. An important challenge is to develop specific drugs for treating TNBC. These cancers are associated with a high response to chemotherapy, but they lack a specific biological therapy and do not present sensitivity to endocrine therapy. Currently, many molecular targets are being evaluated as the standard treatment approach to TNBC (see Section 2 of the Discussion) [128-130].

8. Population-based cancer registries

A population-based cancer registry is an information system which attempts to collect, store, analyse and interpret data for cancer cases diagnosed in a well-defined population, usually residing in a particular geographical area [56, 123, 131]. The main objective is to obtain the epidemiological indicators of a cancer diagnosed in a particular population, allowing the impact of cancer in the population to be evaluated and controlled. Population-based cancer registries are highly necessary in the setting of epidemiology and public health. They are used to obtain incidence, mortality, prevalence and survival rates, as well as their trends over time. Data from a population-based cancer registry are also useful in epidemiological research. They may be a source of information for case-control or cohort studies. Registries are also necessary for evaluating cancer screening programmes and care services, and they can also contribute to promoting preventive initiatives [123].

The first population-based cancer registry was set up in Hamburg in 1926. Currently, more than 200 population-based cancer registries exist worldwide, covering about 5% of the world's population [56]. Some of them cover a specific region or county and others are nationwide, such as those in Denmark, Ireland, Norway or Slovenia. The Cancer Registry of Norway was one of the first European nationwide cancer registries to be implemented, in 1953 [75]. Cancer reporting has been mandatory by law in Norway since 1952 [132]. The unique 11-digit personal identification number assigned to all of the country's inhabitants facilitates links with different sources of information and data collection.

The first Spanish population-based cancer registries were established in 1960 and 1970 in Zaragoza and Navarra, respectively [131]. During the following decades, many other cancer registries were set up and 11 Spanish registries contributed to the last edition of Cancer Incidence in Five Continents (Albacete, Asturias, Basque Country, Canary Islands, Cuenca, Girona, Granada, Murcia, Navarra, Tarragona and Zaragoza) [133]. Spanish cancer registries cover about 25% of the Spanish population but are not random in geographical terms, with information lacking for the two largest cities (Madrid and Barcelona) and the western Autonomous Regions. However, they provide the best possible approximation of Spanish estimates on cancer. This situation is similar to other Mediterranean countries such as France or Italy.

The only two existing Catalan population-based cancer registries were established in 1980, covering Tarragona and Girona provinces. The Girona Cancer Registry was initially created as a monographic breast and gynaecological cancer registry, expanding to cover all cancers in 1994 [134, 135]. This registry is located in the north-east of Catalonia and covers a population of 761,632 inhabitants according to the 2013 census, which represents about 10% of the population of Catalonia [136]. Currently, incident cases are available up to 2011 and data for patients diagnosed in 2012 are being registered.

Cases diagnosed with a malignant tumour and some *in situ* carcinomas are included in the Girona Cancer Registry. In case of bladder and brain tumours, benign and unknown behaviour tumours are also collected. Information sources for the Girona Cancer Registry are public and private hospitals, pathology and haematology departments, medical centres and death certificates. To ensure complete coverage, information regarding cancer cases resident in Girona province are also searched for in centres and hospitals located outside the province, mainly in Barcelona.

Case registration is performed according to the European Network of Cancer Registries (ENCR) and International Association of Cancer Registries (IACR) recommendations [137, 138]. Tumours are encoded following the third edition of the International Classification of Diseases for Oncology (ICD-O-3) for cases diagnosed since 1998 [139]. The second edition of the ICD-O (ICD-O-2) was used previously [140]. To present epidemiological results, the tenth International Statistical Classification of Diseases (ICD-10) is widely used [141]. Currently, the fourth edition of the WHO Classification of Tumours of the Breast is also used to encode breast tumours, as it extensively describes all the histological entities of breast cancer [8]. Prior to 2014, the third edition of the WHO classification was also used [142]. Malignant tumours diagnosed based on exploratory methods and those lacking an anatomopathological report are also included in the database, as well as cases informed by death certificate only (DCO). Percentage of DCOs is a measure of quality in a population-based cancer registry and is recommended to remain low. The percentage varies notably according to tumour site; for example, the percentage of DCOs at the Girona Cancer Registry for 2007 to 2009 was 2.4% for breast cancer but 13.4% for pancreatic cancer [143]. Many other methods of assessing completeness and validity are described by the IARC, including percentage of microscopic verification (MV) and ratio of mortality and incidence (M/I) [133]. MV was 95.9% and M/I corresponded to 22.7% for breast cancer in Girona [143]. Finally, completeness of the Girona Cancer Registry is currently 96.3%.

Hypotheses

1. DCIS incidence and mammographic screening

1.1. The incidence of DCIS in women resident in Girona province has increased in recent decades and stabilized after the implementation of mammographic screening.

1.2. Interval cancers represented a low percentage of all breast cancers diagnosed in Girona province during the years after mammographic screening first started in women aged 50-69.

1.3. During the start-up phase of the mammographic screening programme, more than half of the cancers diagnosed in Girona in women aged 50-69 were screen-detected.

1.4. Screen-detected breast cancers showed a higher proportion of early stage tumours than non-screen-detected and interval breast cancers in women aged 50-69 during the years after mammographic screening first started.

2. Prognostic value of breast cancer molecular subtypes defined by IHC biomarkers

2.1. The distribution of breast cancer molecular subtypes defined by IHC biomarkers diagnosed in recent years in Spain and Norway was similar to that of other European countries.

2.2. Luminal A-like was the most frequent subtype, associated with the most favourable histopathological characteristics and the best survival rate. Contrarily, women diagnosed with TNBC had the lowest survival rate.

2.3. Breast cancer molecular subtype defined by IHC biomarkers provides prognostic value, regardless of histopathological characteristics.

3. Breast cancer survival according to method of detection

3.1. Screen-detected cancers had more favourable histopathological characteristics and a higher survival rate than non-screen-detected cancers recently diagnosed in women aged 50-69 in Norway.

3.2. Screen-detected cancers had a higher proportion of luminal A-like tumours than cancers detected outside screening. Conversely, TNBC were more representative in the group of cancers detected outside screening.

3.3. Method of detection provides prognostic value, regardless of histopathological characteristics, including molecular subtypes defined by IHC biomarkers.

Objectives

In general, this thesis aims to examine various aspects of breast cancer epidemiology related to mammographic screening and molecular subtypes. In order to prove whether the above hypotheses can be confirmed, the following aims were planned:

1. DCIS incidence and mammographic screening

1.1. To analyse incidence trends of DCIS in women resident in Girona province from 1983 to 2007, considering age at diagnosis and use of mammography.

1.2. To identify interval cancers and determine what percentage they represented of all breast cancers diagnosed in 50-69 year-old women in Girona from 2002 to 2006.

1.3. To measure the distribution of breast cancers diagnosed among screened-detected and non-screened detected cancers in women aged 50-69 in Girona from 2002 to 2006.

1.4. To examine tumour stage at diagnosis by method of detection (screen-detected, non-screen-detected and interval cancers) in women aged 50-69 diagnosed from 2002 to 2006.

2. Prognostic value of breast cancer molecular subtypes defined by IHC biomarkers

2.1. To describe the distribution of breast cancer molecular subtypes defined by IHC biomarkers diagnosed in recent years in Spain and Norway.

2.2. To depict histopathological characteristics and estimate breast cancer survival rate according to breast cancer molecular subtypes defined by IHC biomarkers diagnosed in recent years.

2.3. To determine whether the breast cancer molecular subtype defined by IHC biomarkers provides prognostic value, regardless of histopathological characteristics.

3. Breast cancer survival according to method of detection

3.1. To determine histopathological characteristics and estimate breast cancer survival rate according to method of detection in women aged 50-69 diagnosed in Norway from 2005 to 2011.

3.2. To describe proportions of breast cancer molecular subtypes defined by IHC biomarkers according to method of detection.

3.3. To evaluate the prognostic value of method of detection.

Data and methods

Data

The present thesis is composed of four population-based databases containing information about women diagnosed with primary breast cancer. Two of these use data extracted from the Girona Cancer Registry, meaning they include incidence cases for the whole of Girona province. In the first, case selection was restricted to women diagnosed with primary DCIS (ICD-10 classification codes: D050-D059 [141]) during the period 1983 to 2007 (n=416). Cases diagnosed with LCIS, or DCIS and LCIS or intracystic carcinomas were excluded. In the second, women aged 50-69 and diagnosed with an invasive or *in situ* breast cancer (ICD-10 classification codes: C500 – C509 and D050 – D059 [141]) in 1999-2006 were included (n=1254) and classified by detection method: screen-detected cancers, non-screen-detected cancers and interval cancers. Screen-detected cancer was defined as a cancer diagnosed in the PDPCM, non-screen-detected cancers were diagnosed outside the PDPCM either by symptoms or opportunistic screening, and interval cancers were defined as a breast cancer arising after a negative screening episode and before the next scheduled screen round or within 24 months for women who have reached the upper age limit [61]. A link between women participating in the PDPCM and women diagnosed with breast cancer registered in the Girona Cancer Registry was necessary to identify interval cancers. Dalink software was used to this end [144].

Data from the Spanish study were extracted from the ten Spanish Cancer Registries participating in the "Spanish High Resolution Breast Cancer Study" (Albacete, Castellón, Cuenca, Gipuzkoa, Girona, Granada, La Rioja, Murcia, Navarra and Zaragoza). These registries cover about 20% of the Spanish female population and are mainly situated in eastern Spain, as shown in Figure 11. All registries provided data of incident primary invasive breast cancer (ICD-10 classification codes: C500 – C509 [141]) from 2005 and the four registries covering the smallest population (Albacete, Castellón, Cuenca and La Rioja) also included cases diagnosed in 2004. In total, 3480 women were identified. Pathological and clinical records were reviewed to obtain information regarding the characteristics of the women and the tumours involved: ER, PR and HER2 status, age at time of diagnosis, tumour size, stage of tumour at diagnosis, histological grade, multifocality and/or multicentricity of tumour, neoadjuvant treatment (yes/no) and surgery (conservative/mastectomy). The SBR classification modified by Elston and Ellis was used by most hospitals to define histological grade. Five molecular subtypes were defined using information on positivity of ER, PR and HER2, as shown in Table 12. Only 20.4% of the cases corresponded to the unclassified group.

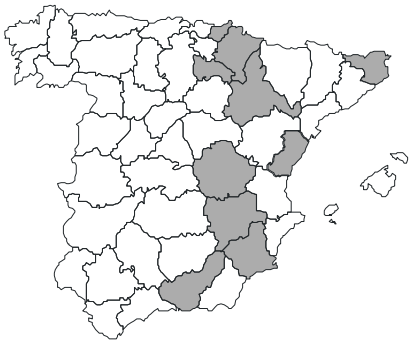

Figure 11. The ten Spanish Cancer Registries participating in the "Spanish High Resolution Breast Cancer Study".

To analyse survival according to detection method data were extracted from the Norwegian Cancer Registry. It contained information on women aged 50-69 diagnosed with a primary invasive breast cancer (ICD-10 classification codes: C500 – C509 [141]) in Norway from 2005 to 2011. Around 2500 cases were excluded, most of them because they were interval cancers and informed consents were not available for all of them. The following information regarding the cases was obtained from the Norwegian Cancer Registry: ER, PR and HER2 status, age at time of diagnosis, tumour size, histological grade, lymph node status and detection method (screen-detected and non-screen-detected cases). Screen-detected cancer was defined as a cancer diagnosed as a result of a positive screening test within three months of screening on the NBCSP. Information on ER, PR and HER2 was used to classify breast cancer into five molecular subtypes, as shown in Table 12.

Study	**Molecular subtypes**	**ER**		**PR**		**HER2**
Spanish	ER+ and/or PR+ and HER2-	+	and/or	+	and	-
	ER+ and/or PR+ and HER2+	+	and/or	+	and	+
	HER2 overexpressed	-	and	-	and	+
	Triple-negative	-	and	-	and	-
	Unclassified	Hormonal receptor or/and HER2 status missing				
Norwegian	Luminal A-like	+	and	+	and	-
	Luminal B-like HER2-	+	and	-	and	-
	Luminal B-like HER2+	+	and	+/-	and	-
	HER2 positive	-	and	-	and	+
	Triple-negative	-	and	-	and	-

Table 5. Classification of molecular subtypes defined by ER, PR and HER2 status in the Spanish and Norwegian study. ER: Estrogen receptor; HER2: Human Epidermal Growth Factor Receptor 2; PR: Progesterone receptor.

ER, PR and HER2 status were assessed by means of IHC in both studies. The cut-off for ER and PR positivity was >10%. IHC for HER2 was considered negative when the score was 0 or 1+ and overexpressed when the score was 3+. FISH was used to evaluate gene amplification in case of a 2+ score.

Statistical analysis

Descriptive analyses were performed and incidence rates, trends in incidence rates and survival rates have been estimated in the present thesis. The population of Girona province was provided by the *Institut d'Estadística de Catalunya* (IDESCAT) to calculate incidence rates [136]. Incidence was estimated as crude rate (CR), age-standardized to the European standard population rate (ASR_E) and age-specific rate for 5-year age per 100,000 women/year. Epidat software was used to compute CR and ASR_E with the 95% CI using the direct method [145].

Joinpoint statistical software was used to quantify the evolution of incidence, as the estimated annual percentage change (EAPC) and to evaluate changes in trends over time [146]. The EAPC was estimated for each period of time between two joinpoints.

Mean age and standard deviation (SD) were calculated for DCIS and invasive breast cancer patients, and the *t* test was used for comparisons. In the Norwegian study, the same test was used to examine mean age (SD) differences between screen-detected and non-screen-detected within molecular subtypes. In the Spanish study, differences in mean age (SD) between molecular subtypes were assessed using one-way analysis of variance (ANOVA) and the Bonferroni method. Differences in histopathological characteristics between molecular subtypes were examined using X^2 for qualitative variables such age groups, menopausal status, tumour size, stage at time of diagnosis, histological grade, multifocality/multicentricity, neoadjuvant treatment, surgery and lymph node status.

Considering survival analyses, DCO and cases diagnosed by autopsy were excluded from the study population. Breast cancer-specific survival was estimated in the Norwegian study. Women were followed from date of breast cancer diagnosis to date of breast cancer death, death due to other reasons, date of emigration, or end of follow-up (31st December 2011), whichever came first. Six-year crude breast cancer survival was estimated using the Kaplan-Meier method. Information on cause of death was not available for the Spanish population. Thus, relative survival rates after 5 years of follow-up were estimated using the Pohar-Perme method as the ratio of observed survival in the study population to expected survival in the general population of the same age, sex, year and province. The end of followed-up was set at 31st December 2010. Survival was estimated according to molecular subtypes and statistical differences between curves were assessed using the log-rank test.

Finally, relative excess risks of death (RER) and Cox proportional hazard models were used to estimate multivariate survival analyses in the Spanish and Norwegian studies, respectively. The

covariables used in the Spanish study were molecular subtype, age at diagnosis, stage at diagnosis and histological grade. In the Norwegian study, the covariables used were detection method, molecular subtype, tumour size, histological grade and lymph node involvement.

Statistical significance was determined at $p < 0.05$ for all analyses. Analyses were performed with SPSS version 18 and 19, and R version 2.14.0 and 3.0.2

Results

The present thesis is composed of four population-based studies. Three of them are published as original research in different peer-reviewed journals. The last article has been recently submitted for publication. Table 5 shows the study periods and populations for each of these four articles. Article 1 focuses on DCIS incidence trends in Girona province from 1983-2007; in article 2, we aimed to study interval cancers, also in Girona province, from 1999 to 2006; in articles 3 and 4 we analysed whether the molecular subtype defined by a panel of IHC biomarkers provides prognostic value regardless of histopathological factors; and in article 4 we also investigated survival by method of cancer detection.

Articles	**Study period**	**Study population**
1. Puig-Vives M *et al*. Breast 2012 Oct;21(5):646-51	1983-2007	416 women diagnosed with DCIS in Girona province
2. Puig-Vives M *et al*. Med Clin (Barc). 2013 Dec 27. In press, corrected proof.	1999-2006	1254 women aged 50-69 diagnosed with invasive or *in situ* breast cancer in Girona province
3. Puig-Vives M *et al*. Gynecol Oncol. 2013 Sep;130(3):609-14.	2004-2005	3480 women diagnosed with invasive breast cancer in ten Spanish Cancer Registries
4. Puig-Vives M *et al*. Submitted in The Breast	2005-2011	7851 women aged 50-69 diagnosed with invasive breast cancer in Norway

Table 6. Study periods and populations of each of the four articles included in the present doctoral thesis.

Article 1:

"Rapid increase in incidence of breast ductal carcinoma *in situ* in Girona, Spain 1983-2007"

M. Puig-Vives,M. Pollan,M. Rue,G. Osca-Gelis,M. Saez,A. Izquierdo,R. Marcos-Gragera. "Rapid increase in incidence of breast ductal carcinoma *in situ* in Girona, Spain 1983–2007". *The breast, vol.*21(5) (2012) : 646-651

DOI: http://dx.doi.org/10.1016/j.breast.2012.01.014

http://www.thebreastonline.com/article/S0960-9776(12)00020-3/abstract

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Abstract

Introduction

The aim of this study was to describe breast ductal carcinoma *in situ* (DCIS) incidence trends in women in the Girona province during a period of 25 years. The influence of age, use of mammography and implementation of the breast cancer screening programs was explored. Incidence of subsequent invasive breast cancers (IBC) and DCIS treatment was also considered.

Materials and methods

Cases diagnosed with primary pure DCIS (n = 416) during 1983–2007 were extracted from the population-based Girona Cancer Registry. The estimated annual percent change was estimated using joinpoint analysis.

Results

DCIS incidence showed a sharp rise until 1997, followed by a less marked upward trend. Among women aged 50–69 the increase was particularly important between 1992 and in 1996, reflecting the spread in mammography use.

Conclusion

The upward trend of DCIS was mainly related to an increase in mammography use either opportunistic or as a result of screening implementation.

Keywords

Ductal carcinoma in situ of the breast (DCIS), Incidence, Screening

Article 2:

“Proportion of breast cancer in women aged 50 to 69 years from Girona, Spain, according to detection method”

Montse Puig-Vives, Gemma Osca-Gelis , Carla Camprubí-Font, M. Loreto Vilardell, Angel Izquierdo , Rafael Marcos-Gragera. **"**Proporción de cáncer de mama en mujeres de 50 a 69 años de Girona según el método de detección. Proportion of breast cancer in women aged 50 to 69 years from Girona, Spain, according to detection method". *Medicina Clínica*, vol. *143 (7) (2014) : 300-302*

DOI: http://dx.doi.org/10.1016/j.medcli.2013.09.042

http://www.elsevier.es/es-revista-medicina-clinica-2-articulo-proporcion-cancer-mama-mujeres-50-90349846

Resumen
Fundamento y objetivo

El objetivo de este estudio fue determinar el estadio tumoral, la proporción de casos y la tasa específica por edad de las pacientes con cáncer de mama (CM) según el método de detección.

Material y método

Los datos se obtuvieron del Registro de Cáncer de Girona de base poblacional. Se incluyeron las mujeres de 50 a 69 años diagnosticadas de CM en la provincia de Girona durante el período 1999-2006 (n = 1.254). Se clasificaron los CM según el método de detección: cáncer de cribado, cáncer de intervalo y otros. Se calculó la proporción de casos y la tasa específica por edad según el método de detección.

Resultados

Durante los años 2002-2006, un 42,2% de los CM diagnosticados en Girona fueron cánceres de cribado, el 52,0% se detectaron fuera del Programa de Detección Precoz del Cáncer de Mama (PDPCM), y el 5,8% fueron cánceres de intervalo. Con la implementación del PDPCM disminuyó la incidencia del CM diagnosticado fuera del programa, aumentó la de los cánceres de cribado y poco después incrementó la de los cánceres de intervalo.

Conclusiones

Durante los primeros años del funcionamiento del PDPCM (2002-2006) los casos de cáncer de intervalo representaron un porcentaje bajo (5,8%) respecto el total de CM diagnosticados en mujeres de 50 a 69 años en la provincia de Girona.

Abstract
Background and objective

The aim of this study was to determine the tumor stage, the proportion of cases and the age specific rate of breast cancer (BC) cases according to detection method.

Material and method

Cases of women aged 50 to 69 years diagnosed with BC in the Girona province during 1999-2006 were extracted from the population-based Girona Cancer Registry (n = 1,254). BC was classified by detection method: screen-detected cancer, interval cancer and others. Proportion of cases and age-specific incidence were calculated according to detection method.

Results

During the period 2002-2006, the proportion of screen-detected cancers, interval cancers and other cancers were 42.2%, 5.8% and 52.2%, respectively. After implementation of the early detection of breast cancer program (PDPCM), the incidence of screen-detected cases raised; thereafter, interval cancers also increased and the rate of other cancers decreased.

Conclusions

In the Girona province during the fully implemented PDPCM period (2002-2006), interval cancers represented a low proportion (5.8%) of women diagnosed with BC at 50 to 69 years old.

Palabras Clave
Cáncer de mama. Programa de cribado. Cáncer de intervalo.

Keywords

Breast cancer. Screening program. Interval cancer.

Article 3:

"Distribution and prognosis of molecular breast cancer subtypes defined by immunohistochemical biomarkers in a Spanish population-based study"

M. Puig-Vives, , M.J. Sánchez, J. Sánchez-Cantalejo, A. Torrella-Ramos, C. Martos, E. Ardanaz, M.D. Chirlaque, J. Perucha, J.M. Díaz, A. Mateos, M. Machón, R. Marcos-Gragera. "Distribution and prognosis of molecular breast cancer subtypes defined by immunohistochemical biomarkers in a Spanish population-based study". *Gynecologic Oncology, vol 130*(3) (Sept. 2013) : 609-614

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DOI: http://dx.doi.org/10.1016/j.ygyno.2013.05.039

http://www.sciencedirect.com/science/article/pii/S0090825813008287

Highlights

This study showed differences in clinicopathological features and survival rates among breast cancer molecular subtypes classified by immunohistochemical biomarkers.

We confirm that molecular subtypes defined by immunohistochemical biomarkers provide useful prognosis information for guiding and evaluating clinical treatment.

The prognosis value of molecular subtype persists when adjusting by age, stage and histological grade.

Abstract

Background

The objective of this study is to analyze the distribution, clinicopathological features, relative survival rate and excess risk of death among females diagnosed with invasive breast cancer and classified by molecular subtype from ten Spanish cancer registries.

Method

Three thousand four hundred and eighty incident cases of women – mostly diagnosed in 2005 – were classified into five molecular subtypes according to immunohistochemical status of hormonal receptors and HER2 (human epidermal growth factor receptor 2): estrogen receptor (ER) and/or progesterone receptor (PR)+ and HER2 –, ER + and/or PR + and HER2 +, HER2-overexpressed (ER –, PR – and HER2 +), triple negative (ER, PR and HER2 –) and unclassified (hormonal receptor or/and HER2 unknown). Relative survival rates at 1, 3 and 5 years and relative excess risks (RER) of death adjusting for molecular subtype, age, stage and histological grade were estimated.

Results

Marked differences in clinicopathological characteristics and relative survival rate were observed between molecular subtypes. Compared with women with ER + and/or PR + and HER2 –, ER + and/or PR + and HER2 + cases had an RER of 1.00 (95% CI: 0.66 to 1.52) after adjusting for age, stage and histological grade, whereas HER2-overexpressed, triple negative and women with unclassified subtypes presented an RER of 1.72 (95% CI: 1.15 to 2.57), 3.16 (95% CI: 2.26 to 4.41) and 2.55 (95% CI: 1.96 to 3.32), respectively.

Conclusion

The prognostic value of molecular subtype persists when adjusting for age, stage and histological grade. Hormone receptor-positive tumors were associated with a better prognosis when compared with HER2-overexpressed and triple negative subtypes. Further research is required to improve triple negative prognosis.

Keywords

Breast cancer; Molecular subtype; Relative survival; Hormonal receptor; HER2

Article 4:

"Molecular subtypes and survival of breast cancer diagnosed within and outside a national mammographic screening program"

Molecular subtypes and survival of breast cancer diagnosed within and outside a national mammographic screening program

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Abstract

Screen-detected breast cancer has favourable prognostic and predictive characteristics compared with symptomatic cancer. We aimed to investigate whether molecular subtype-classification based on registry data could explain the difference in disease specific survival between women with screen-detected and not screen-detected breast cancer. We analysed breast cancer death among 4848 screen-detected and 1914 not screen-detected breast cancer cases diagnosed in Norwegian women aged 50-69 years during 2005-2011. Immunohistochemical markers of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2) were used to classify the tumours into five subtypes: Luminal A-like, Luminal B-like HER2-, Luminal B-like HER2+, HER2 positive, and triple negative breast cancer (TNBC). Kaplan-Meier was used to estimate breast cancer survival, while Cox proportional hazard models were used to estimate the hazard ratio (HR) of breast cancer death associated with detection mode, adjusted for subtype, age at diagnosis, tumour size , histologic grade and lymph node involvement. The risk of dying from breast cancer was higher (HR: 2.7, 95% CI: 1.9-3.7) for women diagnosed with breast cancer outside compared to in the screening program in adjusted analyses. Women diagnosed with Luminal B-like HER2-, Luminal B-like HER2+, HER2 positive and TNBC subtypes of breast cancer had statistically significant higher risk of dying from breast cancer compared to women with Luminal A-like cancer, regardless of detection mode. In conclusion, women diagnosed with screen-detected cancer have better outcome compared with women diagnosed with breast cancer outside the screening program, independent of subtype, tumour size, histologic grade, and lymph node status.

Keywords

Molecular subtypes

Detection mode

Screening program

Breast cancer-specific survival

Discussion

This section is intended as a global discussion of the articles referred to in the present doctoral thesis, while avoiding repetition of what has already been discussed therein. It will therefore build and expand upon previous discussions regarding: 1) DCIS incidence and mammographic screening; 2) the prognostic value of breast cancer molecular breast cancer subtypes defined by IHC biomarkers; and 3) breast cancer survival according to method of detection. In order to analyse these aspects of breast cancer epidemiology, this thesis took advantage of the information collected in ten Spanish Cancer Registries (Albacete, Castelló, Cuenca, Girona, Granada, Guipuzkoa, La Rioja, Navarra, Murcia and Zaragoza) and the Norwegian Cancer Registry, as well as the mammographic screening programmes from Girona (PDPCM) and Norway (NBCSP). Cancer registries provide high-quality population-based data, which are of great necessity in building knowledge on epidemiological indicators of cancer such as incidence and survival rates. In total, four different databases were used to achieve the proposed objectives. DCIS incidence and the impact of mammographic screening programmes were examined using databases covering Girona province (Article 1 and 2). The prognostic value of breast cancer molecular subtypes defined by IHC biomarkers was analysed in two studies (Article 3 and Article 4). Information from ten Spanish Cancer Registries was included in Article 3 and nationwide data from Norway were collected in Article 4, which was also used to investigate method of detection as a prognostic factor.

1. DCIS incidence and mammographic screening

Over recent decades, DCIS has accounted for around 7% of breast carcinoma diseases in Girona province, though this percentage has not remained constant over time. The observed proportion of DCIS among all breast malignancies has risen from <3% throughout the 1980s to >10% in recent years (Figure 11). Our results showed that age-standardized incidence rates of DCIS per 100,000 women/year increased from 1.3 in 1983-1987 to 10.8 in 2003-2007. Incidence markedly increased from 1983 to the end of the 1990s ($EAPC_{1983-1997}$ = 20.1%; 95% CI: 12.7% to 27.9%), remaining stable thereafter ($EAPC_{1998-2007}$ = 3.6%; 95% CI: -1.3% to 8.7%). Following the implementation of PDPCM, trends in DCIS incidence continued to increase, but no longer with statistical significance, even among the target population of the PDPCM (women aged 50–69). In this study, however, the PDPCM had only been functioning for 6 years (2002 – 2007). When analyses were calculated, 2007 was the most recent year with available data in the Girona Cancer Registry, and therefore the last year to be included. Results of updated and expanded data (1983 – 2010) are shown in Figure 12. We observe that by adding three years to the analyses, the EAPC rises to 4.5% (95% CI: 1.3% to 7.9%) from 1997 to 2010, showing an increase in statistically significant incidence. This upward trend in DCIS incidence in recent decades has not been observed exclusively in Girona, but also in other developed countries [18, 36-38, 41, 42]. Sørum *et al.* found that DCIS incidence in Norway increased from 4 to 11 per 100,000 women/year from 1993 to 2007. In most studies, the detected increase was particularly marked in women aged 50-69, usually the target population for mammographic screening.

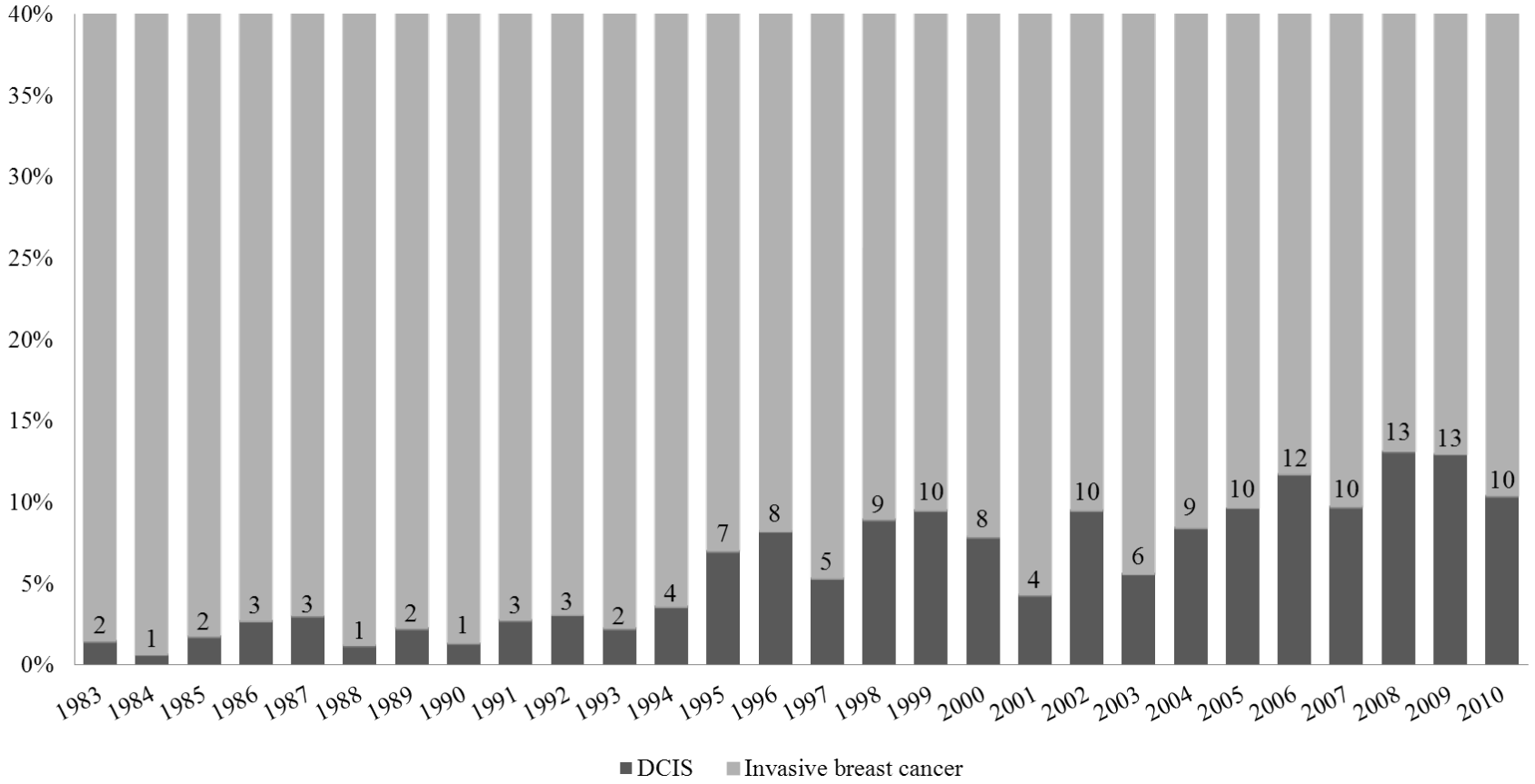

Figure 12. Proportion of invasive breast cancer and breast ductal carcinoma *in situ* (DCIS) in Girona province from 1983 to 2010. Annual percentages of DCIS are represented in numbers.

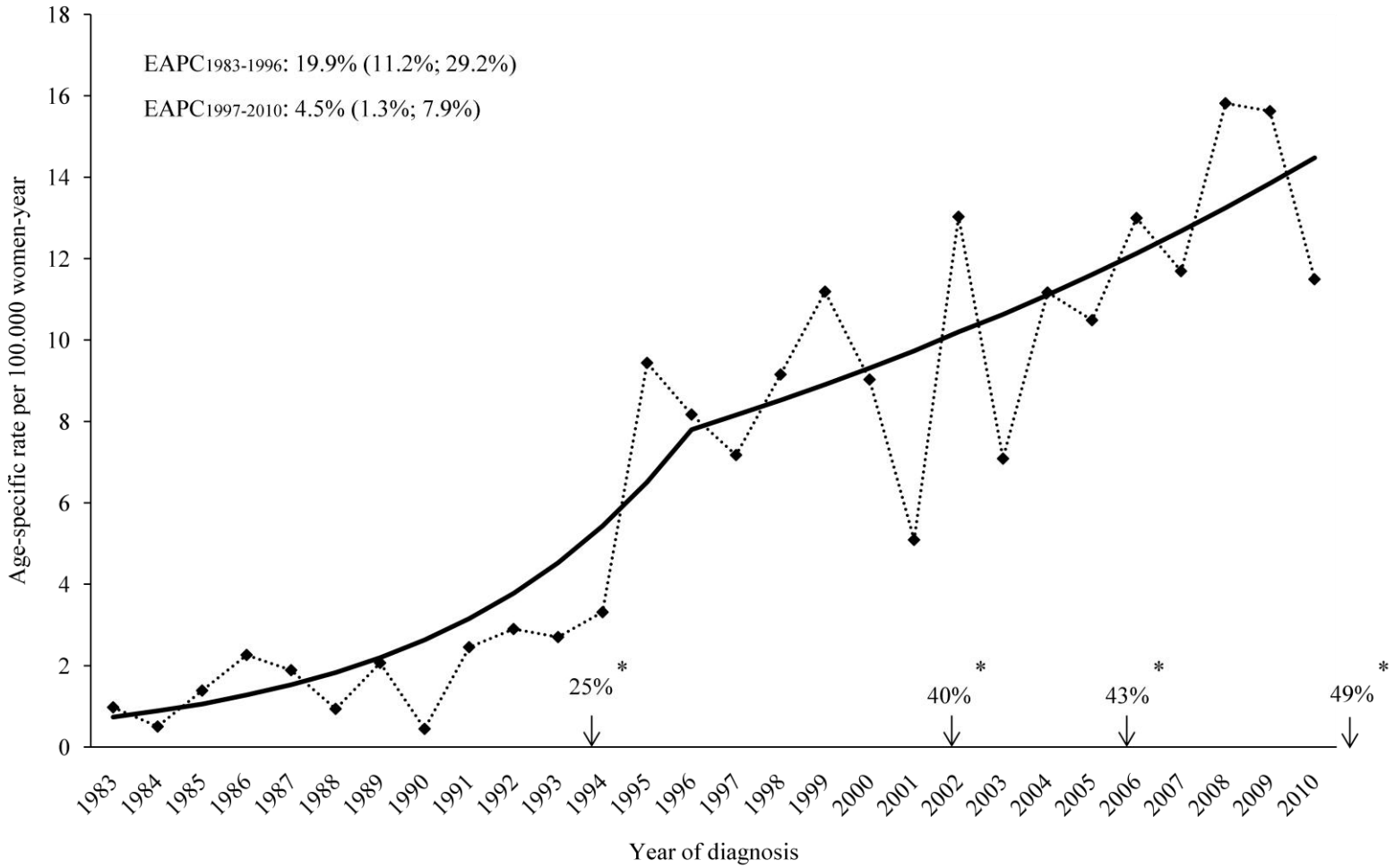

Figure 13. Trends in age-adjusted incidence of breast ductal carcinoma *in situ* in Girona, 1983 – 2010. Dotted lines represent observed rates and estimated rates are shown in solid lines. EAPC: Estimated annual percentage change. * Percentage of women older than 20 years undergoing mammography periodically in Catalonia in 1994, 2002, 2006 and 2012, data from "The Catalan Health Surveys" [86, 87, 89].

Incidence rose from 3 and 10 to 34 and 40 per100,000 women/year in Norway and the southern Netherlands, respectively [36, 38]. In Girona, there was an abrupt but non-statistically significant increase among women aged 50-69 in the mid-1990s. Incidence rose from 2 per 100,000 women/year in 1983 to 30 in 2007.

It is well-known that *in situ* breast cancers are rarely diagnosed clinically but often detectable by mammography. As specified in the Introduction, the number of available mammography devices in Catalonia increased from 10 in 1980 to 134 in 2000 [23]. According to Catalan Health Surveys, the percentage of women periodically undergoing mammographic screening has increased in recent decades, particularly among women aged 50-69 [86, 87, 89]. The widespread adoption of mammographic screening programmes over the past decade has influenced the increase in women undergoing mammography. Consequently, the introduction of mammographic screening has often been used to explain the increase in DCIS incidence. Nevertheless, this increase has also been identified in screen-detected cancers. A recent report showed that in a large cohort of Spanish women diagnosed by screening, rates of DCIS increased steadily from 1992 to 2006, with an average rise of 2.5% (95% CI: 1.3% to 3.8%) [147]. The fact that DCIS incidence also increases for screening participants suggests that changes in the technique used and/or interpretation of mammogrammes may also influence the upward trend described. In Girona, digital mammography was introduced in 2004 as part of the PDPCM to substitute screen-film mammography. This new technique has been evaluated for different population-based screening programmes. Results from a Spanish study show that false-positive rates were higher for screen-film than for digital mammography [148]. Interestingly, another study found that incidence rate of DCIS was higher for digital than screen-film mammography among screen-detected women [149].

As expected, mammographic screening leads to early detection and reduction of the breast cancer mortality rate [59, 64-66, 150]. However, it may also cause overdiagnosis and subsequent overtreatment, thus reducing the overall effectiveness of screening [151, 152]. To understand the possible extent of overdiagnosis in the population it is necessary to have information on DCIS incidence over time. The fact that trends in DCIS incidence continued to increase during the period after PDPCM was implemented may indicate the possible existence of overdiagnosis in Girona. However, there is no single and consistent method for measuring the scale of DCIS overdiagnosis in a population. Contrarily, many mathematical approximations are available for estimating this, resulting in large discrepancies between studies that are difficult to interpret [76, 78-80]. Interest in determining the potential of DCIS in causing overdiagnosis lies in the fact that it has very good survival. It can progress to invasive breast cancer, but does not always evolve. Since the natural history of breast cancer is not well-known, the most effective means of managing DCIS remains uncertain. Many studies support the fact that patients with DCIS are at increased risk of subsequent breast malignancy [6, 12, 153]. They also describe a lower rate of relapse among treated compared to non-treated patients (see

Section 1.4 of the Introduction). This provides further evidence that not all DCIS represent overdiagnosis.

The percentage of invasive and *in situ* screen-detected cancers is used to evaluate mammographic screening. According to the European Guidelines for Quality Assurance in Breast Cancer Screening and Diagnosis, the accepted and desirable levels of *in situ* screen-detected cancers are 10% and 10-20%, respectively [61]. Our results indicated that the PDPCM is within the limits of the desirable level, as 13.4% of screen-detected breast cancers were *in situ* breast carcinomas among women aged 50-69 diagnosed during the years after mammographic screening first started (2002-2006). This percentage was slightly lower than the percentage found in Catalonia for the period 2008-2009 (18.1%), but very similar to Spain (14.3%) [58].

An effective strategy to reduce breast cancer overdiagnosis and overtreatment would be to develop screening approaches based on known risks. Among these risks, breast density and family history of breast cancer have been proposed to tailor screening [85, 154]. Clearly, it is necessary to find a biomarker to definitively distinguish which DCIS would further progress to invasive breast cancer from those which would not progress if left untreated (see Section 1.4 of the Introduction) [6, 11, 13].

Apart from overdiagnosis, another possible adverse effect of mammographic screening is the emergence of interval cancers. Most of these cancers are unavoidable, but it is important to maintain their incidence as low as possible. Interval cancers represented a low percentage (5.8%) of all breast cancers diagnosed in Girona province in women aged 50-69 during the years after mammographic screening first started (2002-2006). Knowing this percentage is essential in the evaluation of screening programmes. If it is too high, screening programme procedures should be reviewed.

Although not included in the present doctoral thesis, in order to further evaluate the PDPCM those interval cancers were stratified into the four categories recommended by European guidelines [61, 155]. This was done by a panel of expert radiologists who regularly interpret mammogrammes for the PDPCM. They reviewed both screening and diagnostic mammogrammes through independent double reading with arbitration. 54.5% were found to be true interval tumours, 13.6% false-negative tumours, 18.2% occult tumours and the remaining 13.6% minimal signs tumours. Comparing these results to a larger Spanish study (n=948), we found a higher proportion of true interval (54.5% versus 48.0%) and occult tumours (18.2% versus 10.9%) and conversely, a lower percentage of false-negative (13.6% *vs*. 23.6%) and minimal signs tumours (13.6% versus 17.5%) [85]. It must be borne in mind that we were able to recover the two mammogrammes (screening and diagnosis) necessary for correct interval cancer classification in 50% of the total number of cases. Regarding the proportion of false-negatives, the PDPCM is within the values recommended by European guidelines, which suggest that false-negative cases should not exceed 20% of the total number of interval cancers [61]. Our result is consistent with previous studies from Navarra, East Anglia and Sabadell,

which reveal a percentage of false-negative breast cancers of 12%, 17% and 21%, respectively [156-158]. False-negative cancers are avoidable because they are visible on the mammography but not diagnosed by screening either due to misinterpretation or technical error. They should therefore be periodically identified in order to ensure the appropriate evaluation of a mammographic screening programme. Finally, the proportional incidence of interval cancer found in the first and the second year was lower than that stipulated by European guidelines (30% and 50%) [61].

Continuing with the evaluation of the PDPCM, 44.8% (n = 341) of all breast cancers diagnosed in Girona in 2002-2006 among women aged 50-69 were screen-detected and the remaining 55.2% (n = 421) were non-screen-detected, excluding interval cancers. Despite the intensive effort to run the programme, half of patients are diagnosed outside of it. As mentioned in Section 4.3 of the Introduction, participation rates in the PDPCM have always remained just below the acceptable level defined by European guidelines [62]. This may explain why screen-detected cancers represent a low percentage of all breast cancers. More data would be needed to determine the reasons why the participation rate is low and whether some initiatives might be needed to increase it.

Some possible reasons why women are non-screen-detected include the fact that women with a history of breast cancer are usually excluded from participating in the programme because they are monitored more exhaustively; however, they only account for a very low percentage. Also, an unknown but probably high proportion of women diagnosed outside the PDPCM will have participated in an opportunistic screening programme. As this information only sometimes appears in clinical reports, it is very difficult to determine this percentage. It would be very interesting to examine whether women who were diagnosed outside the programme belong to a different socioeconomic and/or educational group than those diagnosed within an organized screening programme. Women from higher socioeconomic classes might prefer to attend their private gynaecologist rather than the public health service. It was reported that in Catalonia the introduction of the organized screening programme was associated with a reduction in inequality of access to screening [63]. This is a strong argument to keep the PDPCM ongoing in spite of the associated adverse effects presented here.

A considerably higher proportion of breast cancer cases in women aged 50-69 were diagnosed by the NBCSP (71.7%) from 2005 to 2011 than by the PDPCM (44.8%) from 2002 to 2006. There may be many reasons for this important disparity between Girona and Norway. Most of these may be due to differences between the two countries' public health systems. The number of women attending opportunistic screening in Norway is probably much lower than in Girona. In fact, the percentage of women aged 50-69 regularly undergoing mammography prior to screening started was already higher in Norway (40%, 1996) than in Catalonia (27%, 1994) [86, 159]. Also, the participation rate in Norway is higher than in Girona, around 84% and <70%,

respectively (see Section 4.3 of the Introduction). However, determining the reasons for this difference was not the aim of our study, so it was not analysed further.

The possible adverse effects associated with mammographic screening (mostly overdiagnosis and interval cancers, mostly) in Girona province have been discussed here. To further and definitively evaluate the impact of PDPCM on the Girona population, the reduction of breast cancer mortality due to screening should be analysed in future studies so as to definitively quantify the benefit of screening.

2. The prognostic value of breast cancer molecular subtypes defined by IHC biomarkers

Our studies based on Spanish and Norwegian women have shown that patients with a hormonal receptor positive tumour presented more favourable prognostic tumour characteristics and higher survival rates than HER2 positive and TNBC subtypes. Molecular subtype defined by IHC biomarkers was an independent risk factor for breast cancer in survival analyses adjusted for histopathological characteristics. These results are in accordance with previous studies [70, 117-119, 160]. When multivariate survival analyses were stratified by method of detection in the Norwegian cohort, only luminal B-like HER2- and TNBC showed a statistically significant higher risk of breast cancer death than luminal A-like.

In terms of distribution of molecular subtypes, our studies showed similar percentages, luminal A-like tumours being the most common subtype and the HER2 positive subtype the least frequent. These results are consistent with previous studies from Europe and the US, although differing definitions of molecular subtypes will have contributed to differences in results [116, 118, 160, 161]. The most notable differences regarding distribution of molecular subtypes are found when comparing our results with analyses based on Asians and Pacific Islanders, who have a higher proportion of HER2 positive subtype; and African American women, who exhibit a higher rate of TNBCs [54, 119, 120].

There is currently a lack of an international classification of molecular subtypes, but the most widely used is that proposed by the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer [106, 113, 115]. Biannually, experts on breast cancer review and approve many aspects of the treatment of early invasive breast cancer according to new insights in breast cancer research. With regard to molecular subtype definitions, changes mostly refer to the classification of tumours responsive to endocrine treatment. These variations and the lack of an international consensus cause classification heterogeneity among studies. Table 6 illustrates the definition of luminal subtypes adopted before and in our studies. All of them used ER, PR and HER2, but only some include histological grade or Ki-67 to distinguish luminal A-like from luminal B-like [118, 160, 162]. Few studies separated luminal B-like into two subtypes [118, 160].

Studies	**Luminal A-like**	**Luminal B-like**
Carey *et al*. [54]	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Dawood *et al*. [118]	ER+ and/or PR+ and HER2- and HG 1 or 2	*ER+ and/or PR+ and HER2- and HG 3 ER+ and/or PR+ and HER2+
Domingo *et al*. [85]	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Engstrøm *et al*. [160]	ER+ and/or PR+ and HER2- and Ki-67 <15%	*ER+ and/or PR+ and HER2- and Ki-67 ≥15% ER+ and/or PR+ and HER2+
Haque *et al*. [117]	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Preat *et al*. [162]	ER+ and/or PR+ and HER2- and Ki-67 <14%	ER+ and/or PR+ and HER2+ and/or Ki-67 ≥14%
Sihto *et al*. [70]	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Spitale *et al*. [116]	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Puig-Vives *et al.* Article 3	ER+ and/or PR+ and HER2-	ER+ and/or PR+ and HER2+
Puig-Vives *et al.* Article 4	ER+ and PR+ and HER2-	*ER+ and PR- and HER2- ER+ and PR+/- and HER2+

Table 7. Definition of luminal A-like and luminal B-like used in different studies. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; HG: Histological grade; PR: Progesterone receptor. *Luminal B-like was separated into two subtypes: Luminal B-like HER+ and luminal B-like HER-**.**

For the Norwegian study, we used the molecular subtype classification from the most recent St Gallen report, which for the first time proposed the requirement of substantial PR positivity (≥20%) in the definition of luminal A-like disease and that either a high Ki-67 or low PR value be used to distinguish luminal A-like and luminal B-like HER2- (Table 4 and Table 6) [106]. As Ki-67 status information was not available for most of the cases, PR was used to separate luminal A-like from luminal B-like HER2- in the Norwegian study. A notable strength of our study is its high compliance with information on molecular subtypes (86% of the total cases). We found that women diagnosed with both luminal B-like subtypes had a statistically significant higher risk of breast cancer death than women with luminal A-like cancer. The increased risk remained only for luminal B-like HER2- after adjusting for method of detection, tumour size, histological grade, lymph node involvement and age. PR therefore might play an important role in the outcome of these tumours and deserves further research.

In the Spanish study, carried out prior to the Norwegian one, the classification of molecular subtypes used was based on the St Gallen International Consensus of 2011 (Table 6) [113]. However, as information on Ki-67 was not available here either, we could not apply this definition precisely. In the Spanish study we thus called the luminal molecular subtypes ER+ and/or PR+ and HER2-; and ER+ and/or PR+ and HER2+. Here, no statistically significant differences regarding RER were detected comparing these two subtypes. Using this classification, cases that would belong to the luminal B-like HER2- subtype were included in the ER+ and/or PR+ and HER2- subtype. Luminal B-like HER2- tumours are associated with a more aggressive behaviour than luminal A-like, as shown by the Norwegian study. If tumours showing either a high Ki-67 or low PR value classified as ER+ and/or PR+ and HER2- subtype were reclassified as luminal B-like HER2-, the ER+ and/or PR+ and HER2- subtype might have presented a better outcome.

We have observed that the definition used for luminal subtypes is important in evaluating prognosis and use of the most recent classification is recommended. Luminal B-like appears to have been related with a similar risk of death as luminal A-like in the results from Spain, but the two luminal B-like subtypes (HER2+ and HER2-) showed different outcomes in the Norwegian study. Further investigation is needed to identify molecular subtypes with homogenous clinical and biological behaviour. This would allow clinicians to more accurately estimate the prognosis of a patient, her risk of recurrence and the possible benefits of cytotoxic drug administration. This information will be also a useful tool in helping patients make their own decisions regarding their cancer.

To improve existing molecular subtype classification, Maisonneuve P *et al*. recently proposed a new definition of HER2-negative endocrine responsive breast cancers (Table 7) [163]. They suggest using <14% and ≥20% as thresholds for Ki-67 for the distinction of luminal A-like and luminal B-like HER2- subtypes. When the percentage of Ki-67 positivity is between 14% and 19% (intermediate), a high expression (≥20%) of PR would be required to define luminal A-like

and a low expression (<20%) to identify luminal B-like HER-. As these authors have stated, the main implication of this modification will be the impact on decision-making regarding the possible benefit of adjuvant cytotoxic therapy. In fact, most of the classification changes ever proposed aim to define groups of tumours that respond to specific treatment in order to obtain tailored therapy and avoid overtreatment.

Molecular subtype	**Definition**
Luminal A-like	*all of*: ER+ HER2- *and at least one of:* Ki-67 "low" (<14%) Ki-67 "intermediate" (14-19%) and PR "high" (≥20%)
Luminal B-like HER2-	*all of*: ER+ HER2- *and at least one of:* Ki-67 "intermediate" (14-19%) and PR "negative or low" (<20%) Ki-67 "high" (≥20%)

Table 8. New proposal for surrogate definitions of intrinsic subtypes for HER2-negative endocrine responsive breast cancer. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor. Adapted from Maisonneuve *et al*., 2014 [163]**.**

Regarding the definition of TNBC, the most commonly adopted is the low or lack of expression of ER, PR and HER2, which is the classification recommended by the Panel of Experts of the St Gallen Consensus in recent years [106, 113]. However, as shown in Table 8, this classification is not completely uniform across studies. Although most use only ER, PR and HER2 negativity, others also include CK5 and/or CK6 and/or HER1 positivity in the definition [54, 70, 118, 160] or identify another subtype category, similar to TNBC [70, 160].

The recommended cut-off for ER, PR and HER2 positivity has also changed over the decades. Results from a study including 7 countries (Belgium, France, Italy, Portugal, Spain, Switzerland and Uruguay) confirmed that the criteria for biomarker positivity in breast cancer varied among countries, within countries and even within the area covered by a cancer registry [164]. Although stained tumour sections are reviewed by pathologists in some studies, data on biomarker expression are usually obtained from pathology reports, which serve as a basic data source for population-based cancer registries. In our studies, hormonal receptor status and HER2 overexpression were recorded from pathology and clinical reports, which may have led to diversity in biomarker status information.

Studies	Triple-negative breast cancer / basal-like	Others
Carey *et al*. [54]	ER- and PR- and HER2- and CK5/6+ and/or HER1+	-
Dawood *et al*. [118]	ER- and PR- and HER2- and CK5/6+ and/or HER1+	-
Domingo *et al.* [85]	ER- and PR- and HER2-	
Engstrøm *et al*. [160]	ER- and PR- and HER2- and CK5+ and/or HER1+	5 Negative Phenotype: ER- and PR- and HER2- and CK5- and HER1-
Haque *et al*. [117]	ER- and PR- and HER2-	-
Preat *et al*. [162]	ER- and PR- and HER2-	-
Sihto *et al*. [70]	ER- and PR- and HER2- and CK5/6+ and/or HER1+	Nonexpressor type: ER- and PR- and HER2- and CK5/6- and HER1-
Spitale *et al*. [116]	ER- and PR- and HER2-	-
Puig-Vives *et al.* Article 3	ER- and PR- and HER2-	-
Puig-Vives *et al.* Article 4	ER- and PR- and HER2-	-

Table 9. Definition of triple-negative breast cancer / basal-like subtype used in different studies. CK: Cytokeratin; ER: Estrogen receptor; HER: Human epidermal growth factor receptor; PR: Progesterone receptor.

All the circumstances discussed above, including heterogeneity in molecular subtype definitions and cut-offs for biomarker positivity, make comparisons of results between reports difficult [54, 70, 85, 116-118, 160, 162]. Furthermore, differences in the statistical methodology used to estimate survival, the study period and the size of the study population contribute to complicating comparisons between studies. In relation to our studies, the higher rates of survival of the Norwegian women compared to the Spanish women are partly attributed to differences in survival estimate. Whereas breast cancer-specific survival was calculated in the Norwegian study, relative survival was used in the Spanish study because cause of death was not available. Despite differences in methodology, however, both analyses concluded that risk of death and risk of breast cancer death is higher for the TNBC (RER = 3.2; CI 95%: 2.3 to 4.4 and HR = 2.9; CI 95%: 1.9 to 4.5) and HER2 positive subtypes (RER = 1.7; CI 95%: 1.2 to 2.6 and HR = 2.0; CI 95%: 1.1 to 3.4) than for the ER+ and/or PR+ and HER2-; and luminal A-like subtypes after adjusting for histopathological characteristics. Therefore, molecular subtypes defined by IHC biomarkers are of highly relevant prognostic value for women diagnosed with breast cancer. This information is very valuable for both patients and clinicians alike, particularly in guiding clinical decisions.

Interestingly, HER2 positive and TNBC subtypes showed similar histopathological characteristics in Articles 3 and 4. The proportion of cases with a high histological grade was about 70% for these subtypes in both studies, whereas the percentage was much lower for the ER+ and/or PR+ and HER2- and luminal A-like subtypes (22% and 11%). However, survival rate for the HER2 positive subtype was higher than for TNBC, which agrees with results from studies carried out during the post-trastuzumab era [116, 119]. Contrarily, analyses including incidence cases from the pre-trastuzumab era found that patients with a HER2 positive breast tumour had lower survival than TNBC [117, 118]. This increased survival for women with a tumour overexpressing HER2 may reflect the benefits of trastuzumab treatment. Currently, women diagnosed with a TNBC remain the population with the lowest survival. Women with TNBC do not benefit either from hormonal or HER2-targeted agents because these tumours exhibit a low expression of ER, PR and HER2. Therefore, the approach to treatment is often only based on surgery, radiotherapy and chemotherapy. To find a target therapy for TNBC we must first understand the molecular pathology of this disease. Over the last few years, novel treatment strategies have reached advanced stages of clinical evaluation in TNBC patients [129, 130]. Some of these target angiogenesis (VEGF and VEGFR, vascular endothelial growth factor receptor), DNA repair (PARP1/2, poly (adenosine disphosphate-ribose) polymerase) or cell proliferation (HER1 and mTOR, mammalian target of rapamycin) (Table 9). TNBC is a highly proliferative neoplasm that needs constant angiogenesis to progress, making VEGF and VEGFR therapeutic targets in these types of tumour. PARP plays a key role in pathways involved in repairing DNA damage. By inhibiting this molecule, genomic instability and cell death occur in some TNBC. Although HER2 is not overexpressed in TNBC, HER1 is present in a subset of these tumours and is involved in cancer formation and/or progression. Finally, mTOR is also

involved in cell growth, proliferation and survival. Currently, preclinical and clinical studies have confirmed that the best treatment approach for TNBC patients would be a combination of different target agents or in combination with traditional chemotherapy. In addition, these studies have shown that response to treatment is heterogenic within triple-negative tumours, thus indicating that future biomarkers need to be developed to better define subsets of this molecular subtype.

Therapeutic targets	**Agents**
VEGFR, VEGR	Bevacizumab, sunitinib, sorafenib, apatinib, cediranib
PARP1/2	Olaparib
HER1	Cetuximab, erlotinib, neratinib, lapatinib
mTOR	Temsirolimus, everolimus

Table 10. Some therapeutic targets and related agents under investigation in triple-negative breast cancer. HER1: Human epidermal growth factor receptor 1; mTOR: Mammalian target of rapamycin; PARP: Poly (adenosine disphosphate-ribose) polymerase; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor. Adapted from Crown *et al.*, 2012 and Duffy *et al.*, 2012 [129, 130].

3. Breast cancer survival according to method of detection

The risk of dying from breast cancer remained higher, with an HR of 2.7 (95% CI: 1.9 to 3.7), among Norwegian women with non-screen versus screen-detected breast cancer after adjustment for age at diagnosis, molecular subtype, tumour size, histological grade and lymph node involvement. This finding indicates that other factors such as comorbidity, socioeconomic level, health awareness and symptomatology may explain favourable survival among screen-detected cancers. Our results therefore show the benefits of the NBCSP and provide arguments for retaining it. Further analysis is required on breast cancer survival by method of detection for the Girona population to determine whether the result obtained by the NBCSP can be extrapolated here, and thus demonstrate the benefits of mammographic screening in Girona province.

Women with screen-detected cancers have a strong survival advantage over women with cancers detected without screening [67-72, 165]. Table 10 shows the adjusted HR of non-screen versus screen-detected breast cancers obtained in our and seven other European studies. Most studies conclude that method of cancer detection is a prognostic factor for breast cancer regardless of histopathological characteristics. However, there are differences regarding methodology. We calculated breast cancer-specific survival, whereas others measured distant disease-free survival or survival from any cause of death. Time of follow-up varies from 5 years to 15 years. In addition, studies do not use the same covariates for adjusting the Cox multivariate analysis, or when they do, they are usually categorized differently. For example, Wishart *et al.* adjusted HR for the Nottingham Prognostic Index (NPI, excellent, good, moderate 1, moderate 2 and poor) and age as a continuous variable, whereas Sihto *et al*. added molecular subtypes (luminal A, luminal B, HER2+/ER-, basal-like, nonexpressor), tumour size, number of positive axillary lymph nodes, histological grade (1 and 2-3) and age at diagnosis (<35 and 35-69) to the multivariate analysis [67, 70].

Compared with previous studies, our analysis included a higher number of cases, around 7000; cases were diagnosed more recently, 2005-2011; and data were not derived from a clinical series, but a nationwide population-based Cancer Registry. The fact that cases were diagnosed recently allowed for the evaluation of approaches to treatment used in the last decade. Most previously published articles were based on cancers diagnosed in the 1990s, when targeted therapies were not yet available and mammographic screening programmes were in the start-up phase.

Studies	n	Years of diagnosis	Non-screen *vs.* Screen-detected breast cancers HR (95% CI)
Crispo *et al*. [47]	448	2004-2006	2.7 (0.9 – 7.8)
Dawson *et al*. [48]	1379	1991-1996	1.5 (1.0 – 2.2)
Joensuu *et al*. [49]	1983	1991-1992	1.9 (1.2 – 3.1)
Lehtimäki *et al*. [50]	1934	1991-1992	1.7 (1.1 – 2.7)
Mook *et al*. [51]	978	1997-2000	1.6 (1.0 – 2.5)
Sihto *et al*. [33]	1236	1991-1992	1.8 (1.1 – 2.8)
Wishart e*t al*.[52]	5604	1998-2003	1.3 (1.0 – 1.6)
Puig-Vives *et al.* Article 4	6762	2005-2011	2.7 (1.9 – 3.7)

Table 11. Survival multivariate analysis from seven European studies. CI: Confidence interval; HR: Hazard ratio.

The survival benefit associated with screen-detected cancers is partially due to detection of early-staged and slowly growing tumours. Lead time and length bias represent a concern when comparing survival of screen versus non-screen-detected breast cancers. To minimize the effect of these biases, we adjusted the model for tumour size, lymph node involvement, histological grade, age and molecular subtype, similarly to previous studies, although a complete adjustment is still a challenging task [72, 166]. It has been shown that histological grade and molecular subtype adjustment decreases the influence of length bias [67, 71, 167]. Low histological grade tumours are associated with slowly growing tumours, whereas high histological grade tumours are related to rapidly growing cancers. Molecular subtypes are also associated with different tumour growth. True interval cancers, which are rapidly growing tumours, are more frequently TNBC than screen-detected breast cancers, which are not usually rapidly growing tumours [84, 85]. Considering that lead time bias refers to the detection of tumours in an earlier stage by screening, it is reduced by adjusting for tumour size and lymph node status. Although all these factors diminish potential effects of lead time and length bias, it has to be taken into account that some small residual bias may remain after adjustment.

Screen and non-screen-detected breast cancers are diagnosed in the same hospitals in Norway and women are offered treatment regardless of how the cancer was detected. However, participants on the screening programme are usually more aware of health issues than women who do not attend screening. This may lead to a selection bias that could have an impact on our results.

Finally, we have confirmed that both molecular subtypes and method of detection provide prognostic information regardless of age at diagnosis, histological grade, tumour size and lymph

node involvement in women with invasive breast carcinomas. Consequently, our results suggest that method of detection and molecular subtypes should be used in combination with traditional clinical and pathological factors to estimate prognosis for each individual breast cancer patient.

Conclusions

1. DCIS incidence and mammographic screening

1.1. The incidence of DCIS in women resident in Girona province has increased over recent decades (1983–2007). This upward trend was more pronounced among women aged 50-69, which is the target population of mammographic screening, than among other age groups. This rise was related to an increase in women undergoing mammographic screening.

1.2. Interval cancers represented a low percentage (5.8%) of all breast cancers diagnosed in women aged 50-69 in Girona province during the years after mammographic screening was first implemented (2002–2006).

1.3. Screen-detected breast cancers represented less than half of all breast cancers (44.8%) diagnosed in women aged 50-69 in Girona during the start-up phase of the mammographic screening programme (2002–2006), excluding interval cancers.

1.4. During 2002-2006, screen-detected breast cancers showed a higher proportion of early stage tumours among women aged 50-69 than non-screen-detected and interval cancers.

2. Prognostic value of breast cancer molecular subtypes defined by IHC biomarkers

2.1. The distribution of breast cancer molecular subtypes defined by IHC biomarkers diagnosed in recent years in Spain and Norway did not differ from that of other European countries.

2.2. Luminal A-like was the most frequent subtype, associated with the most favourable histopathological characteristics and the highest survival rate. Conversely, women diagnosed with TNBC had the lowest survival rate.

2.3. Breast cancer molecular subtype defined by IHC biomarkers provides prognostic value, regardless of age, tumour size, histological grade, lymph node involvement and method of detection.

3. Breast cancer survival according to method of detection

3.1. Screen-detected cancers had more favourable histopathological characteristics and higher survival rate than non-screen-detected cancers diagnosed in women aged 50-69 in Norway from 2005 to 2011.

3.2. Screen-detected cancers had a higher proportion of luminal A-like tumours than cancers detected outside screening. Conversely, TNBC were more representative in the group of cancers detected outside screening.

3.3. Method of detection provides prognostic value, regardless of age, tumour size, histological grade, lymph node involvement and breast cancer molecular subtype defined by IHC biomarkers.

Annex

Publications

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