Prevalence of silent breast cancer in autopsy specimens, as studied by the disease being held by image-guided biopsies: The pilot study and literature review

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Received February 22, 2017; Accepted May 10, 2017

DOI: 10.3892/mco.2017.1299

Abstract. Breast cancer epidemiological patterns vary in European countries, which present different incidence rates. Data have suggested that the reduction in breast cancer mortality is not only due to the early detection of the disease, but is, in almost equal part, due to screening and to the advances that have been made in molecular medicine and the development of novel therapies. The aim of the present study is to quantify the actual number of cases of breast cancer present in both of the sexes by calculating the prevalence of silent breast cancer in corpses. To achieve this quantification, bilateral subcutaneous radical mastectomies are performed in corpses of either sex above 40 years of age that lacked any clinical manifestation of the disease, and where the breast cancer or its complications was not the cause of death. Only five publications exist in the international literature based on medico-legal autopsies that were designed to define the 'natural reservoir' of the disease. To the best of our knowledge, the present study is the first one to appraise breast tissue via imaging by means of orienting the biopsy incision. In conclusion, to the best of our knowledge, the design of the present study is the first of its type, where image-guided biopsies are used to define the prevalence of silent breast cancer. The study aims to demonstrate that the 'disease reservoir' is, in reality, higher than was originally considered to be so. Furthermore, the study aims to contribute towards an improved definition of the disease by determining which tumour profiles potentially do not benefit from aggressive treatments (for example, in case where a high prevalence of low-grade ductal carcinoma *in situ* is to be detected). According to our pilot study, this analysis represents a feasible protocol.

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Key words: silent breast cancer, breast cancer prevalence, breast cancer epidemiology, cancer and forensic autopsies, breast cancer overtreatment

Introduction

Breast cancer epidemiological patterns (1) vary in European countries, presenting different incidence rates (49-148 new cases per 100,000 women) with a narrower, but still variable, range of mortality (15-36 new cases per 100,000 women).

In Portugal, there has been a gradual and progressive increase in the incidence of female breast cancer, and a progressive decrease in the mortality rate, which, according to the latest published data, is 118.5 and 30.4 cases per 100,000 women, respectively [statistics provided by the Directorate-General of Health (Direção-General da Saúde), 2016; see https://www.dgs.pt/em-destaque/portugal-doencas-oncologicas-em-numeros-201511.aspx]. According to the same report, the national screening programme covers 67.70% of the target population, with a population adhesion rate of 60.89% (www.dgs.pt.).

Breast cancer incidence and mortality patterns differ significantly among different regions within Portugal. Furthermore, the capital area (Lisbon) of the country is not officially screened, and the majority of the population is followed in private or general practice settings.

Male breast cancer is a very rare disease, comprising ~1% of breast cancers, and data are generally scant. One national study reported the diagnosis and treatment of 166 cases of male patients with breast cancer in Portugal between 1970 and 2013 (https://fenix.tecnico.ulisboa.pt/downloadFile/395145917396/resumo.pdf.). Portugal is a participant in the International Male Breast Cancer Program, coordinated by the European Organization for Research and Treatment of Cancer (EORTC) and run in conjunction with the Breast

International Group (BIG) and the North American Breast Cancer Group (NABCG) networks.

Through increasing public awareness and improving screening programmes, the early detection of breast cancer has been made possible, resulting in an increase in the incidence of small breast tumours. However, the incidence of advanced metastatic breast cancer remains stable. Approximately 10-15% of breast cancers in Portugal are diagnosed at stage IV, and almost one-third of the early breast cancers that are detected eventually relapse. Data have suggested that the reduction in breast cancer mortality is not only due to the early detection of the disease, but is, in almost equal part, a consequence of screening and the advances that have been made in terms of molecular medicine and the development of novel therapies (Clinical Science Symposium: New Insights into Epidemiology and Outcomes, ECCO, abs. no. O-410, 2014; http://ec.europa.eu/eurostat/statistics-explained/index.php/Cancer_statistics.).

The aim of the present study is to quantify the actual number of cases of breast cancer present in both sexes by calculating the prevalence of silent breast cancer in corpses. The intention is to quantify the cases of existing cancers, those not that had clinically manifested themselves. The results of our pilot study are consequently shown in the present case study. In the international literature, there are only five publications based on medico-legal autopsies that were designed to define the 'natural reservoir' of the disease. To the best of our knowledge, the present study is the first one to appraise breast tissue via imaging by means of orienting the biopsy incision.

Patients and methods

Literature review. In the present study, a thorough MEDLINE database search (from 1953 to the present day) was performed using the medical subject heading (MeSH) terms of 'breast' AND 'autopsy/ies', where, after the exclusion of case reports, hospital autopsies, breast benign disease and series over autopsies in patients with breast cancer, five publications (2-6) were identified, one of them being a meta-analysis (4) of the papers published between 1966-1997.

Table I summarises the five relevant studies that were identified. The most recent of the studies, published in 2015 by Stalsberg *et al* (5), did not enable an improved evaluation of the 'silent breast cancer' phenomenon, since it was not designed to characterise the disease 'reservoir' in the study population: The sample remained low, the age limits were outside the target population and the biopsy technique was neither oriented nor extensive.

Although the conclusion indicated that 'to definitively characterise the ductal carcinoma *in situ* (DCis) reservoir, a large prospective study of the age-specific prevalence of occult breast cancer is sorely needed' (4), and, in spite of the controversies of breast cancer screening and eventual overdiagnosis, hardly any studies have been performed since 1987, and the present review highlights the requirement for such a broad study.

Objectives. The present study aims to achieve several objectives, namely: i) to determine silent breast cancer prevalence in both genders; ii) to identify the specific profiles that influence the clinical manifestation of the disease; and iii) to characterise the age distribution of the silent breast cancer in the population

Table I. Literature review.

Year of publication	Authors	Type of study	Women	Men	Ages	Biopsy technique	Sample no.	Cis (%)	IC (%)	AH (%)	(Refs.)
1985	Bhathal *et al*	Forensic	207	None	15-97	Fixation, 3 mm, random	11	12.1	1.4	13	(2)
1987	Bartow *et al*	Forensic	490	None	15-98	Fixation, 5 mm slices, random or selected	9	0	1.8	10	(3)
1987	Nielsen *et al*	Forensic	110	None	20-54	Radiography, fixation, 5 mm slices, gross and histology	275	14.7	0.9	12	(4)
1997	Welch *et al*	Meta-analysis (1966-1997)	852	None	15-98	None		8.9	1.3		(5)
2015	Stalsberg *et al*	Forensic	54	None	15-60	Central sagital fixed, 8 blocks	8	0	0.05	0.01	(6)

Cis, carcinoma *in situ*; IC, invasive carcinoma, AH, atypical hyperplasia.

under study. The following inclusion criterion is implemented: Corpses of both sexes aged 40 or over. The exclusion criteria are mortality due to breast cancer and/or its complications, and breast tissue damage (i.e., violent death with breast injury).

Study design. Since the true prevalence of the disease has yet to be elucidated, the worst-case scenario was considered. Specifically, it was speculated that, if 50% of the studied population had undetected breast cancer, with a 5% error margin, then 384 [$n=P \times (100-P) \times z^2/d^2$, where P is the anticipated prevalence, d is the desired precision, and z is the appropriate value from the normal distribution for the desired confidence] corpses of each of the sexes would subsequently need to be examined. The samples comprising the study population were taken from the National Institute of Legal Medicine and Forensic Science (Lisbon, Portugal), following the proper tissue collection authorisation procedure.

The benefit of forensic autopsies lies in the relatively uniform age distribution of the population under study, unlike hospital samples (Table II). According to the standard procedure, once the eligibility criteria have been fulfilled and the sample collection authorisation cleared, bilateral subcutaneous modified radical mastectomy in each fresh cadaver is performed at the National Institute of Legal Medicine and Forensic Science. Tissues are subsequently transported within an appropriate container to the Hospital São Francisco Xavier (Lisbon, Portugal), and submitted for inspection, palpation, ultrasound and mammography by breast radiologists (Figs. 1-4). The imaging of the collected tissues are performed using the GE Healthcare digital mammography system, Senographe Essential™ (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), using an X-ray beam of 27 kV (range, 60-70 mA) and 10-15 decanewtons (daN) compression, depending on tissue density and size. The visualisation screen comprised 5 megapixels of resolution (GE Healthcare LOGIQ™ S7 *Expert* ultrasound system, with a medium frequency of 9-15 MHz; GE Healthcare Bio-Sciences).

Breast tissue, classified as Breast Imaging Reporting and Data System (BI-RADS) (7) category 3 or higher, is submitted to wire-guided surgical biopsy by a breast surgeon. These samples are subsequently analysed in the pathology department by an experienced hospital breast pathologist and, in case of doubt, a second pathologist, located outside the hospital, also reviews the samples.

The time frame of the study is 24-36 months, depending on the cadaver recruitment. The details of the data collection sheet are shown in Fig. 5; essentially, information concerning the patient's profile (age, ethnicity, cause of mortality, breast screening adhesion, comorbidities, medications and breast cancer risk factors), the gland's characteristics (size, weight and dimensions), the imaging characterisation of suspicious lesions (microcalcifications, stromal distortions, nodules, and so forth), lesion size, histological type, immunohistochemical profile and molecular surrogates were sought afterwards. The data collected are analysed by the hospital's statistical team.

Results and discussion

Pilot study/feasibility report. The pilot study comprised the results of the first 7 of each of the sex-specific, bilateral,

Table II. Age distribution.

Age (years)	No. of corpses
30-39	74
40-49	120
50-59	186
60-69	127
70-79	149
80-89	96
>90	1

Forensic autopsies were performed in 2014 at the National Institute of Forensic Science (Lisbon, Portugal).

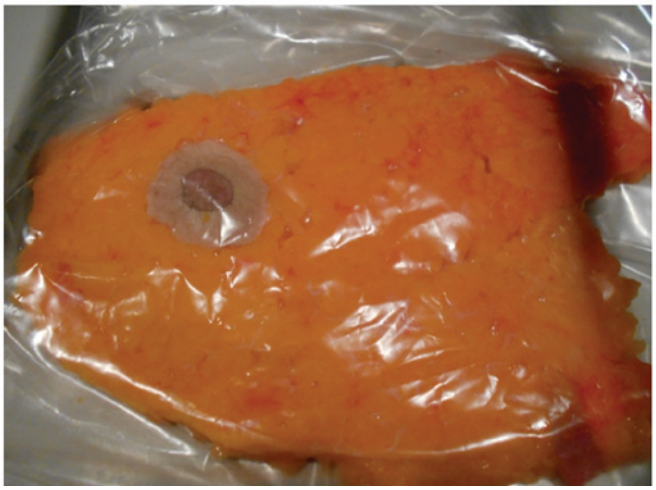

Figure 1. Left female breast sample.

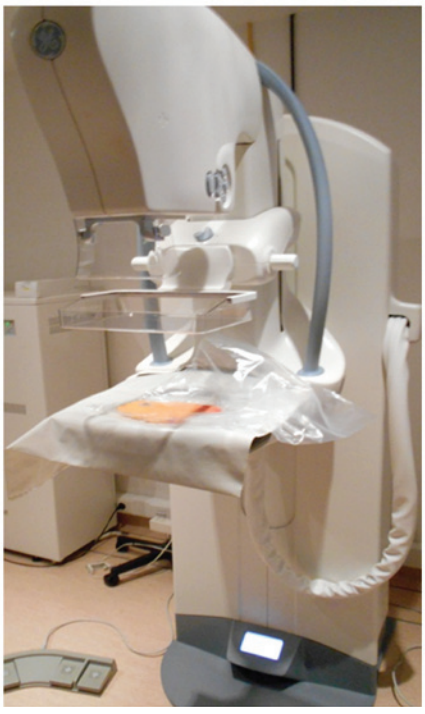

Figure 2. Mammography device and placing.

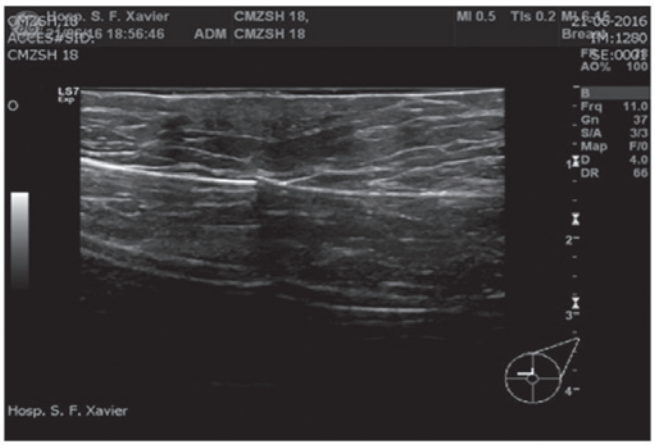

Figure 3. Ultrasonographic aspect of the tissue.

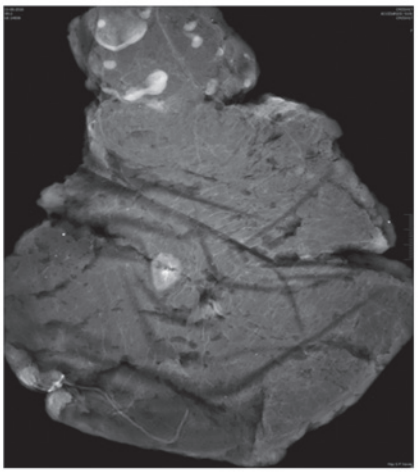

Figure 4. Mammography of the tissue.

DONOR No

Age (years)						
Ethnicity	Caucasian/ White		Negroid/ Black		Asian/ Mongoloid	
Cause of death						
BMI						
Breast screening	YES	Frequency		NO		N/A
Comorbidities						
Medication						

GLAND

	Right	Left
Weight (g)		
Size (cm x cm)		
BI-RADS		
Size of the lesion (cm)		
Characteristics imaging		
Mics		
Nodules		
Architectural distortion		
Other lesions		
Quadrant		
Number of samples		

BIOPSY

	Right	Left
Histology (CDI, CLI, CDis, CLis, other histologies)		
Molecular surrogate (luminal a, luminal b, HER2, triple negative)		
RE		
RP		
Her2		
Ki 67		
Grade		
RA (in triple negative)		

Figure 5. Details of the data collection document. BMI, body mass index; BI-RADS, breast imaging reporting and data system; Mic, microcalcification; CDI, invasive ductal carcinoma; CLI, invasive lobular carcinoma; CDis, ductal carcinoma *in situ*; CLis, lobular carcinoma *in situ*; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor.

modified radical mastectomy performed, and these details are shown in Tables III and IV for women and men, respectively.

It remains too early to present any conclusions regarding our original hypothesis, i.e., that the prevalence of silent breast cancer is going to be higher compared with the actual incidence of breast cancer. However, at the present time, it is possible to state that: (i) it is feasible to execute the prevalence definition by enlarging our time frame up to 36 months, since the

Table III. Data concerning the female corpses.

Autopsy	Age (years)	Ethinicity	Cause of mortality	BMI	Scr/	OBG index	Comorb.	Med.	Breast	Weight (g)	Size	Palp.	BI-RADS	Ecography	Mammogram	Quadrant
2016/M1	61	Caucasian	Tromboembolism	31.1	NA	NA	NA	0	RB	NA	NA	N	2	0	miCs	Disperse
									LB	NA	NA	N	2	0	miCs + macroC	Disperse
2016/M2	85	Caucasian	Intoxic.	30.8	NA	NA	NA	0	RB	NA	NA	N	2	0	miCs	Disperse
									LB	NA	NA	N	2	0	miCs	Disperse
2016/M3	74	Caucasian	W	39.7	NA	NA	Diabetes, Hypertension	Oral antidiabetics	RB	2,500	32x26	N	2	Cysts	plasmacytic mastitis	UI/UE
									LB	1,900	27x24	N	2	Ductal ectasia	miCs	Disperse
2016/M4	61	Caucasian	Heart attack	37.5	NA	NA	Hypertension	Anti-HTA	RB	1,330	27x21	N	1	0	0	
									LB	1,450	28x26	N	1	0	0	
2016/M5	45	Caucasian	W	27.2	NA	NA	NA	0	RB	1,190	29x24	N	1	0	0	
									LB	1,230	28x25	N	2	Microcysts	0	UE
2016/M6	45	Caucasian	Heart attack	30.2	NA	NA	NA	NA	RB	960	30x21	N	1	0	0	
									LB	990	30x20	N	1	0	0	
2016/M7	94	Caucasian	Respiratory failure	22.3	NA	NA	NA	0	RB	420	18x16	N	1	0	0	
									LB	490	23x14	N	1	0	0	

BMI, body mass index; OBG, obstetrics and gynaecology; Comorb., comorbidity; Scr., screening; Med., medication; Palp., palpation; NA, not available; BI-RADS, breast imaging reporting and data system; RB, right breast; LB, left breast; N, normal; miC, microcalcification; macroC, macrocalcification; UE, upper external; UI, upper inner; anti-HTA, anti-hypertensive medication.

Table IV. Data concerning the male corpses.

Autopsy	Age (years)	Ethnicity	Cause of mortality	BMI	Comorb.	Med.	Breast	Weight (g)	Size	Palp.	BI-RADS	Ecography	Mammogram	Quadrant
2016/H1	37	Negroid	Asphyxiation	27.68	NA	NA	RB	NA	17x33	N	2	Intramammary lymph nodes	ID	UE
							LB	NA	23x38	N	2	Intramammary lymph nodes	ID	UT
2016/H2	74	Caucasian	Stroke	NA	Hypertension, diabetes	NA	RB	NA	NA	N	2	Axillary lymph nodes	miCs	Disperse
							LB	NA	NA	N	2	Axillary lymph nodes	miCs	Disperse
2016/H3	86	Caucasian	Peritonitis	27.34	Colorectal Cancer	NA	RB	NA	NA	N	1	0		0
							LB	NA	NA	N	1	0		0
2016/H4	63	Caucasian	Heart attack	25.403	Congestive heart disease	Carvedilol	RB	780	23x23	N	1	0		0
							LB	940	28x25	N	1	0		0
2016/H5	48	Caucasian	Meningitis	23.94	HIV	NA	RB	147	14x13	N	1	0		0
							LB	180	23x16	P	4a	Intramammary lymph nodes		UT
2016/H6	48	Caucasian	Cranial trauma	30.72	NA	NA	RB	NA	NA	N	1	0		0
							LB	NA	NA	N	1	0		0
2016/H7	57	Caucasian	Under investigation	NA	NA	NA	RB	207	17x14	N	1	0		0
							LB	250	26x13	N	1	0		0

BMI, body mass index; Comorb., comorbidity; Med., medication; Palp., palpation; NA, not available; HIV, human immunodeficiency virus; BI-RADS, breast imaging reporting and data system; RB, right breast; LB, left breast; N, normal; P, pathological; miC, microcalcification; ID, the same; UE, upper external; UT, upper transition.

actual rate of recruitment is lower than that which was initially planned; (ii) the tissues collected in the fresh cadaver may be analyzed by means of imaging without tissue degradation, up to 48 h post-collection; and (iii) the corpse's specificities in terms of gynaecological/obstetric or medication and comorbidities profile was not able to be established for legal reasons (no access to personal files). For this reason, the determination of potential protective or harming factors is not possible.

In conclusion, to the best of the authors' knowledge, the design of the present study is the first of its kind, where image-guided biopsies will be used to define the silent breast cancer prevalence. The authors' aim is to contribute towards an improved understanding of the disease and its behaviour. In the case where a high prevalence of the disease is detected, this could imply indolent disease frequently identified in older age, without compromising either the quality of life or the life expectancy of the patient. In the case where the prevalence of the disease is low, it may be argued that this is due to the fact that the disease is extremely aggressive or rare, and therefore screening protocols may benefit from reviewing age boundaries or imaging and timing protocols. The present study aims to demonstrate that the disease 'reservoir' is higher than is actually considered to the case. Another objective of the study is to contribute towards an improved definition of the disease by determining which tumour profiles potentially do not benefit from aggressive treatments (for example, in case where a high prevalence of low-grade ductal carcinoma *in situ* is detected). According to our pilot study, this analysis represents a feasible protocol.

Acknowledgements

The authors would like to thank all the hospital staff that supported this study. The present study was supported by the Hospital São Francisco Xavier, Centro Hospitalar de *Lisboa* Ocidental (CHLO), and by the Instituto Nacional de Medicina Legal Delegação do Sul, Serviço de Clínica e Patologia Forenses (Lisbon, Portugal).

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