Breast cancer characterization in cadavers

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Method Article

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Abstract

This protocol describes a technique for data collection in autopsies corpses, and data analysis of breast cancer epidemiological patterns.

The benefit of forensic autopsies lies in the relatively uniform age distribution of the population under study, unlike hospital samples. Only five publications exist in the international literature based on medico-legal autopsies that were designed to define the ‘natural reservoir’ of the disease.

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. Tissues are subsequently transported within an appropriate container, and submitted for inspection, palpation, ultrasound and mammography by breast radiologists. The imaging of the collected tissues is performed using the GE Healthcare digital mammography system, Senographe Essential™, using an X-ray beam of 27 kV and 10-15 decanewtons (daN) compression, depending on tissue density and size. Breast tissue, classified as Breast Imaging Reporting and Data System (BI-RADS) category 3 or higher, is submitted to wire-guided surgical biopsy by a breast surgeon. These samples are subsequently analysed in the pathology department.

Introduction

The samples comprising the study population were obtained from the National Institute of Legal Medicine and Forensic Science in Lisbon, following a proper tissue collection authorization procedure.

The advantage of forensic autopsies stems from two major factors: unexpected deaths and the relatively uniform age distribution of the population under study, as opposed to hospital samples.

The study employed Cochran's (Cochran, 1977) sample size estimation procedure where the target population is infinite. Therefore, the sample size calculated at 95% confidence interval, 0.12 proportion, and precision level of 0.05 will need an estimated population size of 182 cadavers to achieve the null hypothesis.

Reagents

Equipment

Procedure

Data collection

The data collection process of the cadavers included patients' profile, gland characterization, lesion size, histological type, and molecular surrogates. Cadavers profile included age, ethnicity, comorbidities,
medications, cause of death, breast screening adhesion, and breast cancer risk factors. Gland's characteristics included dimensions, weight, and size.

**Data analysis**

The quantitative statistical method of analysis was based on the overall multi-dimension constructs measurements for every factor, descriptive statistics, regression, and parametric as well as non-parametric tests. The regression statistics was used to determine the correlation between the multi-dimension construct assessment and each factor.

**Eligibility criteria**

The study group consisted of a series of consecutive medico-legal autopsies on fresh Portuguese cadaver performed from July 2016 to December 2019 at the National Institute of Legal Medicine and Forensic Science, Lisbon, Portugal.

The criteria for exclusion were age younger than 40 years, the autopsy performed in less than 48 hours after death, extensive injury to one or both breasts, and known or clinically evident breast cancer. Once the eligibility criteria were met, and the sample collection authorization was obtained, a bilateral subcutaneous modified radical mastectomy (bsMRM) was performed through a Douformentel incision (allowing the subsequent reconstruction, previous to corpses release) in each fresh cadaver at the National Institute of Legal Medicine and Forensic Science.

**Execution**

General information, such as age, height, weight, and body mass index (B.M.I.), was obtained from the cadaver's referring file when available, while past medical history data was not included due to inadequate collection.

Each specimen was properly identified in means of spatial orientation and, after conditioning in sealed bags, was transported within an appropriate container to the Hospital São Francisco Xavier (Lisbon, Portugal), and submitted to measuring (three-dimensions), waiting, inspection, palpation, ultrasound, and mammography by breast radiologists and breast surgeon.

The collected tissues were imaged using the G.E. Healthcare digital mammography system, Senographe EssentialTM (G.E. Healthcare Bio Sciences, Pittsburgh, PA, U.S.A.), with an X-ray beam of 27 kV (range, 60-70 mA) and 10 15 decanewtons (daN) compression, depending on tissue density and size. The
visualization screen had a resolution of five megapixels (G.E. Healthcare LOGIQTM S7 Expert ultrasound system, with a medium frequency of 9 15 MHz; G.E. Healthcare Bio Sciences).

Breast tissue, classified as Breast Imaging Reporting and Data System (BI-RADS) category three or higher, was submitted to wire-guided or direct excisional surgical biopsy by the author. According to the 5th edition of the ACR BI-RADS Atlas, ACR BI-RADS (ACR, s.d.) system has been used.

In the pre-analytical phase, breast biopsies were fixed in 10% buffered formalin (JTBaker) for 24 hours, and lumpectomy specimens were fixed for 48 to 72 hours at room temperature (20°C). Formalin-fixed, paraffin (VWR International, EUA) embedded tissues were processed in Sakura’s "Tissue-Tek VIP" and cut into 3 μm sections, one cut per adhesive slide (Superfrost Plus Gold - Thermo Scientific, EUA), with respective positive control. Tissue section adhesion time and temperature were held constant for 1 hour at 70°C.

Following these procedures, the slide was subjected to labeling by the immunocytochemistry (ICC) method.

The ICC panel of primary antibodies used against Ki67 (clone 30-9, Cat. 790-4286), ER (clone SP1, Cat. 790-4324), and PR (clone 1E2, Cat.790-2223) were performed in the BenchMark ULTRA using Optiview DAB IHC Detection Kit (Cat. 760-700), for Ki67 and Ultraview Universal DAB Detection Kit (Cat. 760-500), for ER and PR, all from Ventana Medical Systems, Tucson, USA.

The slides were observed by a surgical pathologist under an optical microscope.

**Troubleshooting**

**Suggestion**

Future directions should point to a combined autopsy study, which would include a large number of glands and compare imaging findings to the histology analyses. Such a study can, in the end, provide an unblemished answer to the allocated question: "In what grade does breast cancer screening over detect the disease?"