The Breast 69 (2023) 342–348

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

Early increase in tamoxifen dose in CYP2D6 poor metaboliser breast cancer patients and survival: A propensity score matching analysis

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ARTICLE INFO

Keywords:
Breast cancer
Tamoxifen
CYP2D6
Disease-free survival
Overall survival

ABSTRACT

Purpose: Tamoxifen is a drug used for hormone receptor-positive breast cancers, primarily metabolised by the CYP2D6 enzyme into active metabolites such as endoxifen. CYP2D6 displays varying degrees of activity depending on its genotype. This study aims to analyse the effect of an early increase in tamoxifen dose in poor metabolisers (PM) on survival.

Methods: We enrolled 220 patients diagnosed with breast cancer who were treated with tamoxifen. CYP2D6 polymorphisms were determined, and the phenotype was estimated according to the Clinical Pharmacogenetics Implementation Consortium. Disease-free survival (DFS) and overall survival (OS) were analysed considering the entire patient group, and a subgroup of 110 patients selected by Propensity Score Matching (PSM). All women were treated with 20 mg/day of tamoxifen for 5 years, except PM, who initially received 20 mg/day for 4 months, followed by 40 mg/day for 4 months and 60 mg/day for 4 months before returning to the standard dose of 20 mg/day until completing 5 years of treatment.

Results: The analysis of the influence of CYP2D6 polymorphisms in the complete group and in the PSM subgroup revealed no significant differences for DFS or OS. Furthermore, DFS and OS were analysed in relation to various covariates such as age, histological grade, nodal status, tumour size, HER-2, Ki-67, chemotherapy, and radiotherapy. Only age, histological grade, nodal status, and chemotherapy treatment demonstrated statistical significance.

Conclusion: An early increase in tamoxifen dose in PM patients is not associated with survival differences among CYP2D6 phenotypes.

1. Introduction

Currently, breast cancer is the most common malignant neoplasm in women worldwide, followed by colorectal cancer and lung cancer, and it is also the cancer type with the highest mortality rate among women. Despite advances in research and treatment, recent estimates indicate that nearly 700,000 women died in one year from this disease worldwide [1].

Tamoxifen was initially used as a contraceptive in the 1960s, and later as an ovulation inducer [2]. However, it was discovered to suppress carcinogen-induced mammary tumours in rats, leading to its approval for the treatment of breast cancer in the USA in 1977 [3,4].

Tamoxifen is an anti-estrogenic drug used in patients with hormone-positive cancers. It binds to estrogen receptors, preventing hormone-receptor interactions and thereby inhibiting the expression of genes regulated by estrogen. At the cellular level, tamoxifen causes a blockage of the G1 phase of the cell cycle, reducing the cell division rate [5,6].

Adjuvant tamoxifen treatment in women with estrogen receptor

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https://doi.org/10.1016/j.breast.2023.03.012
Received 20 February 2023; Received in revised form 23 March 2023; Accepted 23 March 2023
Available online 23 March 2023

(ER)-positive breast cancer has been shown to reduce 10-year disease recurrence and 15-year mortality rates. The drug is more effective if used for 5 years rather than a shorter period [7]. In addition to ER-positive patients, progesterone receptor (PR)-positive patients also benefit from tamoxifen treatment, even in the apparent absence of estrogen receptors [5].

Cytochrome P450 is a superfamily of haemoproteins that includes several enzymes capable of metabolising tamoxifen, such as CYP2D6, CYP2C19, CYP2B6, CYP2C8, CYP2C9, and CYP3A5, among others. However, CYP2D6 is the primary metabolising enzyme for this drug [8]. Tamoxifen is metabolised into active metabolites such as 4-hydroxy-tamoxifen, N-desmethyltamoxifen, and 4-hydroxy-N-desmethyltamoxifen (endoxifen). Tamoxifen has a low affinity for estrogen receptors; however, its metabolites have a higher affinity. Endoxifen is the main metabolite of tamoxifen and, despite reaching much lower concentrations than tamoxifen, has a 100-fold higher affinity for estrogen receptors [9,10].

The response to tamoxifen may vary among patients due to different metabolisation capacities. Numerous studies have proposed that this difference may depend on the CYP2D6 genotype [11,12]. The CYP2D6 gene has a multitude of variants that have been associated with tamoxifen metabolisation. Our previous studies showed that 4-OH tamoxifen and endoxifen levels in poor metaboliser (PM) patients were four times lower than those in normal metabolisers (NM) [13]. However, in another subsequent study we conducted, we demonstrated that an early increase in the dose of tamoxifen in PM, from an initial 20 mg/day to 40 mg/day for 4 months and then to 60 mg/day for another 4 months, raised the endoxifen concentration to the levels found in NM patients treated with 20 mg/day of tamoxifen [14].

The aim of the present study was to evaluate whether CYP2D6 polymorphisms predict disease-free survival (DFS) and overall survival (OS) in tamoxifen-treated breast cancer patients after an early tamoxifen dose increase in poor metabolisers (PM). For this purpose, we analysed a complete group of 220 patients and a subgroup of 110 patients selected by propensity score matching (PSM), based on demographic and tumour characteristics.

2. Material and methods

2.1. Patients

The study prospectively included 220 patients with breast cancer diagnosed between 2000 and 2010 at Hospital Provincial de Castellón and Hospital Universitario San Cecilio de Granada (Spain). The patients were included if their tumours expressed positivity for estrogen receptor (ER) and/or progesterone receptor (PR), and they were proposed to receive tamoxifen in the adjuvant setting for a minimum period of 5 years. The mean follow-up period was 112.6 months. All women received adjuvant tamoxifen (20 mg/day) for 5 years. However, PM patients initially received a dose of 20 mg/day for 4 months and subsequently received 40 mg/day for 4 months, followed by 60 mg/day for another 4 months, and then the usual dose of 20 mg/day until completing 5 years of treatment. Demographic and clinical data such as age, histological grade, involved nodes, tumour size, HER-2 status, Ki-67 expression, as well as treatment with chemotherapy and/or radiotherapy were collected. The study was approved by the Clinical Research Ethics Committee of both hospitals and by the Agencia Española del Medicamento y Productos Sanitarios (Spanish drug regulatory agency). The study was registered in the European Union Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-002942-40/ES). All participants were informed and signed the consent form.

2.2. Genotyping of CYP2D6 polymorphisms

Genomic DNA was isolated from blood samples of the patients using the Qiagen DNA extraction kit (Qiagen). To determine the CYP2D6 cytochrome genetic variants, the AmpliChip CYP450 test (Roche Diagnostics, Indianapolis, IN, USA) was used, according to the manufacturer's instructions [13,14].

2.3. Estimation of CYP2D6 phenotype and comparative groups

Patients with low or no enzyme activity were considered poor metabolisers (PM); those with reduced activity were considered intermediate metabolisers (IM); those with normal enzyme activity were normal metabolisers (NM); and those with higher than normal enzyme activity were ultra-fast metabolisers (UM). Additionally, we established two groups: the rapid metabolisers (RM), which include UM and NM, and the slow metabolisers (SM), which include PM and IM. The predicted CYP2D6 activity score (AS) was established according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines [15]. PM had an AS of 0, IM had an AS between 0.25 and 1, NM had an AS between 1.25 and 2.25, and UM had an AS greater than 2.25.

To analyse the relationship between CYP2D6 polymorphisms and patients' DFS and OS, we considered different alternatives when segregating patients according to their expected metabolising phenotype [16]. The independent comparisons performed were: 4 groups (UM vs NM vs IM vs PM), 3 groups (RM, including UM and NM, vs IM vs PM), and 2 groups (RM, including UM and NM, vs SM, including IM and PM). In addition, we performed two other independent comparisons: AS $\leq$ x vs AS $>$ x, and AS $=$ 0 vs AS $>$ 0. We considered the analysis of the totality of patients recruited for the study (complete group), and the analysis of a subgroup selected by Propensity Score Matching (PSM) with the aim of achieving a robust adjustment for patient background factors (PSM group).

2.4. Statistical analysis

Baseline characteristics were summarised as frequency and percentage. Categorical variables were compared by the chi-square test or Fisher's exact test. PSM group was selected by logistic regression and the greedy matching method [17] using R software (version 4.2.2). For this purpose, we selected patients 1:1 in each comparison group considering the covariates age, tumour grade, nodal status, chemotherapy, radiotherapy, and tumour size. Survival analysis was performed using the Kaplan-Meier (KM) method and the log-rank test. The hazard ratio (HR) was calculated with Cox regression. Disease-free survival (DFS) was defined as the time from surgery to recurrence, other primary breast cancer, metastasis, all-cause death, or the last follow-up. Overall survival (OS) was defined as the time from surgery to all-cause death or the last follow-up. These statistical tests were performed using IBM SPSS statistics software version 28.0 (SPSS, Inc., Chicago, IL). A two-sided P-value $<$0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

The study included 220 patients, with 173 (78.6%) being 50 years of age or younger and 47 (21.4%) older than 50 years of age. According to the histological grade, there were 37 (20.8%) patients with grade I tumours, 109 (61.2%) with grade II, and 32 (18%) with grade III. 115 patients had tumours classified as N0, 67 had N1 tumours, and 25 patients had N2 tumours. Tumour size was $\leq$2 cm in 107 (53%) patients, $>$2–5 cm in 88 (43.6%) patients, and $>$5 cm in 7 (3.5%) patients. 11 (5.2%) of the patients were ER negative and PR positive, and 202 (94.8%) were positive for ER. 22 (10.4%) patients were PR negative, while 189 (89.6%) were positive. The tumours of 83 (55%) patients expressed less than 20% Ki-67, and in 68 (45%) cases the expression was $\geq$20%. 179 (82.9%) of the patients received chemotherapy treatment for early disease, while 37 (17.1%) did not. Adjuvant radiation therapy

was administered to 154 (71.3%) patients, while 62 (28.7%) did not receive it.

3.2. Genotype and phenotype of CYP2D6

The predominant alleles found among the patients were *1, found in 32% of the cases, followed by *2 (20.9%) and *4 (18.9%). With respect to the complete genotype, the most frequent was *1/*2 (14.5%), followed by *1/*1 (11.4%), *1/*4 (9.5%), and *2/*4 (8.2%). It should be noted that numerous genotypes were represented by a single patient (Table 1). According to the CPIC guidelines [15], the alleles found with normal genotype (wild-type) were *1, *2, and *35; the non-functional alleles were *3, *4, *5, *6, *15, and *31; the *10 allele corresponded to a very reduced activity; the *9, *17, *29, and *41 alleles were associated with reduced activity; and the *1XN, and *2XN were considered as alleles with increased activity (Table 2). Taking the entire genotype into account, 13 of the 220 patients had the PM phenotype (5.9%), 84 were IM (38.2%), 119 were NM (54.1%), and 4 were UM (1.8%) (Table 3). In the complete group of enrolled patients, we found 123 RM patients and 97 SM patients, whereas in the subgroup selected by PSM, there were 55 RM and 55 SM patients. In the complete group and in the PSM group there were no significant differences between RM and SM regarding the following covariates: age, histological grade, nodal status, tumour size, HER-2 status, Ki-67 expression, chemotherapy, and radiotherapy administration.

3.3. Influence of CYP2D6 polymorphism on the survival of breast cancer patients treated with tamoxifen

Considering all the comparisons detailed in Section 2.3 regarding the tamoxifen metabolising phenotype and the two study groups (complete and PSM), we found no significant differences establishing a relationship between CYP2D6 polymorphisms and DFS or OS. For example, regarding DFS in the complete group, we found 19 events (15.4%) among 123 RM patients and 18 (18.6%) events among 97 SM patients (KM: RM 136.6 vs SM 132.7 months, p = 0.47; Cox-HR vs SM 1.27, p = 0.472) (Table 4); regarding OS, 15 (12.2%) RM and 9 (9.3%) SM died (KM: RM 142.4 months vs SM 143.2 months, p = 0.538; Cox-HR RM vs SM 0.77, p = 0.539) (Table 5). Concerning the PSM group, we found 11 (20%) DFS events among the 55 RM patients and 14 (25.5%) within the SM group (KM: RM 133.9 months vs SM 127.9 months, p = 0.431; Cox-HR RM vs SM 1.37, p = 0.434); regarding OS, 7 (12.7%) RM and 6

Table 1
Frequency of alleles and genotypes in the study population.

CYP2D6 allele	%	Genotype	n (%)	Genotype	n (%)
***1**	32	***1/*2**	32 (14.5)	***1/*15**	1 (0.5)
***2**	20.9	***1/*1**	25 (11.4)	***1/*17**	1 (0.5)
***4**	18.9	***1/*4**	21 (9.5)	***1/*1XN**	1 (0.5)
***41**	7.3	***2/*4**	18 (8.2)	***1/*2XN**	1 (0.5)
***35**	5.9	***1/*35**	14 (6.4)	***1/*5**	1 (0.5)
***9**	5	***1/*41**	11 (5.0)	***1/*6**	1 (0.5)
***10**	3.4	***2/*2**	10 (4.5)	***10/*10**	1 (0.5)
***5**	2.5	***4/*4**	9 (4.1)	***10/*35**	1 (0.5)
***1XN**	0.9	***4/*35**	7 (3.2)	***17/*41**	1 (0.5)
***17**	0.7	***4/*41**	6 (2.7)	***1XN/*4**	1 (0.5)
***31**	0.7	***2/*9**	5 (2.3)	***2/*31**	1 (0.5)
***2XN**	0.5	***1/*9**	4 (1.8)	***2/*6**	1 (0.5)
***3**	0.5	***2/*10**	4 (1.8)	***2XN/*4**	1 (0.5)
***6**	0.5	***2/*41**	4 (1.8)	***3/*4**	1 (0.5)
***15**	0.2	***1/*10**	3 (1.4)	***3/*41**	1 (0.5)
***29**	0.2	***2/*5**	3 (1.4)	***4/*17**	1 (0.5)
		***4/*10**	3 (1.4)	***4/*29**	1 (0.5)
		***5/*41**	3 (1.4)	***4/*5**	1 (0.5)
		***9/*9**	3 (1.4)	***5/*10**	1 (0.5)
		***1XN/*2**	2 (0.9)	***5/*35**	1 (0.5)
		***2/*35**	2 (0.9)	***5/*9**	1 (0.5)
		***4/*31**	2 (0.9)	***9/*10**	1 (0.5)
		***4/*9**	2 (0.9)	***9/*35**	1 (0.5)

Table 2
AS* value of CYP2D6 alleles in the study population.

	CYP2D6 alleles	AS value per allele
Wild type	*1, *2, *35	1
Variants		
Unfunctional	*3, *4, *5, *6, *15, *31	0
Very reduced activity	*10	0.25
Reduced activity	*9, *17, *29, *41	0.5
Multiple copies		
Increased activity	*1XN, *2XN	2

*AS: Activity score according to the Clinical Pharmacogenetics Implementation Consortium (CPIC).

Table 3
Frequency of CYP2D6 metabolising phenotypes and AS* values.

CYP2D6 phenotype	n (%)	AS value per genotype
Poor (PM)	13 (5.9%)	0
Intermediate (IM)	84 (38.2%)	$0 < x < 1.25$
Normal (NM)	119 (54.1%)	$1.25 \leq x \leq 2.25$
Ultrarapid (UM)	4 (1.8%)	>2.25

*AS: Activity score according to the Clinical Pharmacogenetics Implementation Consortium (CPIC).

Table 4
Relationship between CYP2D6 polymorphisms and DFS in the complete patient group.

	DFS					
	N	Events (%)	SV (months; CI 95%)	*P*	HR (CI 95%)	*P*
CYP2D6						
RM (UR + NM)	123 (55.9)	19 (15.4)	136.6 (130.7–142.5)	0.47	Reference	
SM (IM + PM)	97 (44.1)	18 (18.6)	132.7 (125.2–140.3)		1.27 (0.67–2.42)	0.472
CYP2D6						
UR	4 (1.8)	1 (25)	125.3 (83.2–167.3)	0.847	Reference	
EM	119 (54.1)	18 (15.1)	137.0 (131.0–142.9)		0.62 (0.08–4.67)	0.644
IM	84 (38.2)	15 (17.9)	133.0 (124.9–141.2)		0.78 (0.10–5.93)	0.812
PM	13 (5.9)	3 (23.1)	131.4 (110.6–152.2)		0.94 (0.10–9.02)	0.955
CYP2D6						
RM (UR + NM)	123 (55.9)	19 (15.4)	136.6 (130.7–142.5)	0.736	Reference	
IM	84 (38.2)	15 (17.9)	133.0 (124.9–141.2)		1.23 (0.63–2.43)	0.546
PM	13 (5.9)	3 (23.1)	131.4 (110.6–152.2)		1.48 (0.44–4.99)	0.531
CYP2D6						
$\leq$ x (AS)	105 (47.7)	19 (18.1)	132.9 (125.5–140.2)	0.542	Reference	
> x (AS)	115 (52.3)	18 (15.7)	136.8 (130.8–142.7)		0.82 (0.43–1.56)	0.543
CYP2D6						
AS = 0	13 (5.9)	3 (23.1)	131.4 (110.6–152.2)	0.614	Reference	
AS > 0	207 (94.1)	34 (16.4)	135.1 (130.3–140.0)		0.74 (0.23–2.41)	0.616

DFS: Disease-Free Survival; CI: Confidence Interval; HR: Hazard Ratio; UM: Ultra-Rapid Metabolisers; NM: Normal Metabolisers; IM: Intermediate Metabolisers; PM: Poor Metabolisers; RM: Rapid Metabolisers (UM + NM); SM: Slow Metabolisers (IM + PM).

Table 5
Relationship between CYP2D6 polymorphisms and OS in the complete patient group.

	OS					
	N	Exitus (%)	SV (months; CI 95%)	*P*	HR (CI 95%)	*P*
CYP2D6						
RM (UR + NM)	123 (55.9)	15 (12.2)	142.4 (138.7–146.2)	0.538	Reference	
SM (IM + PM)	97 (44.1)	9 (9.3)	143.2 (138.6–147.8)		0.77 (0.34–1.76)	0.539
CYP2D6						
UR	4 (1.8)	1 (25.0)	134.3 (107.5–161.0)	0.811	Reference	
EM	119 (54.1)	14 (11.8)	142.7 (139.0–146.5)		0.50 (0.07–3.84)	0.509
IM	84 (38.2)	8 (9.5)	143.6 (139.0–148.1)		0.42 (0.05–3.36)	0.413
PM	13 (5.9)	1 (7.7)	140.6 (122.9–158.3)		0.31 (0.02–4.89)	0.402
CYP2D6						
RM (UR + NM)	123 (55.9)	15 (12.2)	142.4 (138.7–146.2)	0.796	Reference	
IM	84 (38.2)	8 (9.5)	143.6 (139.0–148.1)		0.80 (0.34–1.90)	0.618
PM	13 (5.9)	1 (7.7)	140.6 (122.9–158.3)		0.59 (0.08–4.44)	0.605
CYP2D6						
≤ x (AS)	105 (47.7)	10 (9.5)	142.8 (138.1–147.4)	0.59	Reference	
> x (AS)	115 (52.3)	14 (12.2)	142.8 (139.2–146.5)		1.25 (0.55–2.81)	0.591
CYP2D6						
AS = 0	13 (5.9)	1 (7.7)	140.6 (122.9–158.3)	0.655	Reference	
AS > 0	207 (94.1)	23 (11.1)	142.9 (140.0–145.8)		1.57 (0.21–11.65)	0.657

OS: Overall Survival; CI: Confidence Interval; HR: Hazard Ratio; UM: Ultra-Rapid Metabolisers; NM: Normal Metabolisers; IM: Intermediate Metabolisers; PM: Poor Metabolisers; RM: Rapid Metabolisers (UM + NM); SM: Slow Metabolisers (IM + PM).

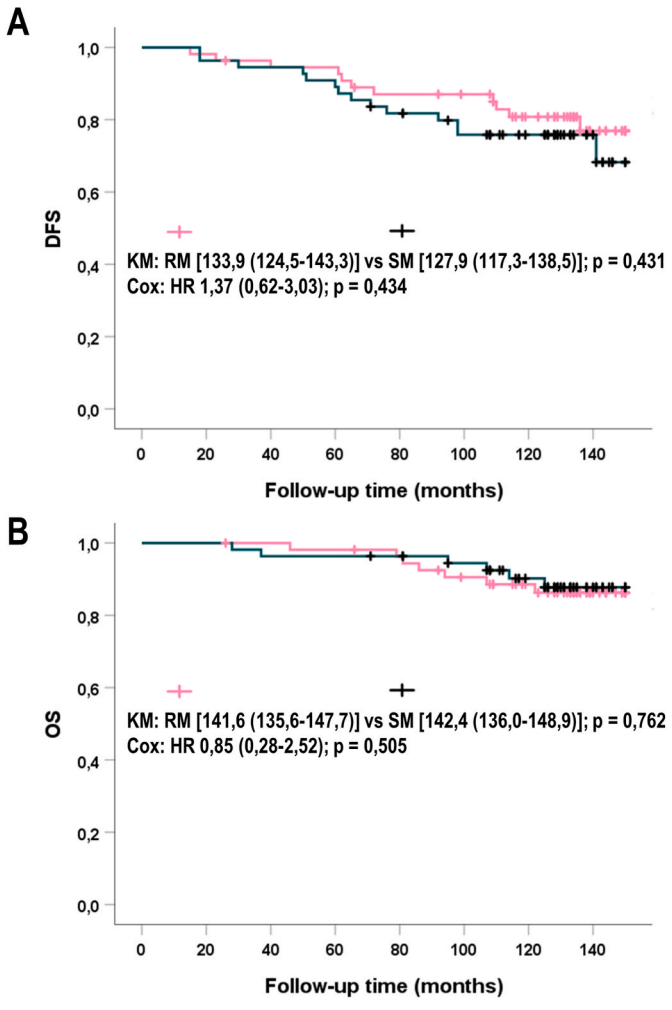

Fig. 1. Survival analysis of the selected patients by PSM. No significant differences were found between the RM and SM groups, classified according to the CYP2D6 polymorphisms and the CPIC guidelines. A) DFS. B) OS. KM: Kaplan-Meier; Cox: Cox regression; RM: rapid metabolisers (UM + NM); SM: slow metabolisers (IM + PM); HR: hazard ratio.

(10.9%) SM died (KM: RM 141.6 months vs SM 142.4 months, p = 0.762; Cox-HR RM vs SM-0.85, p = 0.76) (Fig. 1).

3.4. Influence of clinical-demographic variables on survival in breast cancer patients treated with tamoxifen

Regarding DFS, we found statistical significance in at least one of the tests used for: age (KM 131.9 months in patients ≤50-year-old vs 145.9 months in >50, p = 0.03; Cox-HR 0.29, p = 0.042), histological grade (Cox-HR 4.52/p = 0.04 in grade II vs grade I, and 5.08/p = 0.043 in grade III vs grade I), nodal status (KM: N0 139.9 months, N1 133.1 months, and N2 114.6 months, p = 0.001; Cox-HR N1 vs N0 1.86 p = 0.114, Cox-HR N2 vs N0 4.25 p = 0.001), and chemotherapy (KM 133.6 months in chemotherapy treated patients vs 147.2 months in untreated, p = 0.027) (Table 6). Regarding OS, statistical significance was found for age (KM: 141.4 vs. 148.1 months, p = 0.042), and nodal status (Cox-HR N2 vs. N0 3.21 p = 0.024) (Table 7).

4. Discussion

Tamoxifen is a drug used in adjuvant breast cancer treatment. Its effect on patients' DFS and OS has been demonstrated [5]. The metabolism of tamoxifen depends on certain cytochromes, among which CYP2D6 stands out, and this metabolism is influenced by CYP2D6 gene polymorphisms [8,13,18]. It has been suggested that the rates of DFS and OS in tamoxifen-treated patients may be related to the genotype that the patient possesses. Nevertheless, studies analysing the effect of CYP2D6 polymorphisms on the survival of tamoxifen-treated patients yield conflicting results [16].

Our previous studies showed that increasing the dose of tamoxifen in PM from 20 mg/day to 40 mg/day for 4 months and then to 60 mg/day for another 4 months significantly increased endoxifen levels, matching the levels found in NM after administration of only 20 mg/day [14]. This has also been demonstrated by other authors [19–21]. These findings prompted us to design the current study, which sought to determine the effect of early variations in tamoxifen doses in PM patients on long-term survival outcomes. In our study, we analysed DFS and OS in relation to the different CYP2D6-associated phenotypes, considering that PM received higher than standard doses. Additionally, we used a PSM-based patient selection, which allows us to achieve a robust adjustment for patient background factors. This is a novelty in studies on this topic and enables us to reduce possible bias due to differences in covariate data in our study population, making the comparisons equitable in terms of

Table 6
Relationship between clinical variables and DFS.

	DFS					
	N (%)	Events (%)	SV (months; CI 95%)	*P*	Cox-HR (CI 95%)	*P*
Age (years)						
≤50	173 (78.6)	34 (19.7)	131.9 (126.2–137.7)	0.03	Reference	
>50	47 (21.4)	3 (6.4)	145.9 (140.9–151.0)		0.29 (0.09–0.95)	0.042
Histological Grade						
I	37 (20.8)	2 (5.4)	145.7 (139.4–151.9)	0.066	Reference	
II	109 (61.2)	24 (22.0)	131.5 (124.4–138.5)		4.52 (1.07–19.14)	0.04
III	32 (18.0)	7 (21.9)	124.8 (110.4–139.2)		5.08 (1.05–24.48)	0.043
Nodal						
N0	115 (55.6)	13 (11.3)	139.9 (134.4–145.4)	0.001	Reference	
N1 (1–3)	67 (32.4)	13 (19.4)	133.1 (124.4–141.8)		1.86 (0.86–4.01)	0.114
N2 (4/+)	25 (12.1)	10 (40.0)	114.6 (95.9–133.3)		4.25 (1.86–9.71)	0.001
Tumour size (cm)						
≤2	107 (53.0)	16 (15.0)	138.4 (132.7–144.1)	0.472	Reference	
>2 - 5	88 (43.6)	18 (20.5)	130.3 (121.9–138.8)		1.52 (0.77–2.97)	0.226
>5	7 (3.5)	1 (14.3)	125.0 (101.7–148.3)		1.10 (0.15–8.34)	0.924
HER2						
Negative	190 (89.6)	32 (16.8)	134.6 (129.5–139.8)	0.572	Reference	
Positive	22 (10.4)	5 (22.7)	132.0 (116.7–147.2)		1.31 (0.51–3.37)	0.574
Ki67						
<20%	83 (55.0)	10 (12.0)	138.1 (131.0–145.3)	0.484	Reference	
≥20%	68 (45.0)	11 (16.2)	136.1 (128.1–144.1)		1.36 (0.58–3.19)	0.486
Chemotherapy						
No	37 (17.1)	1 (2.7)	147.2 (141.9–152.6)	0.027	Reference	
Yes	179 (82.9)	34 (19.0)	133.6 (128.3–138.9)		6.91 (0.95–50.48)	0.057
Radiotherapy						
No	62 (28.7)	11 (17.7)	133.5 (124.2–142.7)	0.792	Reference	
Yes	154 (71.3)	24 (15.6)	136.5 (131.3–141.8)		0.91 (0.44–1.86)	0.792

DFS: Disease-Free Survival; SV: Survival estimated by Kaplan-Meier; HR: Hazard Ratio; CI: Confidence Interval.

Table 7
Relationship between clinical variables and OS.

	OS					
	N (%)	Exitus (%)	SV (months; CI 95%)	*P*	Cox-HR (CI 95%)	*P*
Age (years)						
≤50	173 (78.6)	23 (13.3)	141.4 (137.9–144.9)	0.042	Reference	
>50	47 (21.4)	1 (2.1)	148.1 (144.4–151.8)		0.16 (0.02–1.21)	0.076
Histological Grade						
I	37 (20.8)	1 (2.7)	148.5 (145.6–151.4)	0.183	Reference	
II	109 (61.2)	15 (13.8)	141.4 (136.9–145.8)		5.30 (0.70–40.14)	0.106
III	32 (18.0)	4 (12.5)	140.0 (130.6–149.4)		5.64 (0.63–50.47)	0.122
Nodal						
N0	115 (55.6)	10 (8.7)	144.2 (140.5–147.9)	0.053	Reference	
N1 (1–3)	67 (32.4)	7 (10.4)	144.5 (140.6–148.4)		1.25 (0.48–3.28)	0.651
N2 (4/+)	25 (12.1)	6 (24.0)	131.9 (118.6–145.3)		3.21 (1.17–8.84)	0.024
Tumour size (cm)						
≤2	107 (53.0)	8 (7.5)	145.9 (143.1–148.7)	0.193	Reference	
>2 - 5	88 (43.6)	13 (14.8)	139.3 (133.5–145.1)		2.19 (0.91–5.30)	0.08
>5	7 (3.5)	1 (14.3)	133.7 (126.7–140.6)		2.13 (0.27–17.06)	0.475
HER2						
Negative	190 (89.6)	22 (11.6)	142.2 (138.9–145.4)	0.621	Reference	
Positive	22 (10.4)	2 (9.1)	145.8 (140.0–151.6)		0.70 (0.16–2.96)	0.623
Ki67						
<20%	83 (55.0)	7 (8.4)	144.6 (140.5–148.6)	0.631	Reference	
≥20%	68 (45.0)	7 (10.3)	143.1 (137.7–148.5)		1.29 (0.45–3.69)	0.632
Chemotherapy						
No	37 (17.1)	0 (0.0)	–	–	–	–
Yes	179 (82.9)	22 (12.3)	–		–	–
Radiotherapy						
No	62 (28.7)	6 (9.7)	143.0 (137.0–148.9)	0.721	Reference	
Yes	154 (71.3)	16 (10.4)	143.2 (139.9–146.5)		1.19 (0.46–3.04)	0.722

OS: Overall Survival; SV: Survival estimated by Kaplan-Meier; HR: Hazard Ratio; CI: Confidence Interval.

covariates [22,23].

We have analysed two groups (complete and PSM), considering different alternatives to segregate patients according to their expected metabolising phenotypes. However, we have not found significant differences that establish a relationship between CYP2D6 polymorphisms and DFS or OS. There are studies that, having administered the same dose of tamoxifen to all patients during the entire treatment period, found a relationship between the different CYP2D6 phenotypes and survival rates. In a study involving 306 Asian, Middle Eastern, and English patients with a mean age of 39 years, significant differences in distant relapse-free survival (DRFS; defined as the time from diagnosis to distal metastasis or death from any cause) were found between NM +

UM vs PM, classified according to the AS values [24]. In another study involving 141 Indian patients, AS $\leq$ 0.5 vs $\geq$ 1 were compared in relation to relapse free survival (RFS). It was concluded that patients with lower AS had higher rates of RFS [25]. Similarly, Karle J. et al. compared progression-free survival (PFS; time from treatment initiation to tumor progression) and OS (time from treatment initiation to any-cause death) of IM + PM vs NM patients and observed an association between both survival rates and CYP2D6 phenotypes [26].

In a meta-analysis aimed at analysing the relationship between CYP2D6 and survival, Chan CWH. et al. suggest that there is a positive association with OS (defined as the time from surgery, registration, or initiation of tamoxifen treatment until death from any cause), DFS (time from diagnosis, surgery, or recruitment until any type of recurrence occurs, contralateral breast cancer, secondary primary breast cancer, distant metastasis, or death from any cause) and the groups with decreased metabolisers [16]. These studies suggest that survival is affected by the metabolising capacity of tamoxifen. In our study, increasing the dose of tamoxifen in PM resulted in non-significant differences in survival rates among the different phenotypic groups.

However, other studies showed no differences in survival rates according to CYP2D6 phenotypes. For example, a retrospective study evaluating 71 Mexican patients [27], comparing two groups of metabolising patients (NM + UM vs IM + PM), showed that there was no relationship between DFS (considered from the start of tamoxifen treatment) and CYP2D6 phenotypes and, furthermore, that the risk of recurrence was similar for all phenotypes. In another retrospective investigation conducted with 500 USA patients, they observed that there was no association between AS (0 vs > 0) and RFS (relapse-free survival; time from surgery to the patient's first relapse or death) or OS (time from diagnosis to death) [28]. In a randomized study involving 1243 women (98% Caucasian), several comparisons were made (PM vs NM, IM vs NM, and PM + IM vs NM) in relation to the breast cancer-free interval (BCFI) (time to recurrence) and also found no significance in their results [29].

Although no significance was found in our study with respect to CYP2D6 polymorphism, other variables did. Age, histological grade, nodal status, and having been treated with chemotherapy seem to be related to DFS. Our data shows that being $\leq$50 years old is associated with a lower DFS. The effect of age on survival was also studied by Brandt J. et al., who found that women younger than 40 years had a higher probability of exitus than those aged 40 to 49 and those over 80 [30]. In addition to this article, the possible relationship between the early age of breast cancer onset and mortality has also been analysed in other studies [31,32]. Our results show that histological grade may affect DFS, with histological grade III having the worst prognosis, as found in other studies [33–35]. Axillary lymph node metastases can also predict disease status, survival, and recurrence [36,37], as shown by our results regarding N2 (4/+). In addition, another variable affecting DFS seems to be whether the patient has been treated with chemotherapy prior to surgery. However, some studies show that neoadjuvant chemotherapy can decrease the recurrence rate [38] and improve treatment outcomes [39]. The indication for chemotherapy reflects the more advanced stage of the disease, which could justify the results we have found.

One potential limitation of the study is the sample size, as the UM and PM groups included a small number of patients. This renders a robust statistical analysis of survival challenging when comparing four groups based on CYP2D6 polymorphism (UM, NM, IM, and PM). In future research, we believe it is necessary to conduct studies using a treatment scheme similar to that presented here, while incorporating a sufficient number of PM patients to ensure adequate statistical power. Such studies could help determine whether the non-statistical differences in survival among CYP2D6 phenotypic groups are associated with an early increase in tamoxifen dose for PM patients.

Additionally, our investigation focused solely on the CYP2D6 cytochrome. However, other cytochromes are involved in tamoxifen metabolism, and the varying genotypes of these additional cytochromes could influence survival outcomes. Moreover, some data were not collected from certain patients, leading to missing values for specific covariates. Lastly, another potential limitation may be the low ethnic heterogeneity of the study population.

5. Conclusions

An early increase in tamoxifen dose in PM patients is not associated with survival differences among CYP2D6 phenotypes. However, there seems to be a relationship between DFS and age, histological grade, nodal status, and chemotherapy treatment, and between OS and age.

Funding source

This work was supported by grants from the Consejería de Salud y Familias-Junta de Andalucía (PECART-0207-2020), Fundación Hospital Provincial de Castellón, Centre for Industrial Technological Development (CDTI, IDI 20080842), and by an unrestricted grant from Porcelanosa® and Roche Diagnostics.

Declaration of competing interest

Isabel Blancas received grants and support by Roche.

Acknowledgments

The content of this article is part of the PhD thesis of Marina Linares-Rodríguez within the Doctoral Program in Biomedicine of the University of Granada, Spain.

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