ASPIRIN USE IS ASSOCIATED WITH IMPROVED OUTCOMES IN INFLAMMATORY BREAST CANCER PATIENTS

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ABSTRACT

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Purpose: Inflammatory breast cancer (IBC) is the most aggressive form of breast cancer and has a high propensity for distant metastases. Our previous data suggested that aspirin (ASA) use may be associated with reduced risk of distant metastases in aggressive BC; however, there are no reported studies on the potential benefit of ASA use in patients with IBC.

Methods: Data from patients with non-metastatic IBC treated between 2000-2017 at two institutions, were reviewed. Overall survival (OS), disease-free survival (DFS), and distant metastasis-free survival (DMFS) were performed using Kaplan-Meier analysis. Univariate and multivariable logistic regression models were used to identify significant associated factors.

Results: Of 59 patients meeting the criteria for analysis and available for review, 14 ASA users were identified. ASA users demonstrated increased OS (p=.03) and DMFS (p=.02), with 5-year OS and DMFS of 92% (p=.01) and 85% (p=.01) compared to 51% and 43%, respectively, for non-aspirin users. In univariate analysis, pT stage, pN stage, and aspirin use were significantly correlated ($p < .05$) with OS and DFS. On multivariable analysis, ASA use (HR=.11, CI 0.01-0.8) and lymph node stage (HR=5.9, CI 1.4-25.9) remained significant for OS and DFS (aspirin use (HR =0.13, CI 0.03-0.56) and lymph node stage (HR=5.6, CI 1.9-16.4).

Conclusion: ASA use during remission was associated with significantly improved OS and DMFS in patients with IBC. These results suggest that ASA may provide survival benefits to patients with IBC. Prospective clinical trials of ASA use in patients with high-risk IBC in remission should be considered.

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CHAPTER 1

INTRODUCTION

It was estimated that there would be 281,550 new cases of female breast cancer (BC) in the United States in the year 2021; additionally, it was estimated that there would be 43,600 deaths in women with BC [1]. In 2017, when incidence data were analyzed, BC was the leading new cancer diagnosis in females, more than twice as frequently as the second leading diagnosis, cancer of the lung and bronchus [1]. Of all breast cancers, inflammatory breast cancer (IBC) is estimated to make up 1%-5% [2]. Although somewhat rare, IBC, which is one of the more aggressive types of BC, tends to occur in younger women, and grows and spreads rapidly [3]. There is often no underlying mass and the peau d'orange erythematous and edematous appearance typically cover more than half of the breast. Dermal lymphatic invasion is seen in approximately 75% of cases but is not required for diagnosis. However, while dermal lymphatic invasion is not required for diagnosis, pathologic confirmation of malignancy is. Compared to other BC, IBC has a higher incidence in African American women and typically occurs at a younger age [4]. Interestingly, it has been observed that women with IBC have had children at younger ages than those with non-aggressive BC [5]. High body mass index is an additional risk factor for IBC. Patients will typically receive neoadjuvant chemotherapy followed by surgery and radiation therapy. IBC is typically first diagnosed at a locally advanced stage (stage III), and one-third of patients with IBC have distant metastases at the time of diagnosis [6].

For patients with stage III disease, the 5-year survival rate was 56%. If the cancer has spread to a distant part of the body (one-third of those diagnosed), the 5-year survival rate is reduced to 19% [7, 8]. In comparison, this is a significantly worse outcome compared to other

locally advanced or metastatic BC patients which have a 5-year survival rate of 86% and 28% respectively [7].

As expected, metastasis accounts for the majority of BC [9]. As a result, significant efforts have been made to develop new therapeutic approaches for the treatment and prevention of metastases.

It is widely known that the coagulation system is activated in most cancer patients [10]. The role of this system in tumor progression has been an area of ongoing research for several years. Thus far, evidence has suggested that the body's hemostatic system is involved in multiple stages of tumor progression, including proliferation, angiogenesis, metastasis, and modulation of innate immune responses [11-15]. Further research suggests that platelets play a crucial and supportive role in metastasis [16-18]. One can postulate that platelet inhibition may interfere with tumor metastasis, and several studies have suggested such a relationship [18] via both COX and non-COX mechanisms [19-22].

Additionally, several preclinical and clinical studies have evaluated the benefits of antiplatelet therapy, specifically aspirin (ASA). ASA use has been found to be associated with improved outcomes and risk of distant metastasis in prostate [23, 24], colon [25], and breast cancers [26-33]. Furthermore, results from a large multicenter prospective trial of ASA use in high-risk BC in the United States are soon to be reported, but official findings are not yet available [34]. Prospective trials are ongoing in other countries [35]. Unfortunately, no trial has focused exclusively on patients with IBC.

Based on reports from our institution and others, we hypothesized that ASA use in patients with IBC will also reduce distant metastases and improve overall survival. In our study, we set out to determine whether patients with IBC, taking ASA in the remission period

(beginning within 2 years of diagnosis and lasting for a minimum of 60 days) and at high risk for distant metastasis would have improved outcomes compared to non-aspirin users.

CHAPTER 2

METHODS

Study populations

The Institutional Review Board (IRB) approved the registry protocol (STU 052012-019) at UT Southwestern, which permitted this study. A retrospective review of all patients diagnosed with and treated for non-metastatic IBC at UT Southwestern Medical Center and Parkland Hospital between 2000-2017 was performed.

To confirm the IBC diagnosis, all patients included in the analysis had cT4d stage disease at presentation or recurrence (or documentation of characteristics of the inflammatory stage), determined by individual chart review and confirmed by positive identification based on their clinical documentation from all available and searchable medical records [3]. Patients who did not respond to treatment and who had actively progressing cancer were excluded. Only patients who had no evidence or progression of disease on imaging and clinical evaluation between the end of therapy and the first follow-up period were considered to be in remission and were analyzed. Charts were reviewed and data were recorded for each patient's age, race, staging, tumor markers, type of surgery, chemotherapy use, radiation therapy use, medication information including ASA use, and other pertinent clinical data. HER2 positivity was defined as immunohistochemistry (IHC) 3+ or IHC 2+ with concurrent fluorescence in situ hybridization (FISH) amplification of HER2. Estrogen receptor (ER) and progesterone receptor (PR) were considered positive with any IHC staining (e.g. 1-100%). All patients were staged according to the American Joint Committee on Cancer (AJCC) 7th edition guideline. Disease free survival (DFS), distant metastasis free survival (DMFS), and overall survival (OS) were determined based on the time from the completion of the final therapy, excluding hormone therapy (e.g., end

radiation, chemotherapy, or surgery). OS was measured as the time from the last day of treatment to death or last follow-up, and DFS was calculated from the last day of treatment to the confirmation of recurrent disease in the ipsilateral breast, regional, or distant site (for DMFS). For patients who remained alive and recurrence-free, data were censored on the date of the last follow-up [28].

ASA use was the independent variable of interest. Patients were assigned to the ASA group if more than 60 days of ASA use was found in their medical records between the date of their diagnosis and two years thereafter. Those who used ASA prior to diagnosis and those who started more than two years after diagnosis were not included, and were placed in the reference group.

The criteria for ASA use for 60 days were made to identify patients consistently using ASA and omit those taking it intermittently. Additionally, two years following diagnosis was the time allowed to begin ASA use to provide adequate time for patients to start, but not too much time, since this would introduce a confounding variable given the aggressiveness of IBC.

Statistical analysis

Comparisons between the ASA and reference groups were made using the χ^2 test or Fisher's exact test for categorical variables, and the t-test for continuous variables. Kaplan–Meier survival curves for OS, DFS, and DMFS were compared using log-rank tests. Univariate analysis was performed using the Cox proportional hazards regression model with accepted major prognostic factors including age, race, T-stage, N-stage, lymphovascular invasion, Ki-67, and ASA use. Multivariable Cox proportional hazards regression analyses were conducted in a stepwise fashion using the backward selection approach, in which candidate variables with $p <$

0.10 on univariate analyses were included in the initial multivariable models. The assumption of proportionality was evaluated and was met for all variables in the Cox regression analysis. The starting point of data collection was the date of treatment completion. Data were censored for patients who were alive and recurrence-free at the last follow-up. Data were analyzed using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were 2-sided and significant was set at $p < 0.05$.

CHAPTER 3

RESULTS

Patient Characteristics

The clinical and pathological characteristics of the 59 patients with IBC are described and shown (Table 1a). A total of 45 patients did not meet the ASA criteria and were included in the control group. Fourteen patients met the ASA criteria and were included in the ASA group. Of the 14 ASA users, the median time that ASA use was started after diagnosis was 450 days (range–20-625 days). Of the 14 patients who were aspirin users, information on the reason for use was available for nine patients. Five patients were on ASA for cardiovascular issues, three for cerebrovascular issues, one for arthritis and five were unknown. The most common dose was 81 mg, although three patients received 325 mg. The duration of ASA use varied, ranging from a few months to many years. Six ASA users were administered ASA for cardiovascular diseases. ASA has been shown to prolong survival in cardiovascular disease; however, the presence of cardiovascular disease among some ASA users was not thought to be a confounding factor that would improve survival.

The median age of the control group was 53.6, and 49.0 years for the ASA group. The median (IQR) follow-up was 31.3 (14.9, 50.5) months for the control group and 62.6 (39.7, 110.5) months for the ASA group. Patient characteristics were similarly distributed between the two groups, with no statistically significant differences. Systemic therapy regimens are described and shown (Table 1b).

Effect of ASA use on DFS and OS

Univariate analysis was performed using known prognostic factors and ASA use to investigate the potential impact of ASA use on DFS and OS (Table 2). For DFS, higher pT stage

(p=.022), higher pN stage (p=.019), and lymph node positive on biopsy (cN+) (p=.006) were significantly associated with worse outcomes when evaluating typical prognostic factors. However, there was a statistically significant improvement in DFS with ASA administration (p=.017, Figure 1). In the ASA group, only two patients had recurrence (14.3%) and 26 patients (57.8%) in the control group.

There was also a significant difference in OS according to ASA use (Figure 1). In the ASA group, there was only one death (7.1%), as opposed to 21 deaths in the control group (46.7%). A significant reduction in the 5-year overall survival rate was observed in the ASA group (92% in the ASA group vs. 51% in the control group, p=.01). For OS on univariate analysis, higher pN stage (p=.011) and the presence of clinically positive lymph nodes on biopsy (p=.011) were significantly associated with worse outcomes, whereas ASA use (p=.027) was associated with improved OS. No other prognostic factors were significantly associated with OS. From the univariate analysis, factors with p-values less than 0.1(PR, pT stage, pN stage, LN positive on biopsy (cN+), and aspirin use), were further included in the multivariable Cox regression analysis. ASA use was associated with significantly improved OS (p=.029) and DFS (p=.008) in the multivariable analysis (Table 3). Multivariable analysis also demonstrated that clinical node positive disease (positive lymph nodes on biopsy) status remained a significant risk factor associated with worse DFS (p=.001) and OS (p=.007) (Table 3). Of the 59 patients, 22 died during the study period. Nineteen of the deaths were BC-specific.

Effect of ASA use on DMFS

Univariate analysis was performed to compare distant metastasis rates between the ASA and control groups. A significant reduction in the 5-year rate of distant metastases was found in the ASA group (85% vs. 43% in the ASA and control groups, respectively). A Kaplan-Meier

DMFS curve was generated, showing this significant improvement (p=.01, Figure 1). A multivariable analysis of DMFS was not performed. A total of 25 patients had distant metastases.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

Since the 1970s breast cancer therapy has continued to evolve. One of the areas of ongoing research is the relationship between tumor growth, metastasis and anticoagulation, more specifically anti-platelet medications or ASA. This relationship is based on the theory that platelet aggregation assists metastasis by increasing the arrest of tumor cell emboli in the circulation, as well as protecting tumor cells from immunological assault [36]. Thus, it is postulated that ASA, by inhibiting COX-1, can theoretically block adhesion between platelets, circulating tumor cells and the endothelium [37]. Further, additional non-COX effects are theorized to be beneficial such as the effect ASA has on heparanase and as a result the potential inhibition of metastasis and angiogenesis [38]

As evidence supports the role of platelets in supporting tumor growth and metastasis, research into anti-platelet agents such as ASA, including low-dose ASA, to improve the outcome of patients at high risk for distant metastases should be considered.

As early as the late 1960s, a link between cancer and platelets was noted [39] and the potential role of platelets in promoting tumor growth and metastasis has been appreciated [18, 40-46]. Tumor cells have been shown to activate platelets, resulting in tumor cell–induced platelet aggregation [47]. This collection of platelets around tumor cells may support growth and metastasis. Following activation, through aggregation by tumor cells, and exposure to shear stress, platelets release their stored contents, including proangiogenic factors such as vascular endothelial growth factor (VEGF), platelet–derived growth factor (PDGF), and transforming growth factor beta-1 (TGFβ1). These factors play a significant role in angiogenesis and, ultimately, invasive tumor growth and metastasis [46-52].

Platelets can also protect tumor cells from immune destruction through a variety of ways, including protection from NK cell-mediated lysis [15, 53], and transferring platelet major histocompatibility complex class I molecules to tumor cells [54], suggesting a potential role for ASA, or anti-platelet agents, in improving patients' response to immune-mediated therapies.

Although different doses of ASA are often used in the clinical setting, the main function of ASA when administered at lower doses (<100 mg) is platelet inactivation. Given that many patients were being treated for the purpose of anti-platelet therapy, (the primary use in our patient population) [55], this also supports that the improvement of outcome with ASA use seen in our patients could be due to the contributory mechanism of limiting metastasis in aggressive BC through platelet inhibition.

We have previously shown an association between aspirin use and outcomes in cancer patients at risk for distant metastases. In 2013, and 2014 our group reported that ASA use was associated with improved overall survival in patients with high-risk prostate cancer [23, 24] and triple-negative BC. These effects were not noted in patients with breast or prostate cancer with a lower risk of distant metastases. Similarly, improved results following regular ASA use after the diagnosis of colorectal cancer have also been reported [25]. Additionally, in 2010, in over 4000 women diagnosed with stage I, II, or III BC and living at least one year after BC diagnosis, ASA use was associated with a decreased risk of distant recurrence and BC death [26].

In this study, we examined a very high-risk BC population, specifically those with a diagnosis of IBC. Our data showed that ASA use was associated with a reduction in distant metastasis and was further associated with improvements in OS in a BC population at a higher risk of distant metastases. Given the improvements seen in the 5-year distant metastasis rate in

the ASA group, our findings offer further support for considering future studies of antiplatelet therapy, specifically ASA, as an adjunctive therapy for IBC.

Many questions remain regarding aspirin use, some of which are being addressed in randomized clinical trials [34, 35]. However, questions specific to the role of aspirin in inflammatory breast cancer remain, including the dosage of aspirin needed in this population, duration of use, optimal timing of therapy administration, and potential interactions with systemic therapy. Biomarkers that may further predict the selectivity of aspirin is another potential future area of study.

A significant limitation of our study was the small sample size of patients, given the rare nature of the disease. Another limitation of this study is that it is retrospective, and this study has the best hypothesis generation. We are reliant upon accurate documentation in the electronic medical record. While this is not as much of an issue for certain objective measures, it is certainly a challenge for subjective measures, such as recalling medication lists and ASA use. Additionally, we were analyzing charts from up to 16 years ago which enhances the difficulty of acquiring needed information. We hope that this and other studies will provide a basis for consideration of prospective clinical trials that include and focus on ASA use in locally advanced, non-metastatic IBC patients.

Another limitation in our study, is the nature of ASA use. Not only is it difficult due to the retrospective aspect, but also because of the inability to ensure adherence. We relied on documentation of compliance, but even when it was thoroughly documented, there is no way to be certain that the ASA was actually administered or taken, or the frequency with which it was taken. There is a spectrum of confidence in the actual amount of ASA use, depending on the different situations of the patients, that is hard to accurately depict and measure.

CONCLUSION

ASA administration initiated during the remission period was associated with significantly improved OS, DFS, and DMFS in patients with IBC. These results suggest that aspirin use may provide survival benefits for IBC patients at risk of distant metastases. Prospective clinical trials of augmented ASA use in patients with high-risk IBC in remission should be considered for development.

List of Tables

Table 1a Patient and tumor characteristics

Variable	Aspirin use after diagnosis		
	Yes (n = 14)	**No (n = 45)**	***P* value**
Age at diagnosis (years)			
Mean±SD	50.5±14.9	53.2±11	0.541
Race			
Black	4 (28.6)	9 (20.0)	0.881
White	6 (42.9)	23 (51.1)	
Hispanic	3 (21.4)	8 (17.8)	
Asian	1 (7.1)	5 (11.1)	
Race (dichotomous)			
Black	4 (28.6)	9 (20.0)	0.485
Non-Black	10(71.4)	36 (80.0)	
BMI			
Mean±SD	33.1±8.4	29.6±7	0.172
Alcohol history			
Current	7 (53.8)	10 (23.8)	0.082
Never	6 (46.2)	32 (76.2)	
Tobacco history			
Current	2 (15.4)	4 (9.5)	0.512
Never	8 (61.5)	32 (76.2)	
Past	3 (23.1)	6 (14.3)	
ER			
Positive	7 (50.0)	20 (45.5)	1
Negative	7 (50.0)	24 (54.5)	
PR			
Positive	7 (50.0)	17 (38.6)	0.593
Negative	7 (50.0)	27 (61.4)	
Her2 IHC (dichotomous)*			
Negative	7 (58.3)	32 (74.4)	0.3
Positive	5 (41.7)	11 (25.6)	
Triple negative			
Yes	2 (14.3)	12 (27.3)	0.48
No	12 (85.7)	32 (72.7)	
Ki-67 > 35%			
Yes	8 (57.1)	32 (71.1)	0.345
No	6 (42.9)	13 (28.9)	
LVI (biopsy)			

Yes	5 (50.0)	21 (72.4)	0.254
No	5 (50.0)	8 (27.6)	
cT stage			
T4d	13 (100.0)	39 (100.0)	N/A
cN stage			
N0	0 (0.0)	3 (7.8)	0.839
N1	10 (83.4)	22 (56.4)	
N2	1 (8.3)	7 (17.9)	
N3	1 (8.3)	7 (17.9)	
cN stage (dichotomous)			
N0	0 (0.0)	3 (7.7)	1
N1/N2/N3	12 (100.0)	36 (92.3)	
Stage (Clinical)			
IIIB	13 (92.9)	36 (87.8)	1
IIIC	1 (7.1)	5 (12.2)	
pT stage (dichotomous)			
T0/Tis	4 (30.8)	15 (35.7)	1
T1/T2/T3/T4	9 (69.2)	27 (64.3)	
pN stage			
N0	5 (38.5)	16 (38.1)	0.727
N1	4 (30.7)	8 (19.0)	
N2	3 (23.1)	10 (23.9)	
N3	1 (7.7)	8 (19.0)	
pN stage (dichotomous)			
N0	5 (38.5)	16 (38.1)	1
N1/N2/N3	8 (61.5)	26 (61.9)	
Complete response			
CR (pT0/pTis and pN0)	3 (23.1)	13 (37.1)	0.497
Non-CR (others)	10 (76.9)	22 (62.9)	
Chemotherapy			
Neoadjuvant	10 (76.9)	29 (65.9)	0.773
Adjuvant	1 (7.7)	3 (6.8)	
Both	2 (15.4)	12 (27.3)	
Hormones			
Yes	7 (58.3)	14 (34.1)	0.183
No	5 (41.7)	27 (65.9)	

(In parentheses are percentages)
LVI=Lymphovascular Invasion; CR=complete response
* Her2 positive was referred to a patient with Her2 IHC 3+ and/or Her2 Fish amplified.
† LN positive was referred to the number of LN greater than 0.

Table 1b Systemic therapy regimens of all patients

Variable	Aspirin use after diagnosis		
	Yes (n=14)	**No (n=45)**	***P* value**
NA AC+Taxol	7 (24.1)	20 (23.0)	0.462
NA FEC	1 (3.4)	3 (3.4)	
NA TC	3 (10.3)	12 (13.8)	
NA Carboplatin based	0 (0.0)	3 (3.4)	
NA Other	0 (0.0)	2 (2.3)	
NA Trastuzumab+Pertuzumab	2 (6.9)	10 (11.5)	
NA Trastuzumab	3 (10.3)	2 (2.3)	
Adjuvant AC+Taxol	1 (3.4)	0 (0.0)	
Adjuvant TC	0 (0.0)	2 (2.3)	
Adjuvant Capecitabine	0 (0.0)	7 (8.0)	
Adjuvant Carboplatin based	0 (0.0)	1 (1.5)	
Adjuvant Trastuzumab	5 (17.2)	12 (13.8)	
Hormones	7 (24.1)	14 (16.1)	

(In parentheses are percentages)
NA=neoadjuvant; AC=Adriamycin/cyclophosphamide; FEC=5-fluorouracil/epirubicin/cyclophosphamide; TC=Taxotere/cyclophosphamide

Table 2 Univariate analysis of major prognostic factors and aspirin use with respect to DFS and OS

Variable	Disease Free Survival		Overall Survival	
	Hazard Ratio (95% CI)	***P* value**	**Hazard Ratio (95% CI)**	***P* value**
Age at diagnosis (years)				
Age	1.006 (0.975, 1.038)	0.715	1.007 (0.974, 1.042)	0.669
Race (dichotomous)				
Non-Black	Reference	-	Reference	-
Black	0.761 (0.286, 2.022)	0.584	0.731 (0.247, 2.168)	0.573
BMI				
BMI	0.966 (0.916, 1.019)	0.204	0.965 (0.910, 1.023)	0.228
Alcohol history				
Never	Reference	-	Reference	-
Current	0.874 (0.360, 2.127)	0.767	0.636 (0.231, 1.754)	0.382
Tobacco history				
Never	Reference	-	Reference	-
Current	0.756 (0.174, 3.277)	0.709	0.506 (0.067, 3.837)	0.51
Past	1.131 (0.380, 3.366)	0.825	1.126 (0.371, 3.415)	0.834
ER				
Negative	Reference	-	Reference	-
Positive	1.286 (0.586, 2.820)	0.531	1.671 (0.701, 3.983)	0.247
PR				
Negative	Reference	-	Reference	-
Positive	1.710 (0.777, 3.763)	0.182	2.159 (0.902, 5.166)	0.084
Her2 (dichotomous)				
Negative	Reference	-	Reference	-
Positive	0.604 (0.224, 1.632)	0.32	0.373 (0.109, 1.283)	0.118
Triple negative				
No	Reference	-	Reference	-
Yes	1.137 (0.452, 2.860)	0.785	1.375 (0.530, 3.567)	0.512
Ki-67 > 35%				
No	Reference	-	Reference	-
Yes	0.644 (0.295, 1.402)	0.267	0.637 (0.271, 1.496)	0.3
LVI (biopsy)				
No	Reference	-	Reference	-
Yes	1.655 (0.526, 5.206)	0.389	1.610 (0.427, 6.075)	0.482
pT stage (dichotomous)				

T0/Tis	Reference	-	Reference	-
T1/T2/T3/T4	3.517 (1.200, 10.310)	0.022	3.271 (0.955, 11.200)	0.059
pN stage (dichotomous)				
N0	Reference	-	Reference	-
N1/N2/N3	3.267 (1.218, 8.767)	0.019	6.675 (1.545, 28.850)	0.011
Hormones				
No	Reference	-	Reference	-
Yes	1.142 (0.500, 2.606)	0.753	1.231 (0.531, 2.858)	0.628
Clinically LN positive				
No	Reference	-	Reference	-
Yes	4.531 (1.551, 13.230)	0.006	6.716 (1.562, 28.870)	0.011
Aspirin use after diagnosis				
No	Reference	-	Reference	-
Yes	0.171 (0.040, 0.727)	0.017	0.103 (0.014, 0.769)	0.027

DFS=disease free survival; OS=overall survival; CI=confidence interval; LVI=lymphovascular invasion; LN=lymph node

Table 3 Multivariate analysis of major prognostic factors and aspirin use with respect to DFS and OS

	DFS		**OS**	
Variable	**Hazard Ratio (95% CI)**	***P* value**	**Hazard Ratio (95% CI)**	***P* value**
cN+	6.165 (2.071, 18.351)	0.001	7.697 (1.761, 33.640)	0.007
Aspirin use	0.140 (0.032, 0.603)	0.008	0.106 (0.014, 0.797)	0.029

DFS=disease free survival; OS=overall survival; CI=confidence interval; LN=lymph node

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Figure 1 The effect of aspirin use on disease free survival

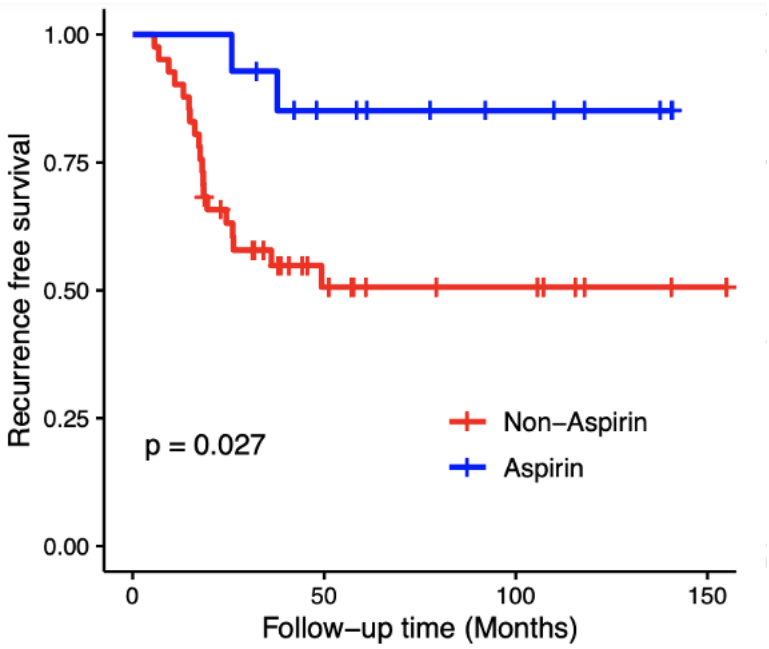


Figure 2 The effect of aspirin use on distant metastases free survival

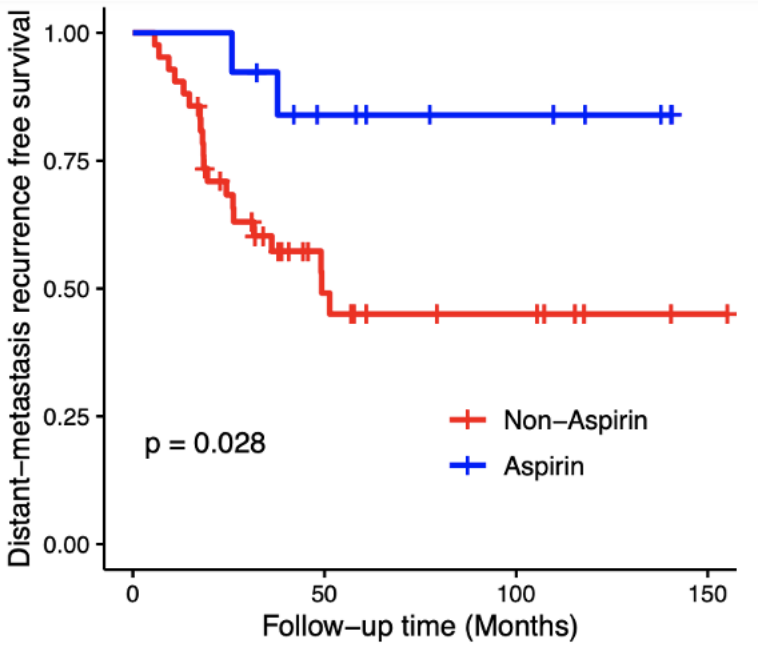
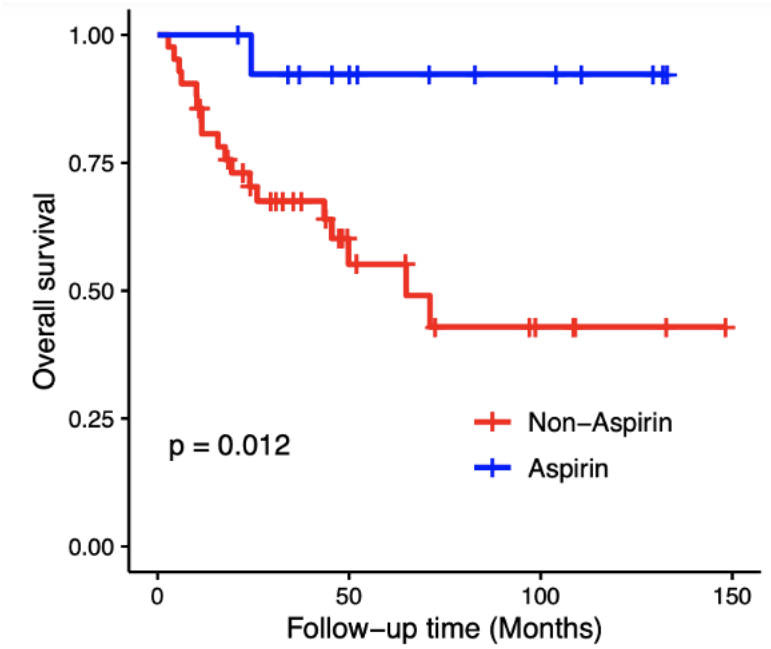


Figure 3 The effect of aspirin use on overall survival



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VITAE

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